

Identification of Race-Specific Resistance in North American *Vitis* spp. Limiting *Erysiphe necator* Hyphal Growth

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ABSTRACT

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Race-specific resistance against powdery mildews is well documented in small grains but, in other crops such as grapevine, controlled analysis of host–pathogen interactions on resistant plants is uncommon. In the current study, we attempted to confirm powdery mildew resistance phenotypes through vineyard, greenhouse, and in vitro inoculations for test cross-mapping populations for two resistance sources: (i) a complex hybrid breeding line, ‘Bloodworth 81-107-11’, of at least *Vitis rotundifolia*, *V. vinifera*, *V. berlandieri*, *V. rupestris*, *V. labrusca*, and *V. aestivalis* background; and (ii) *Vitis* hybrid ‘Tamiami’ of *V. aestivalis* and *V. vinifera* origin. Statistical analysis of vineyard resistance data suggested the segregation of two and three race-specific resistance genes from the two sources, respectively. However, in each population, some resistant

progeny were susceptible in greenhouse or in vitro screens, which suggested the presence of *Erysiphe necator* isolates virulent on progeny segregating for one or more resistance genes. Controlled inoculation of resistant and susceptible progeny with a diverse set of *E. necator* isolates clearly demonstrated the presence of fungal races differentially interacting with race-specific resistance genes, providing proof of race specificity in the grape powdery mildew pathosystem. Consistent with known race-specific resistance mechanisms, both resistance sources were characterized by programmed cell death of host epidermal cells under appressoria, which arrested or slowed hyphal growth; this response was also accompanied by collapse of conidia, germ tubes, appressoria, and secondary hyphae. The observation of prevalent isolates virulent on progeny with multiple race-specific resistance genes before resistance gene deployment has implications for grape breeding strategies. We suggest that grape breeders should characterize the mechanisms of resistance and pyramid multiple resistance genes with different mechanisms for improved durability.

Effector triggered immunity (ETI), sometimes called resistance (*R*)-gene-mediated or gene-for-gene resistance, is a common form of disease resistance that is dominantly inherited and straightforward to select in a breeding program (27). However, this type of resistance is typically short lived in the field due to the evolution of races that can overcome a specific resistance. The ETI resistance mechanism is characterized by plants recognizing pathogen effector molecules, either directly or indirectly, via receptors or nucleotide-binding leucine-rich repeat (NB-LRR) *R* genes and responding by triggering programmed cell death (i.e., a hypersensitive response). If a pathogen can evolve effectors that

evade this recognition, a new pathogen race emerges, and the resistance is overcome.

The plant pathogens causing powdery mildews are a collection of obligately biotrophic fungi in the order Erysiphales causing host-specific disease on ≈10,000 plant species, including many agriculturally and horticulturally important crops (23). In many powdery mildew pathosystems, pathogen races have evolved that are virulent on resistant host genotypes (2,8,15,28–30,32,35), several of which have been shown to fit the classic gene-for-gene resistance model proposed by Flor decades ago (17). No races are known in the grape powdery mildew pathosystem, caused by *Erysiphe necator* (syn. *Uncinula necator*) on *Vitis* spp. and other members of the Vitaceae (20). The prominent reliance on fungicide application rather than host resistance to manage grape powdery mildew in commercial production may have obscured any observation of race-specific resistance (20). However, as a result of repeated failures of targeted fungicide classes (14), there is a growing interest in grape cultivars with powdery mildew resistance to complement existing management tactics (20).

In grapevine, the best characterized resistances, *Run1* and *Ren1*, have hallmarks of ETI genes, based on their dominant gene action that maps to NB-LRR-encoding regions of the genome, with the resistance phenotype being associated with accelerated single-cell necrosis (3,9). Although not yet deployed in com-

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mercial cultivars, *Run1* has been introgressed into *Vitis vinifera* from *V. rotundifolia*, whereas *Ren1* is a rare example of powdery mildew resistance naturally occurring in *V. vinifera* in a few cultivars planted in Central Asia (25).

Eastern North America is likely the center of origin for *E. necator* (4,19). Whereas variation for resistance to powdery mildew exists within all gene pools of the Vitaceae (6), the North American *Vitis* spp. have been of particular interest. In addition to *Run1* from *V. rotundifolia*, mapping and quantitative trait loci studies arising from breeding populations derived from North American germplasm have identified *Ren2* from *V. cinerea* (10) and *Ren3* from the *Vitis* interspecific hybrid 'Regent' (1,16,26, 40). The prevalence and variation for resistance to powdery mildew is indicative of the coevolution of this host and pathogen in North America. In addition to inter- and intraspecific variation in susceptibility, the inconsistency of ratings between environments has been noted (6). For example, *V. labrusca* accessions planted near Lake Erie, where they were surrounded by juice-grape production (primarily *V. labrusca* interspecific hybrid cultivars), were more susceptible than those planted only 200 km away in the Finger Lakes region of New York, where they were surrounded by wine-grape production (predominantly *V. vinifera*) (6). One explanation for differential responses to these nearby, similar environments could be the presence of *E. necator* races in the two different areas, a situation that can be unintentionally selected by long-term monoculture farming systems and one that has been well documented in other powdery mildew pathosystems (15). Although no studies have directly demonstrated differential virulence of *E. necator* isolates on single resistance genes, host specialization has been suggested (18,21), in which isolates were shown to have different host ranges. However, because only a single host genotype was used to represent each *Vitis* sp., the differential susceptibility described could have been either at the host species level (host specialization) or single-resistance gene level (race specificity) (20).

A consistent, broad-spectrum resistance phenotype conferred by *Ren4* was recently described (34) and mapped to *V. vinifera* chromosome 18 (33). In the current study, we evaluated progeny segregating for powdery mildew resistance from two additional sources: 'Bloodworth 81-107-11' and 'Tamiami', each backcrossed to susceptible *V. vinifera*. Individual progeny of the backcross populations had inconsistent resistance phenotypes, which is in contrast to results in side-by-side tests with progeny segregating for *Ren4*, for which resistance was consistent (34). This led us to test directly the hypothesis that these two breeding populations were segregating for ETI that could be specific to races of the pathogen present in different locations. We addressed this hypothesis with two objectives: (i) to identify isolates of the pathogen differentially recognized by each of the two resistance sources and (ii) to provide quantitative and descriptive analysis of early pathogenesis on each resistance source, as a reference for future studies.

MATERIALS AND METHODS

Germplasm. The complex hybrid Bloodworth 81-107-11 was obtained from Jeff Bloodworth, Hillsborough, NC. Its parentage includes at least *V. rotundifolia*, *V. vinifera*, *V. berlandieri*, *V. rupestris*, *V. labrusca*, and *V. aestivalis* (Supplemental Figure 1). *Vitis* hybrid Tamiami is an F₁ hybrid resulting from the cross 'Fennell 6' (*V. aestivalis*) × 'Malaga' (*V. vinifera*). These accessions were selected for the breeding program based on their resistance to natural infection by *E. necator* when grown in Parlier, CA. The segregating F₁ populations 02-3517 and 03-3513 were developed in 2002 and 2003 by the cross Bloodworth 81-107-11 × 'C67-120' (*V. vinifera*), which resulted in 9 and 15 segregating progeny, respectively, used in the current study. In 2002, the segregating modified-backcross₁ (mBC₁) 02-3512 population with

Tamiami resistance was developed by the crossing B37-28 (Tamiami × *V. vinifera*) × C56-11 (*V. vinifera*), resulting in 94 segregating progeny in the current study. C67-120 (susceptible), B37-28 (resistant), and C56-11 (susceptible) are breeding parents in the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) grape breeding program at Parlier, CA.

