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# Powdery Mildew-resistant “Renstack” Vines Released to the Public Domain

Tim Martinson, Bruce Reisch, Matthew Clark, Craig Ledbetter, Surya Sapkota, and Lance Cadle-Davidson

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**PUBLIC AND PRIVATE GRAPE BREEDERS** will soon have access to new powdery mildew-resistant grapevines produced by the USDA-funded *VitisGen2* project. Eight “Renstack” vines that incorporate four to six different powdery mildew resistance loci have been released to Foundation Plant Services at the University of California, Davis (**FIGURE 1, FIGURE 2**).

These eight vines are the tangible product of a decade of progress in incorporating advanced DNA sequencing to identify DNA markers associated with powdery mildew resistance and use them for marker-assisted selection. Breeders have used resistant sources for variety improvement for more than a century but have not had an efficient way to screen new seedlings to identify the resistant ones without introducing the pathogen.

“Before marker-assisted selection, we could only observe whether or not powdery mildew was infecting our grapevine selections,” said Bruce Reisch, Cornell University grape breeder and leader of the *VitisGen2* project. “Reliable DNA markers allow us to know which resistance genes are present in each seedling we test.”

Using marker-assisted selection, breeders are now able to “stack” several different loci and combine them into vines that have multiple sources of powdery mildew resistance. This strategy, it is thought, should make the resistance more durable (as each gene has a unique mechanism) and less likely to be affected by the chance occurrence of novel powdery mildew strains that can overcome the resistance.

## Resistance Markers

To date, 15 powdery mildew resistance loci have been identified, originating from several different *Vitis* species native to the Caucasus regions of Europe, North America, and Asia (**FIGURE 3**).<sup>1</sup> Studies from 24 F1 mapping populations (siblings of crosses between susceptible and resistant vines) have localized these distinct loci on eight of the 19 grapevine chromosomes (chromosomes 2, 9, 12, 13, 14, 15, 18, and 19).

A marker for the first resistance locus, called Run1, was identified in 2000.<sup>2</sup> “Run” refers to **R**esistance to *U*ncinula *n*ecator. Following the revision of the scientific name to *Erysiphe necator*, subsequent resistance loci were identified as “Ren” followed by a number. As DNA sequencing techniques and mapping populations have progressed over the last 15 years, the rate of discovery of additional loci has accelerated, with the most recent locus described in September 2021 called Ren11.<sup>3</sup>

## VitisGen2 Collaboration to Produce Renstack Vines

The *VitisGen2* project brought together grape breeding programs at four institutions to make the crosses that produced the *Renstack* vines: Bruce Reisch’s program at Cornell University, Craig Ledbetter’s table grape breeding program at USDA-ARS Parlier, Andy Walker’s program at University of California, Davis, and Matthew Clark’s program at the University of Minnesota (**FIGURE 4**).<sup>4,5</sup>



BRUCE REISCH

**FIGURE 1:** Post-Doctoral research associate Surya Sapkota with a vine used to make cuttings at Cornell AgriTech in Geneva, NY before shipping to the Foundation Plant Services at the University of California, Davis.



TIM MARTINSON

**FIGURE 2:** Vines regenerated from Cornell cuttings at Foundation Plant Service’s greenhouse, on the campus of UC Davis in Davis, Calif.

