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# Natural infection of *Run1*-positive vines by naïve genotypes of *Erysiphe necator*

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## Summary

**The *Run*1 locus for dominant resistance to powdery mildew (*Erysiphe necator*) has been successfully introgressed into Euvitis from *Vitis rotundifolia*. In the current study, *Run*1 vines were hybridized with breeding lines at Cornell University, and the presence of the locus was assayed using the markers GLP1-12 and VMC8g9. Signs of powdery mildew were observed on 14 of 113 Run1-positive seedlings in October 2010 in Geneva, N.Y. Severity of infection was lower for *Run*1-positive than for *Run*1-negative seedlings. Presence of mature cleistothecia suggested infection by at least two pathogen genotypes, which since *V. rotundifolia* is not grown within 800+ km of Geneva, N.Y., evolved from a pathogen population naïve to *Run*1 resistance. Therefore, caution in the deployment of the *Run*1 locus in new resistant cultivars is suggested so the effectiveness of *Run*1 does not diminish over time.**

K e y w o r d s : powdery mildew, disease resistance, molecular markers.

## Introduction

Resistance to grape powdery mildew (*Erysiphe necator)* can be categorized as follows: penetration resistance that prevents haustorial formation; R-gene mediated resistance associated with programmed cell death (PCD); or quantitative resistance that reduces pathogen fecundity. Studies of resistance to powdery mildew in cereals indicate that: 1) dominant, major genes associated with PCD are not durable; and 2) stacking multiple major genes will improve durability, though not indefinitely (Brown 1994). While all commercially relevant *Vitis vinifera* cultivars are susceptible to powdery mildew, many grape breeders are introgressing resistance from wild *Vitis* spp. Thus, knowledge from other powdery mildew pathosystems should contribute to strategies for introgression; evaluation and selection; cultivar release; and disease management.

The dominant powdery mildew resistance gene *Run1* from muscadine grapes (*V. rotundifolia*) confers PCD resistance and has been highly effective where evaluated and in all genetic backgrounds evaluated, even by itself (Pauquet *et al.* 2001). Thus, grape breeders are introgressing *Run1* into breeding lines, and several are combining *Run1* with other resistance genes to improve durability (Eibach *et al.* 2007). However, given the lack of durability of similar resistance genes from other crops, the question still looms: Will *Run1* remain effective in commercial vineyards? To address this question, we monitored a vineyard of breeding lines known to have *Run1* resistance. A distinguishing aspect of this vineyard is its location in a major viticultural region within the presumed center of origin of *E. necator* (Gadoury *et al.* 2011), but one where the cold-sensitive *V. rotundifolia* does not survive.

## Material and Methods

Seed from the cross Eger 99-11 (VRH 3082-1-42 ($BC_4$) X SK 90-2-19) (*V. rotundifolia* backcrossed into Euvitis species) were received in 2000 from P. Kozma, Eger, Hungary, and resulting seedlings grown under fungicide-free conditions. The female parent, VRH 3082-1-42 ($BC_4$), has the *Run1* gene (Katula-Debreceni *et al.* 2010). Vines were selected for powdery mildew resistance and planted to a permanent vineyard. In 2006, eleven crosses were made between individual Eger 99-11 vines and locally-adapted wine grapes, from which 1,002 seedlings were grown fungicide-free, and selected visually for powdery mildew resistance. Selected vines were planted in three adjacent rows in a permanent vineyard in 2008. Within each ~415 m row, a set of four powdery mildew susceptible control vines were planted. In addition, susceptible vines in adjacent rows provided large amounts of inoculum. Vines were trained to single trunks and were cane-pruned.

DNA was isolated from 171 selected progeny in total from the eleven populations using a modified CTAB extraction method (Mahanil *et al.* 2007). The presence of the *Run1* locus was assayed using the markers GLP1-12 (Donald *et al.* 2002) and VMC8g9 (Barker *et al.* 2005).