Segregating progeny were established in the greenhouse to characterize powdery mildew resistance. For each segregant, 14 dormant cuttings were prepared with the goal of obtaining seven healthy plants: three plants for greenhouse evaluation, two for microscopy, and two for field planting. Cuttings were rooted by dipping in Hormex number 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. The above crosses were repeated in 2007, resulting in Bloodworth 81-107-11-derived population 07-3510 and Tamiami-derived population 07-3509 for testing the hypothesis of race-specific resistance and characterizing the resistance mechanism by light microscopy in Geneva, NY.

Greenhouse disease evaluation. Progeny of 02-3517, 03-3513, and 02-3512 were screened for resistance to powdery mildew as young vines in USDA-ARS, Parlier, CA greenhouses because disease incidence on rooted cuttings can predict the future incidence and severity of mildew infections on mature vines in the vineyard (31). Dormant grapevine cuttings taken in January were rooted and established in the greenhouse in February 2006. Vines in the greenhouse that were several months to 1 year old were grown in Anderson pots, 6.35 cm square by 17.8 cm high (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 randomly placed test vines to provide the natural inoculum source for the test vines. The growing conditions in the greenhouse were 29°C during the day and 24°C at night with a 12-h photoperiod maintained by supplemental lighting from 6:00 a.m. to 6:00 p.m. Plants were irrigated as needed and fertilized every 2 weeks. Symptoms were evaluated when 70% of susceptible control Ruby Seedless leaves exhibited conidiating 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 (percent leaves with one or more powdery mildew colonies) and severity (percent foliage covered by powdery mildew colonies) scores were recorded separately, then averaged and categorized as resistant (R), 0 to 19%; moderate (M), 20 to 39%; and susceptible (S), 40 to 100%. The higher index rating from the two dates is presented here.

A similar approach was used to screen for resistance on young vines in USDA-ARS, Geneva, NY greenhouses, in order to identify resistant seedlings for testing the hypothesis of race specificity and for characterizing resistance mechanisms microscopically. Seed of 07-3510 and 07-3509 progeny were germinated in December 2007, resulting in evaluation of 213 and 49 seedlings from the two populations, respectively. Seedlings were maintained in pots, 8.26 cm square by 8.26 cm tall (T. O. Plastics, Clearwater, MN). Foliar disease incidence and disease severity ratings were recorded separately when >70% of leaves on susceptible 'Chardonnay' seedlings exhibited conidiating colonies. After the first evaluation in August 2008, the epidemic was allowed to progress further for a second evaluation in September 2008. Progeny with no macroscopically visible disease were selected for light microscopy and differential inoculation experiments described here.

Vineyard disease evaluation. Progeny of 02-3517, 03-3513, and 02-3512 were grown at USDA-ARS San Joaquin Valley Agricultural Sciences Center, Parlier, CA. The soil is a fine sandy loam and the vines were drip irrigated. Vines were grown on their own roots at 4-by-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. Highly susceptible *V. vinifera* Ruby Seedless plants were planted 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, once the plants started fruiting (in their third growing season). Foliar disease severity ratings were recorded based on percent of foliage covered by powdery mildew colonies: 1 = no visible infection (R), 2 = very few small colonies (M), 3 = <50% coverage (S), and 4 = >50% coverage (S). In all cases, the susceptible Ruby Seedless vines were rated susceptible.

Laboratory disease evaluation. Detached leaves were collected on 12 September 2006, from disease-free potted vines of populations 02-3517, 03-3513, and 02-3512 from the USDA-ARS, 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 were surface sterilized in calcium hypochlorite at 0.88 g/liter as described previously (6). The leaves were plated adaxial side up onto 100-by-15-mm petri dishes containing 18 ml of 1% water agar amended with nystatin at 0.01 g/liter (Haorui Pharma-Chem, Edison, NJ). Residual water was evaporated and inoculation was conducted using isolate 10-18-1 collected from a Chardonnay plant in Dresden, NY in 2003 (6). Spore suspensions were made by shaking leaves with conidiating powdery mildew colonies in 40 ml of distilled water with 0.001% (vol/vol) Tween 20 (Sigma-Aldrich, St. Louis), and the concentration was adjusted to 5×10^4 conidia/ml using a hemocytometer. Leaves were inoculated using a portable paint sprayer (Preval, Coal City, IL), then placed into a $20 \pm 2^\circ\text{C}$ growth chamber with a 12-h photoperiod. Severity was rated visually at 21 days postinoculation as the percentage of leaf area with powdery mildew mycelia in 10% increments. Resistance responses were categorized as described above.

Resistance characterization. The *V. vinifera* susceptible parent in each test cross population is known to be homozygous recessive for powdery mildew resistance, as crosses among susceptible parents result in all susceptible progeny. Test cross progeny segregate for resistance when the resistant parent is heterozygous for resistance. To determine the number of genes segregating in a given population, phenotypic classes were tested with χ^2 goodness-of-fit tests against five predicted ratios, each tested at $\alpha = 0.05$: 1 resistant (R) to 1 susceptible (S); that is, 1R:1S, indicating a single dominant resistance gene; 3R:1S, indicating two dominant genes, either of which alone is sufficient to confer resistance; 7R:1S, indicating three dominant genes, as above, any one of which alone is sufficient to confer resistance; 1R:3S, indicating two genes, both of which are required for resistance; and 1R:7S, indicating three genes required for resistance. Because some progeny exhibited moderate disease phenotypes, we tested the two extreme scenarios: either all of the moderates are resistant or all are susceptible. This enabled us to empirically determine the maximum effect that improper resistance categorization could have on inferences of segregation ratios.

Low-temperature scanning electron microscopy. A separate set of disease-free, greenhouse-grown plants were maintained for low-temperature scanning electron microscopy (LTSEM). The youngest fully expanded grape leaves were excised from Bloodworth 81-107-11, from *Vitis* hybrid Tamiami, and from susceptible *Vitis* progeny 03-3004-16, from a population segregating for *Ren4* (34). Each leaf was rinsed in a gentle stream of deionized water and gently blotted dry with a paper towel. Several millimeters of each petiole was removed and the exposed end was inserted into a 17-by-100-mm disposable culture tube filled with deionized water and having a stopper with a hole large enough to allow the petiole to be inserted. Several areas on each leaf were

marked with the inked dull end of a cork borer having a 10-mm diameter. The delineated areas on the leaves were inoculated with conidia of *E. necator*. Conidia were collected by lightly drawing a camel-hair brush over sporulating colonies on leaves of Ruby Seedless grape. The conidia were transferred to the marked areas by gently tapping and rolling the tip of the brush on the grape leaf. The inoculated grape leaves were placed in a plastic box with a false bottom that was suspended over 200 ml of a saturated solution of potassium chloride. The chamber humidity was measured with a PTH-1X temperature humidity meter (Omega Engineering, Inc., Stamford, CT) prior to the introduction of the grape leaves and found to obtain a maximum of 88% at 24°C. The lid was placed on the box, the box was placed in a polyethylene bag, and the contents were incubated at 24°C for 48 h before examination by microscopy.