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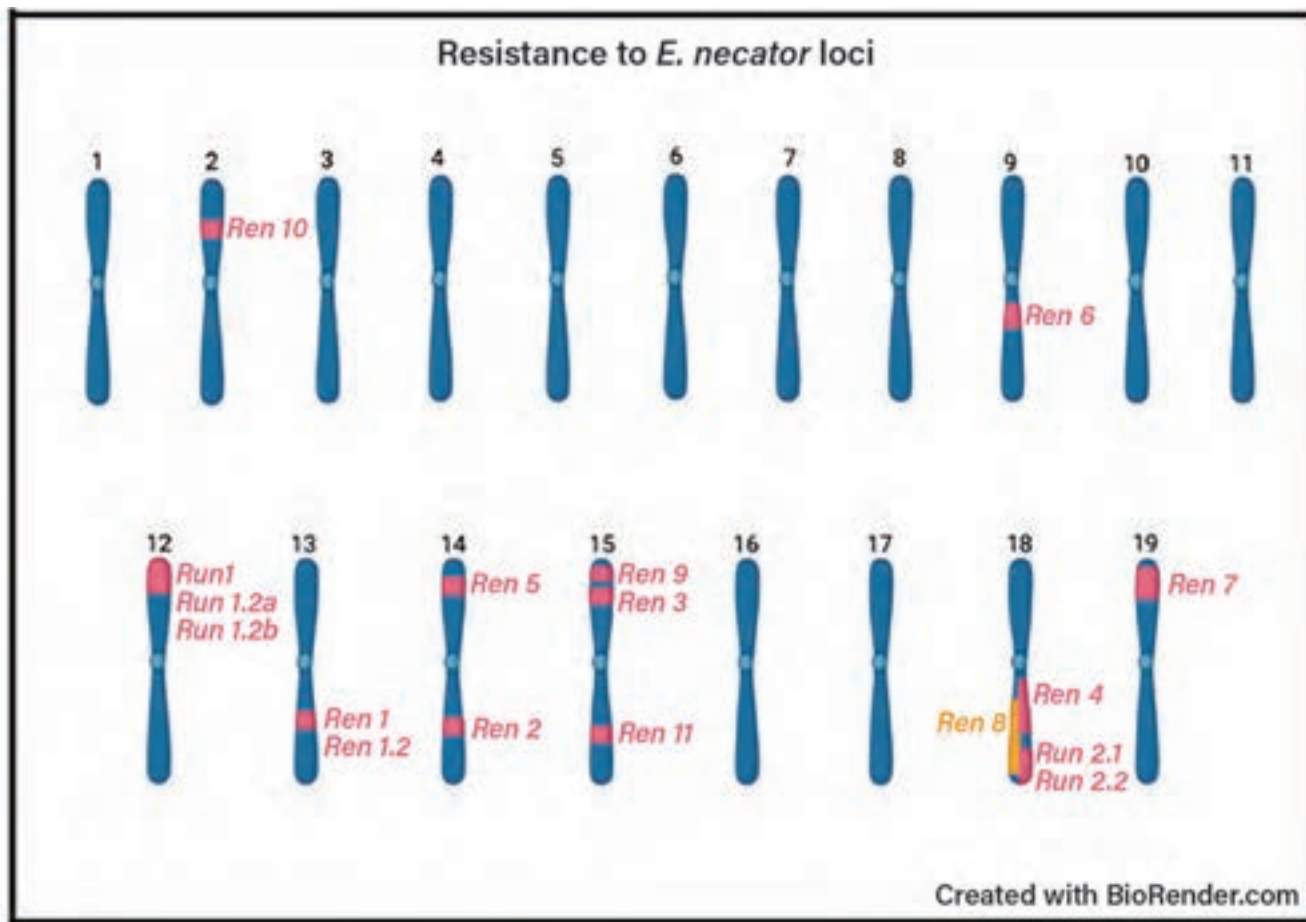


FIGURE 3: Location of 15 known powdery mildew resistance loci in the grapevine genome.

## RENSTACK1

Reisch’s program at Cornell supplied a vine with Run1 and Ren1 loci selected from seeds obtained from Dr. Pal Kozma, a Hungarian grape breeder at the University of Pecs. Walker’s UC Davis program provided the other parent, containing the Ren6 and Ren7 loci from *V. piasezkii*, an accession from China. Walker and Dario Cantu’s programs identified the two novel loci in 2016.<sup>6</sup> The cross, made at Cornell AgriTech, resulted in four *Renstack1* sibling vines containing all four resistance loci.

## RENSTACK2

The Ledbetter program supplied a vine resulting from a 2014 cross that combined the Run1 and Ren1 loci with the Ren4 locus, identified by Ramming, et al in *V. romanetii*, another wild species from China.<sup>4</sup> This was crossed with the University of Minnesota’s MN1264, which

included the Ren10 locus (discovered by the first *VitisGen* project from the interspecific hybrid variety Seyval Blanc<sup>5</sup>) and the Ren3 and Ren9 loci originating in the German-bred cultivar Regent.

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**FIGURE 4:** Powdery-mildew susceptible (left) and resistant (right) vines resulting from a *Vitis vinifera* x *Vitis rotundifolia* cross show the impact of the Ren4 locus on powdery mildew development.

The cross, made at the University of Minnesota, resulted in four *Renstack2* sibling vines containing all six resistance loci.

## Testing and Verification

Seeds resulting from the two crosses at AgriTech and the University of Minnesota were planted, and the resulting seedlings were tested with the *VitisGen*-developed rhAmpSeq platform to confirm the presence of markers for the four (*Renstack1*) or six (*Renstack2*) resistance loci. Four sibling vines from each of the two crosses were then grown in the greenhouse.

### VARIABLE PATHOGENS

To confirm that the vines were indeed resistant to powdery mildew, researchers Lance Cadle-Davidson and Surya Sapkota inoculated leaf tissue with 10 different isolates of powdery mildew and tracked the response with precision leaf disk and computer vision tests.<sup>7</sup>

The 10 isolates represented different strains of *Erysiphe necator* collected in Eastern North America, the center of origin for the powdery mildew pathogen.<sup>8</sup> It was important to test the *Renstack* vines with these different strains, because some of them had been able to overcome resistance and reproduce in vines with just the Run1 locus, which is one of the earliest and strongest resistance loci identified.

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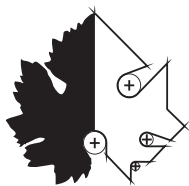
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### ATTAINING DURABLE POWDERY MILDEW RESISTANCE

Incorporating disease resistance into grapevines is a challenge in that the resistance must last the life of a vineyard, which can extend for 30 years or more. Any one source of resistance will exert strong selection pressure on the pathogen and the powdery mildew pathogen, *Erysiphe necator*, is well equipped to evolve rapidly with a short generation time (7 to 10 days) and thousands of spores produced on each infected leaf. From the introduction of the benzimidazole fungicides in 1973, resistance to each new class of fungicides (DMI fungicides, strobilurins) has been detected within 4 to 6 years of their introduction. Resistant varieties need to last much longer, and the selection imposed by resistant varieties may be even more intense than that of fungicides, because it is always ‘on.’