O b s e r v a t i o n s a n d m i c r o s c o p y : Vines and controls were rated in October, 2008-2010 by estimating percentage leaf area visibly infected with powdery or downy mildew. Leaves were collected from *Run1*-positive vines on 13 October 2010 and incubated at 22 °C under natural light conditions in double Petri dishes (Gadoury and Pearson 1988). Leaf blades and petioles were observed under light microscopy at 10x to 40x magnification to visualize hyphae, conidia, and cleistothecia.

## Results and Discussion

From the 1,002 seedlings planted in 2007, 306 were chosen for further testing based primarily on resistance to both powdery and downy mildew. From these 306 vines,

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150 were subjected to marker analysis in 2009. We confirmed the presence of both *Run1* markers (GLP1-12 and VMC8g9-156) in 126 progeny (84 %). In addition, twelve progeny tested positive for the VMC8g9-156 allele but negative for GLP1-12, and twelve progeny tested negative for both markers. For the purpose of this study, we considered vines as containing *Run1* only when both markers were positive.

From 8 to 13 October 2010, signs of powdery mildew infection were readily observed on 14 of 113 progeny testing positive for *Run1* (12.4 %) versus 100 % incidence on vines lacking *Run1* (Table). For vines infected by powdery mildew, average disease severity was also lower for *Run1*-positive progeny (2.5) than for *Run1*-negative seedlings (3.8) or for susceptible controls (4.0-4.8, Table). Leaves were collected from four symptomatic *Run1*-positive vines on 13 October 2010 for inspection by microscopy. Powdery mildew severity (leaf coverage) up to 60 % was observed on infected leaves, with abundant immature and mature cleistothecia (Figure). Conidia were not observed immediately upon leaf collection but developed after several days' incubation.

*Erysiphe necator* has a bipolar heterothallic mating system, thus two genotypes representing compatible mating types must be present on the same leaf for cleistothecial initiation (Gadoury *et al.* 2011). Under optimal conditions, cleistothecia of *E. necator* require at minimum 4 weeks to reach morphological maturity (Gadoury and Pearson 1988). Presence of mature cleistothecia on *Run1* leaves by 13 October suggests that at least two pathogen genotypes had colonized leaves as early as 13 September, and probably much earlier, as temperatures were frequently below optimum (Gadoury and Pearson 1988) for the pathogen.

*Run1* was introgressed from *V. rotundifolia*, which is not grown within 800 km of Geneva, N.Y. It is unlikely that local *E. necator* populations encountered any *V. rotundifolia* resistance prior to our planting of *Run1* vines in 2001. Thus, multiple isolates able to grow upon *Run1* plants were not only selected from a naïve pathogen population in less than a decade, but the isolates were sufficiently virulent to produce the sexual stage of the pathogen required for overwintering. Late season assessments failed to confirm the presence of powdery mildew infection on the *Run1* vines

Table

Incidence and severity of macroscopically apparent powdery mildew on grapevine progeny with and without *Run1*

| GLP1-12[a] | VMC8g9[a] | 2010 Powdery mildew | | |
|---|---|---|---|---|
| | | n[b] | Incidence (%)[c] | Average severity[d] |
| + | + | 113 | 12.4 | 2.5 |
| - | + | 11 | 45.5 | 2.4 |
| - | - | 10 | 100.0 | 3.8 |
| Susceptible Controls: | | | | |
| Chancellor (Seibel 7053) | | 4 | 100.0 | 4.8 |
| Concord | | 4 | 100.0 | 4.3 |
| Steuben | | 4 | 100.0 | 4.5 |
| Yugoslav 5-24 (PI200569) | | 4 | 100.0 | 4.0 |

[a] Markers GLP1-12 and VMC8g9 were interpreted as positive based on the presence of 700 bp and 156 bp alleles, respectively. In this study, progeny were considered positive for *Run1* only when both markers were positive. Five progeny for which VMC8g9 failed to amplify are not shown here. The four susceptible controls are presumed to lack *Run1*.

[b] n = number of progeny with severity ratings.

[c] The incidence of vines with powdery mildew severity ratings of 2 to 5. Positive ratings were successfully confirmed by microscopy in 2010.