Pieces of the inoculated grape leaves, 5 to 6 mm on a side, were excised from the inoculated leaf area and mounted on cryo stubs. The mounted leaves were plunge frozen in a liquid nitrogen slurry and transferred to a PP2000 cryo-preparation chamber (Quorum Technologies Ltd., Ringmer, UK). The specimens were heated to sublimate surface ice, coated with gold-palladium, and then transferred to the cryo-stage at a temperature of -178 to -180°C for examination with a Hitachi S3500-N LTSEM (Hitachi High Technologies America, Inc., Pleasanton, CA). Specimens were examined at 5 kV and images digitally recorded. Spore germination, infection structure formation, the presence or absence of host hypersensitive reaction, and colony development were observed on the inoculated grapevine leaves.

Quantification of penetration and microcolony formation. Incompatible resistance phenotypes were quantified at the cell level microscopically, using Coomassie blue staining as previously described (34). The resistant breeding lines 07-3509-109 and 07-3509-162 from Tamiami and 07-3510-14 from Bloodworth 81-107-11 were compared with the susceptible check *V. vinifera* 'Riesling'. The third- and fourth-youngest leaves were detached, surface sterilized as described above, and placed on 1% agar for all assays. Leaves with actively conidiating colonies of an incompatible isolate were used for inoculation by touching conidiating colonies directly to the inoculated leaf. Plates containing inoculated leaves were incubated at 20°C for 3 days. Four discs were collected from each leaf for staining with Coomassie blue (12) and the experiment was conducted twice. Leaf discs (1 cm²) were collected by 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 in 50% ethanol for long-term storage. The solution was replaced with Coomassie blue stain (Coomassie Brilliant Blue R-250 at 0.12 g/liter in an aqueous solution containing 50% [vol/vol] methanol and 10% [vol/vol] glacial acetic acid) for 5 to 10 s to stain the mycelium, then rinsed with several water changes and mounted for viewing on a microscope slide in 50% glycerol.

We quantified penetration and microcolony formation by randomly sampling ≥ 50 germinated conidia per leaf disc with a compound light microscope at $\times 100$ to 200 magnification. Each germinated conidium was categorized 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 was quantified as the proportion of spores in categories ii and iii, because secondary hyphae only develop after successful penetration; microcolony formation was quantified as the proportion of spores in category iii. The total numbers of conidia observed for quantifying penetration and microcolony success on each seedling were 954 for 07-3509-109, 235 for 07-3509-162, 964 for 07-3510-14, and 1,014 for Riesling. Confidence limits (95%) for the proportion of successful conidia were determined as previously described (34). For each leaf disc, 10 conidia that successfully penetrated were selected and, for each conidium, the total hyphal length was

measured by ocular micrometer. The numbers of conidia observed for quantification of hyphal length on each seedling were 74 for 07-3509-109, 17 for 07-3509-162, 45 for 07-3510-14, and 58 for Riesling. Average hyphal length was analyzed using analysis of variance with the predictors: genotype, leaf age, disc, and genotype–leaf age.

Fungal isolate screening. *E. necator* isolates were collected from several *Vitis* hosts growing wild or cultivated in diverse locations in the eastern half of the United States (4,19), and single-conidium colonies were prepared as described by Brewer and Milgroom (4). Isolates used here included BIMtnt2 (from *V. aestivalis*), LICY (*V. vinifera* Chardonnay), PUMOChn (*Vitis* hybrid ‘Chambourcin’), WVMONT (*Vitis* hybrid ‘Norton’), PCTH (*V. rotundifolia*), LNYM (*V. vinifera* ‘Merlot’), G9 (*Vitis* hybrid ‘Rosette’), and SHNC1 (*Vitis* hybrid Chambourcin), each belonging to a different haplotype based on a multilocus sequencing (4). Several progeny from the populations of Tamiami (07-3509-109, -162, -178, -191, -215, and susceptible checks -40 and -96) and

Bloodworth 81-107-11 (07-3510-70 and susceptible check -31) were screened for resistance against each isolate using a detached leaf assay described by Frenkel et al. (18). Resistant Bloodworth 81-107-11 progeny 07-3510-03 and -32 were also screened and supported the results of 07-3510-70 but were returned to the breeding program in Parlier, CA, before the experiment could be repeated in Geneva, NY. Dry inoculation was conducted by applying ≈200 conidia to the center of the leaf with a sterile pipette tip. Inoculation experiments were repeated a minimum of three times, and isolates were judged compatible when they successfully sporulated, as observed under ×10 to 20 magnification, by 16 days postinoculation.

RESULTS

Progeny segregation due to heterozygosity of resistant parents. In the breeding vineyard in Parlier, CA, progeny of the Tamiami test cross B37-28 × C56-11 segregated across four

TABLE 1. Powdery mildew disease ratings on progeny from a *Vitis* interspecific hybrid ‘Tamiami’ × *Vitis vinifera* modified-backcross under greenhouse, vineyard, or laboratory conditions

Progeny	California greenhouse ^a		California vineyard ^b		New York laboratory ^c	
	Index	Rating	Severity	Rating	Coverage	Rating
C83-235	0	R	1	R	0	R
C84-66	0	R	1	R	0	R
C84-90	0	R	1	R	nd	nd
C83-233	2	R	1	R	60	S
C83-262	5	R	1	R	80	S
C83-238	5	R	1	R	nd	*
C84-39	6	R	1	R	0	R
C83-254	6	R	1	R	nd	nd
C83-256	7	R	1	R	20	M
C83-259	8	R	1	R	0	R
C84-26	8	R	1	R	60	S
C84-02	9	R	1	R	0	R
C84-55	9	R	1	R	40	S
C84-15	10	R	1	R	0	R
C83-237	11	R	1	R	nd	nd
C83-245	12	R	1	R	80	S
C83-251	13	R	1	R	60	S
C83-246	14	R	1	R	70	S
C84-10	15	R	1	R	20	M
C83-243	15	R	1	R	nd	nd
C84-04	16	R	1	R	0	R
C84-91	21 (0)	M	1	R	0	R
C83-257	22	M	1	R	70	S
C84-05	23	M	1	R	40	S
C84-12	24	M	1	R	nd	*
C83-253	25	M	1	R	nd	*
C83-250	26 (0)	M	1	R	20	M
C84-13	26 (0)	M	1	R	20	M
C83-236	26 (0)	M	1	R	30	M
C84-73	28	M	1	R	nd	*
C83-263	28 (0)	M	1	R	90	S
C84-37	29	M	1	R	nd	nd
C84-01	29 (0)	M	1	R	20	M
C84-56	30	M	1	R	20	M
C84-17	30 (0)	M	1	R	60	S
C84-72	32	M	1	R	0	R
C84-81	32	M	3	S	0	R
C84-43	32	M	1	R	nd	nd
C83-241	32 (0)	M	1	R	20	M
C84-57	32 (0)	M	1	R	nd	*
C83-247	33	M	1	R	30	M

(continued on next page)

^a California greenhouse ratings of natural epidemics were recorded in September and November 2006. Incidence and severity scores were averaged here and reported as an index, with the higher index rating from the two dates being presented here. When the index increased by ≥20% from September to November, the September index rating is included in parentheses. Ratings in bold italics denote vines on which powdery mildew colonies were observed on senescing leaves; nd = not determined. Index ratings were categorized as 0 to 19% = resistant (R), 20 to 39% = moderate (M), and 40 to 100% = susceptible (S).