Therefore, pyramiding or “stacking” several different sources of resistance is important. One resistance mechanism can be overcome by a single mutation. However, multiple sources of resistance that involve different mechanisms will require multiple genetic changes in the pathogen to overcome the resistance and make it dramatically less likely to happen. The *Renstack* vines exemplify this approach.

### WHEN WILL THE RENSTACK VINES BECOME AVAILABLE TO BREEDERS?

Budwood cuttings from the *Renstack* vines were transferred to Foundation Plant Service (FPS) at the University of California, Davis in September 2021. According to FPS director Maher Al Rwahnih, six of the eight accessions have been successfully propagated, and plant material or pollen should become available to public and private grape breeding programs in two to three years.

It is important to stress that the *Renstack* vines do not represent new varietal releases for planting by growers, but rather are resources that will be available to breeders who want to incorporate robust powdery mildew resistance into their breeding programs. Aside from the stacked resistance genes, the *Renstack* vines have not been characterized for other important horticultural traits.

### Current and Future Impact

The *VitisGen2* project brought together scientists from throughout the United States to discover DNA markers associated with powdery mildew resistance and evaluate them using advanced high-throughput phenotyping (evaluation) techniques. A key advance that made this possible was development of robust genetic maps that identified 2,000 DNA markers, covering 98 percent of the grape genome.<sup>9</sup> This platform, along with 16 mapping populations, allowed researchers to associate DNA markers and traits and then test seedlings to determine which markers (traits) they contain.<sup>10</sup>

The resulting ability of marker-assisted selection is what allowed breeders to “stack” several resistance traits in the crosses, resulting in the *Renstack1* and *Renstack2* vines. For the first time, different modes of powdery mildew resistance originating in Asia, eastern Europe and North America have been brought together in the *Renstack1* and *Renstack2* vines.

Varieties with durable powdery mildew resistance offer a solution to an intractable issue with traditional European grape varieties—their extreme susceptibility to that disease. Following the introduction of *Erysiphe necator* to Europe as an invasive species in the mid-1800s, producers were forced to adopt multiple sprays of sulfur, at 7 to 10 day intervals, to manage powdery mildew. For over 150 years, grape production depended on widespread use of sulfur and other fungicides.

A recent study estimated that powdery mildew represented 74 percent of pesticide use in grapes.<sup>11</sup> California growers were estimated to spend \$239 million to manage powdery mildew, with up to 14 sprays applied, including

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31 million pounds (about 15 tons) of elemental sulfur.<sup>12</sup> Widespread adoption of powdery mildew-resistant varieties would offer potential savings of \$63 million to \$150 million annually on California’s 590,000 acres of grapes.

In Europe, political demands for reducing pesticide use are prompting reconsideration of government regulations that dictate which varieties of grapes are used for wine production. The European Union’s Farm to Fork policy envisions a 50 percent reduction in pesticide use by 2030. Following 70 years of restricting interspecific hybrids, authorities are starting to embrace new disease-resistant varieties as a strategy for meeting this goal. This includes four varieties that were recently released by France’s INRA (National Institute for Agricultural Research) and incorporate two downy mildew and two powdery mildew resistance genes. Management recommendations are for two fungicide applications rather than the typical 10 to 15 applied to Chardonnay.

However, the goal of reducing vineyard inputs is hampered by the inherent disease susceptibility of international *V. vinifera* wine varieties, along with market demands for traditional varieties with name recognition. Whether growers are conventional, organic, or biodynamic, they all are faced with the need to apply inputs to manage powdery mildew and other diseases. Disease resistance offers great potential in decreasing the number of sprays and making the wine and grape industry more sustainable.

Marker-assisted selection has offered grape breeders new tools to incorporate disease resistance into high-quality wine and table grape cultivars. But breeding new varieties is still inherently a long-term process, requiring a minimum of 10 to 15 years from the initial cross to release. The payoff to widespread adoption by growers may span decades.

For these reasons, it is important to note the tangible and enduring new resource represented by the *Renstack* vines. Grape breeders will be able to use pollen from these vines to introgress disease resistance into their breeding programs for adaptation to their regional or market goals. They will have access to germplasm that brings together multiple resistance genes from three continents and several wild *Vitis* species.

Collaboration by the four breeding programs at USDA-ARS Parlier, UC Davis, the University of Minnesota and Cornell University, along with supporting bioinformatics at Cornell and the Geneva-based USDA Grape Genetics Research Unit, made this possible.

In addition to a pipeline of advanced breeding lines that may become cultivars or parents of cultivars, the *Renstack1* and *Renstack2* vines are the most tangible “sticks in the ground” product of the 10 year *VitisGen* and *VitisGen2* projects funded by the USDA Specialty Crops Research Initiative.<sup>13</sup> They are now in the public domain and will serve as an enduring resource to public and private breeders and geneticists for decades to come. **WBM**

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