[d] The average severity on vines that were symptomatic. A scale of 1 to 5 was used as follows: 1 = 0 - 3 % infected leaf surface; 2 = 3 - 12 %; 3 = 12 - 25 %; 4 = 25 - 50 %; and 5 > 50 %.

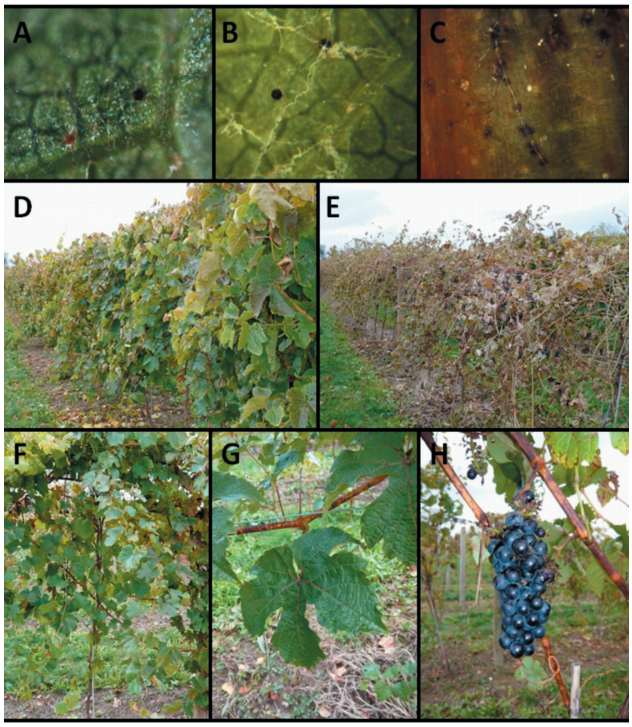


Figure: Growth and development of *Erysiphe necator* on naturally-infected vines grown under no-spray conditions. **A-C**) Observations under light microscopy of infected leaves collected 13 October 2010 from *Run1*-positive vines. **A**, **B**) Mature (dark spheres) and immature (light brown) cleistothecia were commonly observed on leaf blades. Networks of powdery mildew hyphae are crossing the leaf vein in the lower portion of (**A**). Partially pigmented, uncinate cleistothecial appendages can be seen in (**B**), characteristic of *E. necator*. **C**) Typical appearance of infected leaf petioles, here with a long hypha seen vertically, subtended by necrotic host epidermal cells and with several short hyphal branches. **D-H**) Field observations of vine growth and disease severity on 16 October 2009. **D**) Vines growing in row 7, previously selected for disease resistance, most of which have the *Run1* gene for powdery mildew resistance. Vines were photographed following -2 C temperature the previous night, hence some foliar frost damage is evident. **E**) Vines growing in row 6, a population not previously selected for disease resistance. **F-H**) Close-up of individual *Run1*-positive vines. Even on *Run1*-positive vines with disease-free leaf blades, signs of severe powdery mildew infection of canes are readily apparent, seen here as net-like patterns of host necrosis.

until 2010. Thus, the visible manifestation of reduced resistance was observed within a single season.

Our observations indicate that *Run1* resistance has diminished prior to commercial deployment. However, concurrent observations indicate that *Run1* resistance retains considerable commercial value. Eastern North America is the center of diversity for *E. necator* (Gadoury *et al.* 2011), which likely accelerates selection for virulence in genetically fit pathogen backgrounds. In addition, the experimental vineyard was never treated with fungicides and was surrounded by blocks of heavily-diseased vines (Figure), thereby providing ideal conditions for selection of virulent isolates. Finally, despite the presence of individual leaves that were severely diseased, the *Run1* vines retained nearly a full complement of foliage in mid-October, unlike adjacent *Run1*-negative vines that were severely defoliated (Figure).

Given the knowledge of powdery mildew resistance gene durability from other crops, observing virulent populations of powdery mildew growing on a typical *R*-gene with PCD-mediated resistance is not novel. Our goal in communicating these results quickly and directly to the grape community is to encourage breeders, pathologists, and growers to work together to protect *Run1* for future use by considering these results in light of long-term breeding strategies, commercial deployment of resistance genes, and virulence management on resistant vines.

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