^b California vineyard ratings of natural foliar disease severity were recorded and categorized as 1 = no infection (R), 2 = very few small colonies (M), 3 = <50% coverage (S), and 4 = >50% coverage (S).

^c Detached leaves in petri dishes were inoculated in laboratory conditions with a single-spored isolate from New York, 10-18-1. Maximum percent leaf coverage was rated in 10% increments and categorized as: 0 to 19% = R, 20 to 39% = M, and 40 to 100% = S; nd = not determined.

categories of foliar disease severity (1 = no visible infection, R; 2 = very few small colonies, M; 3 = <50% coverage, S; and 4 = >50% severity, S) as 84:3:4:3, respectively (Table 1). The cuttings of the test cross population Bloodworth 81-107-11 × C67-120 rooted poorly, resulting in the small number of progeny (24 individuals). Progeny of this population also segregated across the spectrum of resistance categories as 17:1:1:5 (Table 2). All susceptible checks were rated susceptible, indicating that resistance was not due to escape from infection. By inference, segregation in each test cross suggested that the resistant parent was heterozygous for at least one dominant resistance gene. Goodness-of-fit tests indicated that segregation ratios from Tamiami were consistent with 7:1, suggesting segregation of three dominant resistance genes (Table 3). Bloodworth 81-107-11 progeny segregated 3:1, suggesting two dominant resistance genes (Table 4).

Inconsistency of ratings by resistance screen. In each population, vegetatively propagated cuttings of some individual progeny

had different resistance ratings for each resistance screen, resulting in resistance ratios that differed for each screen. Although the 7:1 vineyard ratio for Tamiami progeny had suggested segregation of three independent resistance genes, any of which was sufficient for resistance, ratios of 1:1 or 1:3 in the single-isolate and greenhouse screens (Table 3) suggested that subsets of resistance genes were not sufficient for resistance in all screens. Similarly, whereas the 3:1 vineyard ratio for Bloodworth 81-107-11 progeny had suggested the segregation of at least two independent resistance genes, any of which was sufficient for resistance, ratios of 1:3 and 1:1 for the greenhouse and single-isolate screens suggested that subsets of resistance genes were not sufficient for resistance in all screens (Table 4).

This inconsistency by resistance screen is apparent in Tables 1 and 2. For example, individual Tamiami progeny C84-18 was susceptible to natural infection in the Parlier, CA greenhouse (64%) but resistant to natural infection in the vineyard (no visible

TABLE 1. (continued from preceding page)

Progeny	California greenhouse ^a		California vineyard ^b		New York laboratory ^c	
	Index	Rating	Severity	Rating	Coverage	Rating
C83-248	34 (0)	M	1	R	50	S
C83-249	35	M	1	R	50	S
C84-88	35	M	1	R	nd	*
C84-32	36	M	1	R	0	R
C84-44	36	M	1	R	0	R
C84-63	36	M	1	R	20	M
C84-87	37	M	1	R	90	S
C84-50	38	M	1	R	nd	*
C84-83	38 (0)	M	1	R	0	R
C84-54	39	M	1	R	0	R
C84-61	40	S	1	R	0	R
C84-21	40	S	1	R	40	S
C84-51	41	S	1	R	0	R
C83-244	41 (18)	S	1	R	50	S
C84-11	41 (5)	S	1	R	nd	*
C83-258	42 (6)	S	2	M	50	S
C84-98	43 (20)	S	1	R	20	M
C84-24	44	S	1	R	0	R
C84-31	45	S	1	R	100	S
C84-14	45 (0)	S	1	R	nd	*
C84-53	46	S	3	S	0	R
C84-30	46 (22)	S	1	R	0	R
C84-82	47	S	1	R	50	S
C84-19	47 (21)	S	2	M	40	S
C84-29	48 (19)	S	1	R	90	S
C84-84	50	S	1	R	30	M
C84-59	51 (31)	S	1	R	nd	*
C84-22	52	S	1	R	0	R
C84-35	52 (22)	S	1	R	nd	*
C84-47	52 (29)	S	1	R	60	S
C84-06	53 (27)	S	1	R	0	R
C84-28	54 (10)	S	1	R	90	S
C84-68	55	S	1	R	nd	*
C84-07	57	S	1	R	nd	nd
C84-75	58	S	4	S	30	M
C84-36	58	S	1	R	nd	*
C84-34	58 (11)	S	1	R	nd	*
C84-25	60	S	1	R	0	R
C84-69	63 (18)	S	1	R	nd	*
C84-18	64 (28)	S	1	R	0	R
C84-42	68	S	1	R	nd	*
C84-77	68	S	3	S	nd	*
C84-78	69	S	1	R	60	S
C84-38	70 (26)	S	1	R	nd	nd
C84-49	72 (43)	S	1	R	nd	*
C84-33	77 (26)	S	1	R	0	R
C84-76	78 (46)	S	3	S	70	S
C84-70	81 (38)	S	1	R	nd	*
C84-79	82	S	4	S	nd	nd
C84-41	84 (29)	S	1	R	0	R
C84-95	91	S	4	S	30	M
C84-45	92	S	1	R	nd	nd
C84-09	92 (55)	S	2	M	nd	nd

infection) or to inoculation with isolate 10-18-1 (0%) (Table 1). Bloodworth 81-107-11 progeny C84-252 was susceptible to natural infection in the Parlier, CA greenhouse (85%) but resistant to natural infection in the vineyard (no visible infection) and had a moderate response to inoculation with isolate 10-18-1 (20%) (Table 2). A testable hypothesis was that inconsistency in resistance phenotype among screens was the result of differences in the pathogen genotypes with respect to race specificity, though this inconsistency could be due to other experimental or environmental differences.

Evidence of race specificity by differential infection. Given the inconsistency among screens in the inferred models for the inheritance of resistance, we directly tested the hypothesis that progeny segregated for race-specific resistance. Five single-conidium *E. necator* isolates were inoculated on segregants from the Tamiami and Bloodworth 81-107-11 modified-backcross populations. These isolates were differential in their ability to form colonies and sporulate (Table 5). We tested five Tamiami

progeny previously shown to be resistant to natural infection, and all five supported sporulation by either isolate BIMtnt2 (collected from *V. aestivalis*), isolate WVMONT (from the interspecific *V. aestivalis* hybrid Norton), or both. Two progeny supported sporulation by LICY and PUMOChn (collected from *V. vinifera* Chardonnay and *Vitis* hybrid Chambourcin, respectively) in some replicates.

A Bloodworth 81-107-11 progeny that was resistant to natural infection was also resistant to several isolates but supported sporulation by isolate PUMOChn in multiple experiments (Table 5) and by isolate PCTH (collected from *V. rotundifolia*) in one experiment before isolate PCTH was contaminated by mycoparasites. Supporting these results, resistant Bloodworth 81-107-11 progeny 07-3510-03 and -32 were differentially infected by *E. necator* isolates, with BIMtnt2 and LICY compatible and WVMONT incompatible (data not shown). Isolates compatible with Bloodworth 81-107-11 progeny elicited visible host epidermal necrosis underlying each appressorium, but this slow, “trailing” necrosis could not halt colony development (Fig. 1).

TABLE 2. Powdery mildew disease ratings on progeny from a complex hybrid ‘Bloodworth 81-107-11’ modified-backcross under greenhouse, vineyard, or laboratory conditions

Progeny	California greenhouse ^a		California vineyard ^b		New York laboratory ^c	
	Index	Rating	Severity	Rating	Coverage	Rating
C84-229	0	R	1	R	0	R
Y313-60	0	R	1	R	0	R
Y313-64	0	R	1	R	nd	nd
C84-225	6	R	1	R	40	S
C84-224	14	R	1	R	40	S
Y313-63	17	R	1	R	20	M
C84-237	30	M	1	R	30	M
Y313-61	40	S	1	R	80	S
C84-228	52	S	1	R	nd	nd
Y313-49	56	S	2	M	60	S
Y313-54	59	S	1	R	20	M
Y313-46	59	S	1	R	70	S
C84-232	61 (10)	S	1	R	60	S
Y313-55	64	S	1	R	70	S
Y313-62	64 (7)	S	1	R	30	M
Y313-59	65	S	1	R	90	S
Y313-70	72 (27)	S	3	S	0	R
Y313-65	81	S	4	S	70	S
C84-246	81	S	4	S	90	S
C84-252	85	S	1	R	20	M
Y313-66	91	S	4	S	40	S
Y313-67	93	S	4	S	70	S
Y313-51	96	S	4	S	nd	nd
C84-234	nd	nd	1	R	20	M

^a California greenhouse ratings of natural epidemics were recorded in September and November 2006. Incidence and severity scores were averaged here and reported as an index, with the higher index rating from the two dates being presented here. When the index increased by $\geq 20\%$ from September to November, the September index rating is included in parentheses. Ratings in bold italics denote vines on which powdery mildew colonies were observed on senescing leaves; nd = not determined. Index ratings were categorized as 0 to 19% = resistant (R), 20 to 39% = moderate (M), and 40 to 100% = susceptible (S).

^b California vineyard ratings of natural foliar disease severity were recorded and categorized as 1 = no infection (R), 2 = very few small colonies (M), 3 = <50% coverage (S), and 4 = >50% coverage (S).

^c Detached leaves in petri dishes were inoculated in laboratory conditions with a single-spored isolate from New York, 10-18-1. Maximum percent leaf coverage was rated in 10% increments and categorized as 0 to 19% = R, 20 to 39% = M, and 40 to 100% = S; nd = not determined.

TABLE 3. Segregation ratios and χ^2 goodness-of-fit test suggesting race specificity of *Vitis* hybrid ‘Tamiami’ resistance in the cross B37-28 \times C56-11

Location	Threshold ^a	Proportion of progeny in each class		<i>P</i> value for χ^2 goodness-of-fit test per model ^b				
		Resistant	Susceptible	7:1	3:1	1:1	1:3	1:7
California vineyard	2	0.89	0.11	0.5852	0.0013	0.0001	0.0001	0.0001
	3	0.93	0.07	0.1385	0.0001	0.0001	0.0001	0.0001
California greenhouse	20%	0.22	0.78	0.0001	0.0001	0.0001	0.5515	0.0039
	40%	0.54	0.46	0.0001	0.0001	0.4093	0.0001	0.0001
New York laboratory	20%	0.39	0.61	0.0001	0.0001	0.0801	0.0008	0.0001
	40%	0.61	0.39	0.0001	0.0008	0.0801	0.0001	0.0001

^a Testing standard models of Mendelian inheritance of qualitative resistance required categorization of phenotypes as either resistant or susceptible. Because categorization could result in different segregation ratios depending on the threshold selected for susceptibility, we analyzed ratios either including progeny with moderate disease (2 = very few small colonies) as susceptible (lower threshold) or including them as resistant (higher threshold).

^b Models with *P* values < 0.05 are rejected and in italics; the most likely model is highlighted in bold.

Mechanism of resistance. Race-specific resistances to powdery mildews typically allow penetration and formation of secondary hyphae before restricting hyphal growth. As quantified using light microscopy, resistant progeny from each population had moderately reduced levels of penetration and microcolony formation relative to the susceptible Riesling (Fig. 2). However, hyphal length on susceptible and resistant genotypes showed pronounced and significant ($P < 0.0001$) differences (Figs. 3 and 4). Examination of the inoculated grape leaves by LTSEM showed that, on a susceptible genotype, *E. necator* conidia germinated (often forming two germ tubes), formed appressoria, and, as the germ tubes continued to grow, formed appressoria at frequent intervals along the hyphae (Fig. 5). In contrast, on leaves of Tamiami and Bloodworth 81-107-11, conidia germinated and formed a single germ tube and primary appressorium. Conidia, germ tubes, and appressoria collapsed on the resistant leaves, consistent with moderate penetration resistance observed using light microscopy. When further hyphal growth occurred, the hypha did not form additional appressoria and also collapsed within 48 h postinoculation, consistent with short hyphal length observed using light microscopy.

DISCUSSION

The existence of races of *E. necator* has been hypothesized and supported anecdotally for decades (6,13,37,39). The data pre-

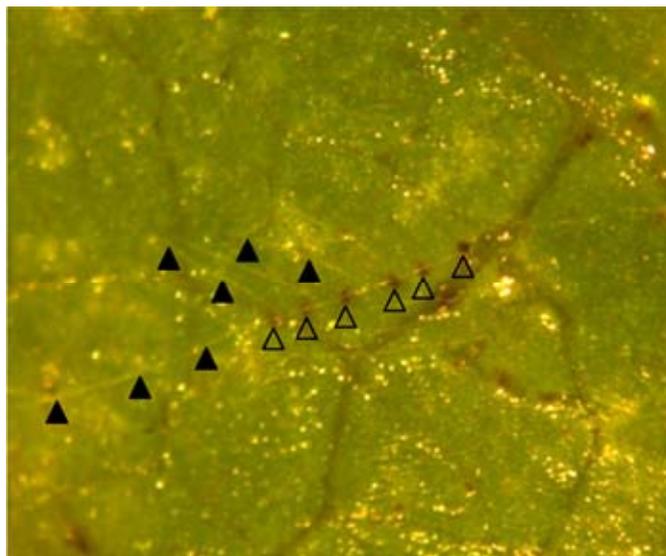


Fig. 1. Microcolony development on a resistant segregant of ‘Bloodworth 81-107-11’ population 07-3510 when inoculated with a virulent isolate. For isolates that overcome resistance from Bloodworth 81-107-11, host necrosis (subtended by open triangles) is still observed but is not rapid enough to prevent colony development and, thus, appears to “trail” the leading edge of hyphal growth (subtended by filled triangles), a phenotype referred to here as “trailing necrosis.”

TABLE 4. Segregation ratios and χ^2 goodness-of-fit test suggesting race specificity of *Vitis* hybrid ‘Bloodworth 81-107-11’ resistance in the cross Bloodworth 81-107-11 \times C67-120

Location	Threshold ^a	Proportion of progeny in each class		P value for χ^2 goodness-of-fit test per model ^b				
		Resistant	Susceptible	7:1	3:1	1:1	1:3	1:7
California vineyard	2	0.71	0.29	<i>0.0136</i>	0.6374	<i>0.0412</i>	<i>0.0001</i>	<i>0.0001</i>
	3	0.75	0.25	<i>0.0641</i>	1.0000	<i>0.0143</i>	<i>0.0001</i>	<i>0.0001</i>
California greenhouse	20%	0.26	0.74	<i>0.0001</i>	<i>0.0001</i>	<i>0.0218</i>	0.9042	<i>0.0488</i>
	40%	0.30	0.70	<i>0.0001</i>	<i>0.0001</i>	<i>0.0606</i>	0.5472	<i>0.0093</i>
New York laboratory	20%	0.14	0.86	<i>0.0001</i>	<i>0.0001</i>	<i>0.0011</i>	0.2568	<i>0.0610</i>
	40%	0.43	0.57	<i>0.0001</i>	<i>0.0007</i>	0.4290	<i>0.0588</i>	<i>0.0001</i>

^a Models with P values < 0.05 are rejected and in italics; the most likely model is highlighted in bold.

^b Testing standard models of Mendelian inheritance of qualitative resistance required categorization of phenotypes as either resistant or susceptible. Because categorization could result in different segregation ratios depending on the threshold selected for susceptibility, we analyzed ratios either including moderate (M) progeny as susceptible (susceptible thresholds = 2 [very few small colonies] for California vineyard, 20% index for California greenhouse, and 20% coverage New York Single isolate) or including moderate (M) progeny as resistant (susceptible thresholds = 3 [$< 50\%$ coverage] for California vineyard, 40% index for California greenhouse, and 40% coverage for New York single isolate).

TABLE 5. Differential compatibility of *Erysiphe necator* isolates on progeny carrying resistance from *Vitis* hybrid ‘Tamiami’ or the *Vitis* hybrid ‘Bloodworth 81-107-11’

Population, progeny	Rating ^a	<i>E. necator</i> isolate ^b							
		BIMtnt2	WVMONT*	LICY	LNYM	G9	SHNC1	PUMOChn	PCTH*
Tamiami (07-3509-)									
162	R	±	+	–	–	–
215	R	+	+	–	–	–
178	R	+	+	–*	–*	–
109	R	+	–	±	±	–
191	R	+	+	±	±	–
Susceptible	S	+	+	+	+	+
Bloodworth 81-107-11 (07-3510-)									
70	R	–	–	–	+	+
Susceptible	S	+	+	+	+	+

^a Disease ratings as scored in Geneva, NY on naturally infected greenhouse plants. All resistant (R) individuals shown here had no disease; susceptible (S) checks were susceptible full-sibling progeny.

^b *E. necator* isolates were collected as follows and single spored to obtain pure cultures (4,18): BIMtnt2, September 2008 on wild *Vitis aestivalis* in Blood Mountain, GA; LICY, October 2008 on *V. vinifera* ‘Chardonnay’ in Riverhead, NY; PUMOChn, October 2008 on *Vitis* interspecific hybrid ‘Chambourcin’ in Purdy, MO; WVMONT, September 2008 on *Vitis* interspecific hybrid ‘Norton’ in Waverly, MO; PCTH, May 2009 on wild *V. rotundifolia* in Panther Creek Recreational Area, GA; LNYM, October 2008 on *V. vinifera* ‘Merlot’ in Lockport, NY; G9, June 2007 on *Vitis* interspecific hybrid ‘Rosette’ in Geneva, NY; and SHNC1, September 2008 on *Vitis* hybrid Chambourcin in Pittsboro, NC. Symbols: Asterisks (*) denote isolates or individual observations that were not repeated in an independent experiment, + indicates compatible interactions in which the *E. necator* isolate formed a colony and sporulated following dry pipette tip transfer of conidia, – indicates incompatible interactions, and ± indicates that a compatible interaction was observed in some but not all independent experiments.

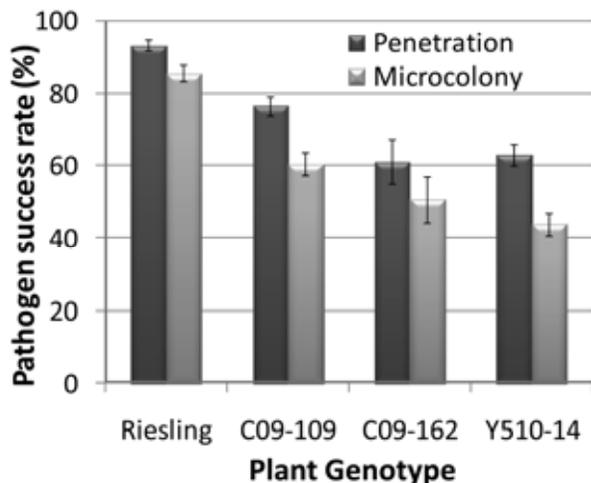


Fig. 2. Percentage of successful penetration and microcolony formation by germinated conidia of an *Erysiphe necator* isolate incompatible on *Vitis* hybrid ‘Tamiami’ and ‘Bloodworth 81-107-11’ resistance sources. *Vitis vinifera* ‘Riesling’ is a susceptible control. C09-109 and -162 are resistant full-sibs derived from Tamiami population 07-3509. Y510-14 is a resistant progeny of Bloodworth 81-107-11 population 07-3510. Error bars represent 95% confidence limits.

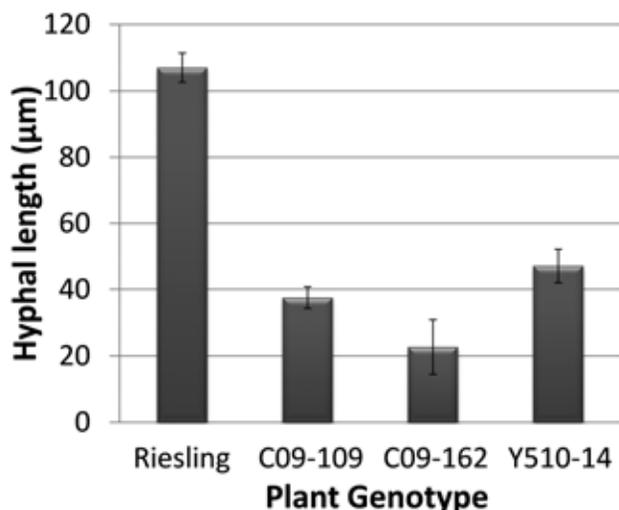


Fig. 3. Hyphal length for an *Erysiphe necator* isolate incompatible on *Vitis* hybrid ‘Tamiami’ and ‘Bloodworth 81-107-11’ resistance sources. *Vitis vinifera* ‘Riesling’ is a susceptible control. C09-109 and -162 are resistant full-sib progeny in Tamiami population 07-3509. Y510-14 is a resistant progeny of Bloodworth 81-107-11 population 07-3510. Colonies were analyzed following 72 h incubation at 20°C. Error bars represent 95% confidence limits.

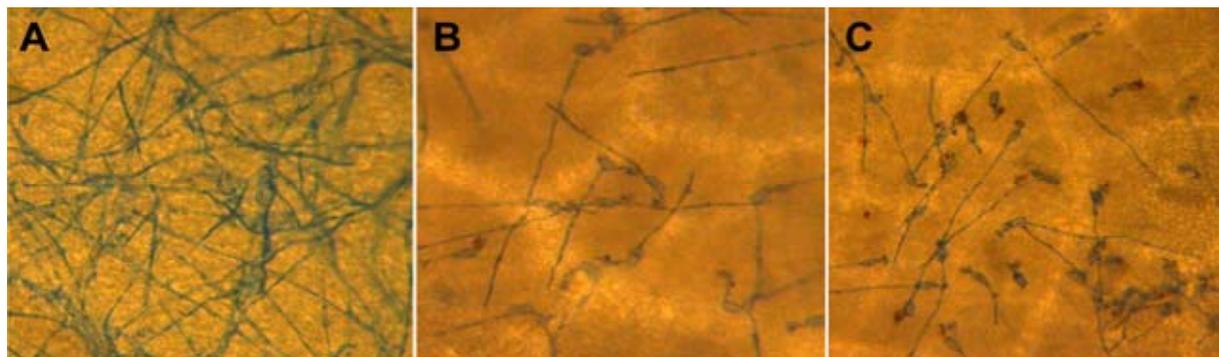


Fig. 4. Colony development for an *Erysiphe necator* isolate incompatible on characteristic progeny of *Vitis* hybrid ‘Tamiami’ and ‘Bloodworth 81-107-11’ resistance sources. The incompatible isolate was inoculated onto *Vitis* leaves and visualized with Coomassie blue. **A**, Hyphal growth on susceptible *Vitis vinifera* ‘Riesling’. **B and C**, Germinated conidia with secondary hyphae and with appressoria subtended by epidermal cell necrosis on incompatible **B**, Tamiami-resistant progeny C09-109 and **C**, Bloodworth 81-107-11-resistant progeny Y510-14.

sented here first implicate, then demonstrate, the presence of multiple race-specific resistance genes in the Tamiami and Bloodworth 81-107-11 resistance sources, with resistance hallmarks suggesting that Flor’s gene-for-gene model may apply here (17).

In attempting to confirm powdery mildew resistance ratings in mapping populations, we found that ratings for individual progeny were inconsistent and depended on the pathogen genotype or other experimental parameters used for phenotyping. This inconsistency was in stark contrast to parallel results that we obtained in the same experiments with a population segregating for *Ren4* resistance, in which individual progeny were either resistant in all three screens or susceptible in all three screens (34). A simple explanation of Mendelian segregation ratios that significantly differed among screens (Tables 3 and 4) is that the inoculum varied, with different races in each pathogen population or isolate used. Experiments testing several single-conidium pathogen isolates on select segregants from each population then provided conclusive evidence of *E. necator* races differentially compatible on resistant *Vitis* leaves in these populations (Table 5). Following the classical definition of a fungal race, that a single race is virulent on certain host genotypes and not others (11), we have demonstrated race specificity in the *E. necator* pathosystem.

Isolates collected from wild *V. aestivalis* or the interspecific *V. aestivalis* hybrid Norton (38) sporulated on progeny of the *V. aestivalis* resistance source Tamiami. Similarly, on resistant progeny from the complex hybrid Bloodworth 81-107-11 of at least *V. rotundifolia*, *V. vinifera*, *V. berlandieri*, *V. rupestris*, *V. labrusca*, and *V. aestivalis* background, the isolates that sporulated in repeated experiments were from wild *V. rotundifolia* and the complex hybrid Chambourcin—which contains, in its pedigree, most of the wild *Vitis* spp. of Bloodworth 81-107-11 except *V. rotundifolia* (22). Although intuitive, this apparent host specialization was somewhat surprising, because we previously observed a lack of genetic differentiation among *E. necator* isolates collected from several *Vitis* spp. (19) and only minor quantitative variation in host specialization (18), with the notable exception of isolates from *V. rotundifolia* (18,19). Indeed, even in California, where pathogen diversity is low (4,19), we showed here that virulent isolates could be detected by natural infection (e.g., California greenhouse), even within one growing season of selection. Thus, even in the absence of diversity in single-nucleotide protein (4) and simple-sequence repeat markers (19), functional diversity in effector genes appears to be common, which has major implications for the breeding and commercialization of resistant cultivars.

Cellular interactions of the host and pathogen further supported the model of race-specific resistance in each breeding population. Microscopic analyses revealed the ability of conidia landing on resistant genotypes to germinate and penetrate host epidermal cells, as indicated by formation of a secondary hypha (Figs. 2 and

4). Each secondary hypha typically elongated more than twice the length of the source conidium to form a microcolony within 3 days of inoculation. No differences were observed in the rates of successful penetration and microcolony formation between resistant progeny from Tamiami and Bloodworth 81-107-11, and resistant progeny from each population allowed only slightly less penetration and microcolony formation than the susceptible control Riesling (Fig. 2). Instead, the major differences between susceptible and resistant genotypes were in hyphal length (Fig. 3), which was restricted and associated with host necrosis in resistant

progeny (Fig. 4). Interestingly, the clear differences between resistance and susceptibility would have been underestimated if our screen had looked at penetration and microcolony formation alone and not included measurements of hyphal length. LTSEM data contributed to defining the mechanism of these similar resistances, with conidia, appressoria, and hyphae collapsing shortly after initiation of the secondary hypha (Fig. 5). Together, these data suggest that resistance is acting after formation of the haustorium and is associated with cell death much like the previously described *Run1* and *Ren1* powdery mildew resistance genes in

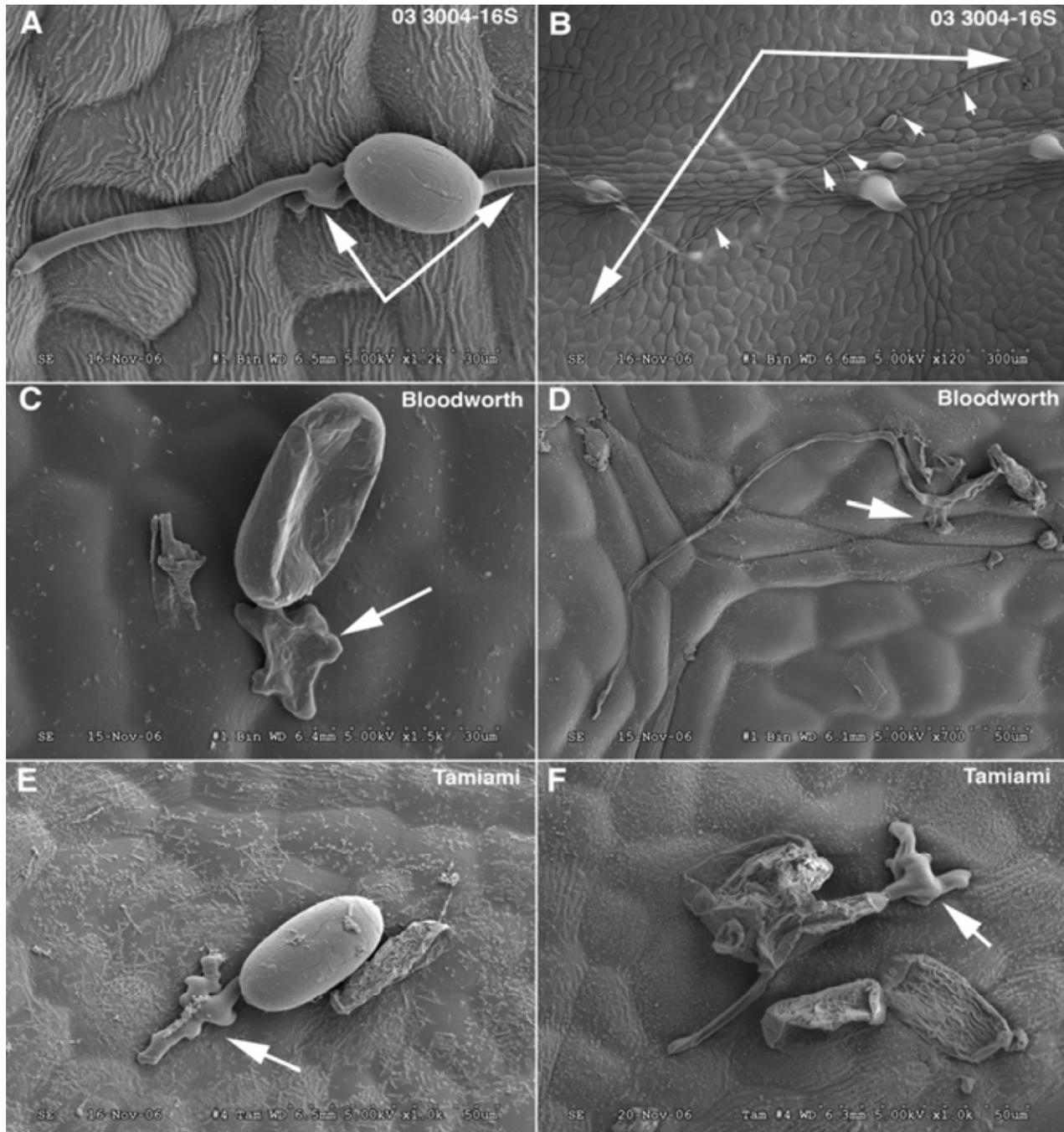


Fig. 5. Low-temperature scanning electron microscopy micrographs of germinated conidia of *Erysiphe necator*. Each frame is labeled in the upper right with the host genotype name. **A and B**, Germinated conidia on susceptible *Vitis* progeny 03-3004-16 from a population segregating for *Ren4* (34). **A**, The conidium has two germ tubes (both arrows) and has formed an appressorium (left arrow). **B**, A germinated conidium with two long hyphae (larger upper arrows) and five appressoria (smaller lower arrows). Note that the conidia and hyphae in A and B are turgid. **C and D**, Germinated conidia on seedling of complex hybrid ‘Bloodworth 81-107-11’. **C**, Germinated conidium showing partial collapse of conidium and appressorium (arrow). **D**, Germinated conidium which formed a long hypha (+250 μ m) and an appressorium (arrow) but failed to establish a colony. Note the collapsed hypha and appressorium. **E and F**, Germinated conidia on seedling of *Vitis* hybrid ‘Tamiami’. **E**, Germinated conidium with a germ tube and the start of an appressorium (arrow). Both conidium and germ tube are turgid. **F**, Germinated conidia that failed to infect the host. The conidia and one of the germ tubes have collapsed; an appressorium is still partially turgid (arrow).

Vitis (3,9), and suggest that this host cell death is associated with the rapid collapse of all superficial fungal tissues.

For both resistance sources studied here, powdery mildew incidence and severity increased on some progeny late in the growing season in the Parlier, CA greenhouse, including on old and senescing leaves (Tables 1 and 2). Late-season epidemics on senescing leaves of otherwise resistant progeny are commonly observed in the Parlier, CA vineyard, typically in October and November (D. W. Ramming, unpublished data). These observations lead to two testable hypotheses: (i) virulent isolates are rare early in the season, resulting in low incidence and severity on resistant vines early in the season but high incidence and severity after natural selection throughout the season for virulence; or (ii) when resistant vines begin to enter dormancy and leaves begin to senesce, the action of these powdery mildew resistance genes becomes less effective. However, neither increasing disease ratings nor increasing growth on older leaves in the Parlier greenhouse correlated with resistance or race specificity in the other resistance screens (Tables 1 and 2).

Recently, resistance from Bloodworth 81-107-11 was mapped to the *Run2.1* locus on chromosome 18 (36). This locus explained 53% of the phenotypic variation in foliar powdery mildew severity following natural infections in a Davis, CA greenhouse for a mBC₁ population. However, in two F₁ populations, the frequency of resistance was more common among progeny than the frequency observed in the mBC₁, and no loci—not even *Run2.1*—significantly predicted foliar resistance in either F₁ population (36). The increased frequency of resistant progeny could be due to multiple resistance genes, as seen in the half-sibling F₁ population here, and it appears their mBC₁ population harbors only one of the two resistance loci we putatively identified as segregating, which are most likely *Run2.1* and an unlinked locus. Our variable phenotypic data suggest that race specificity in this resistance source could be a further complicating factor when mapping resistance based on uncontrolled, natural infections. These studies suggest an advantage to using controlled inoculations with differential isolates to develop molecular markers linked with each resistance gene before applying markers to maintain multiple resistance genes in breeding populations.

The identification of several new resistance genes already introgressed from wild species into advanced breeding lines would seem to be a discovery of significant magnitude for the future of resistance breeding, particularly in a crop with few characterized resistance genes. However, this type of resistance can be rapidly overcome by new races of the pathogen shortly after cultivar release (7,24,27) and, in this case, even in segregating populations early in the breeding and evaluation process. As indicated in Tables 3 and 4, each of the up to five putative resistance genes were overcome at least individually and, in some cases, even pairs of resistance genes were overcome, as evidenced by 1:3 segregation ratios. Given that sexual reproduction by *E. necator* occurs frequently worldwide and is required for winter survival in cold climates (20,21), races that are compatible with host genotypes with the putative pyramid of three resistance genes, as in some Tamiami progeny, would likely arise as a result of recombination. Subsequent selection for compatible races on these resistant cultivars would follow and would eventually result in the breakdown of resistance, as experienced multiple times with well-characterized race-specific powdery mildew resistance genes in cereals (15). Some grapevine progeny found to be resistant in California screens were susceptible in the New York screen (Tables 1 and 2), or vice versa, reinforcing the need to test putatively resistant individuals in multiple environments, which is logical but seldom done in woody perennial breeding programs, due in part to the expense of establishing and maintaining plantings. For controlled inoculations, given the low genetic diversity of isolates outside of the eastern United States (4,19), centralized

phenotyping with characterized reference isolates of *E. necator* in the eastern United States is more desirable than dissemination of reference isolates.

We have detected multiple race-specific powdery mildew resistance genes introgressed from several different North American species as well as races of *E. necator* capable of overcoming them. Although incorporating resistances into breeding material is desirable, the mechanism of the genes identified here is sub-optimal. Their sustainable use in grapevine cultivars could be more effective when pyramided, a condition that can be facilitated by developing markers for marker-assisted selection. However, as demonstrated in barley, pyramiding two race-specific resistance genes typically only increased durability by 1 or 2 years compared with a single gene (5). Additional studies are needed to test the effect of combined resistance mechanisms (prehaustorial, posthaustorial, and quantitative) in the development of durable resistance. Therefore, we recommend that new and existing powdery mildew resistances in all crops be mechanistically characterized by quantitative phenotyping, such as with the Coomassie blue protocols described here. This will not only add standardized information to our growing knowledge base but also streamline approaches to the effective and prolonged use of resistance genes in the field.

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