# The Phenotyping Bottleneck

## How grape breeders link desired traits to DNA markers

By Tim Martinson and Lance Cadle-Davidson

Before inexpensive DNA sequencing was available, grape breeders had to rely solely on traits observed in the field (phenotypes) to decide which new seedlings produced through crosses to keep and which to discard.

For disease resistance, this meant tossing out any plants that showed powdery mildew or downy mildew infections at the seedling stage during the first year of growth. Although they kept those that didn't show any symptoms, this method of field phenotyping didn't help them much in determining which and how many genes were involved and whether or not the trait would survive a subsequent round of breeding intact. DNA markers and more extensive use of mapping populations have changed all that.

Since about 2000, genetics researchers have been busy linking DNA sequences (called markers) to specific locations on grape chromosomes (loci) that are associated with observed traits of grapevines in the field (phenotypes). To date, they have identified markers for at least 13 loci for powdery mildew resistance and 27 for downy mildew resistance (Figure 1).

Now that they have these markers, grape breeders can test seedlings for the presence of specific genes or loci and know what and how many loci for disease resistance they have in their new seedlings (see "Grape breeders no longer flying blind," *Wines & Vines*, March 2018,).

This is a powerful new approach that gives breeders the means to reliably incorporate desirable traits into breeding lines. For example, having DNA markers for a strong locus for Pierce's disease resistance (named Pdr1 for Pierce's disease resistance 1) allowed University of California, Davis professor Andy Walker to pass through five successive "backcross cycles" confidently and quickly, to incorporate Pdr1 (from wild *Vitis arizonica*) in a 98% *Vitis vinifera* background, resulting in new varieties that are resistant to Pierce's disease.

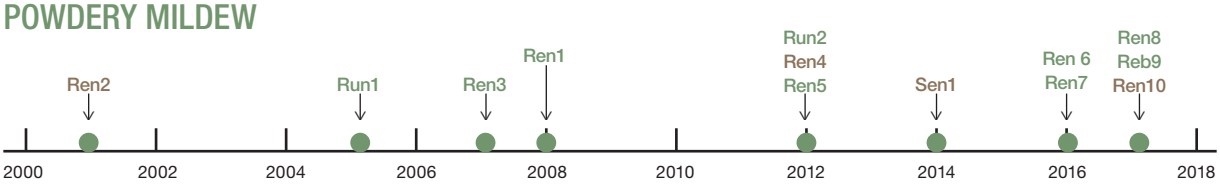
### Mapping populations and markers

How do breeders and geneticists find these DNA markers among the approximately 500 million DNA base pairs and 19 chromosomes in the grapevine genome?

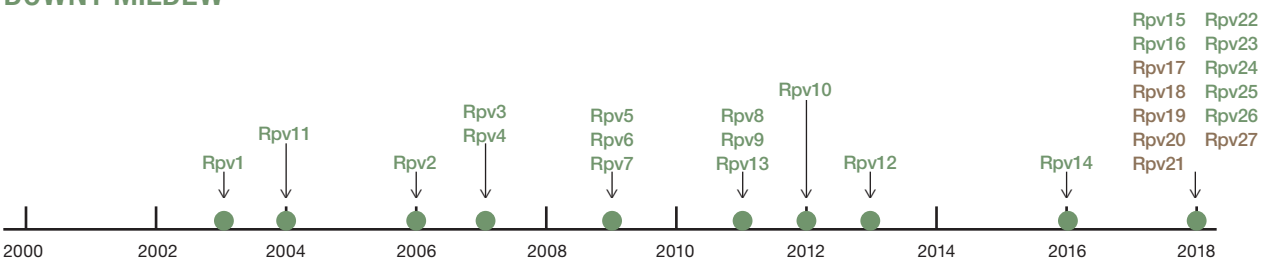
The answer is that they have to make special crosses between two existing varieties or wild accessions and place the resulting seedlings into a special planting called a "mapping population." Mapping populations (there are 12 in the VitisGen2 project) typically consist of 200-300 full-sibling progeny of these crosses, grown in the field. These siblings share half of each parent's DNA, and each vine carries a unique set of traits.

The next step is to use DNA sequencing techniques to identify snippets of DNA sequences (markers) that vary among the two parents. Using a technique called genotyping by sequencing (GBS), VitisGen researchers were able to identify roughly 2,000 DNA markers (~100 for each of the 19 chromosomes) at a cost of $15 for each individual vine tested. Obtaining a detailed genetic map of 200 unique individuals, each with 2,000 markers, used to be very ex-

FIGURE 1:
POWDERY MILDEW




DOWNY MILDEW




Disease resistance loci identified since 2000 for powdery mildew and downy mildew. Black loci denote those that have been identified by scientists in the VitisGen projects.

pensive but is now very affordable at a total cost of $3,000, or less than a penny per marker.

The map of each chromosome is very much like a roadmap with mile markers that show the distance between locations. Once the mapping population and genetic mileposts are in place, researchers then measure the desired trait or phenotype (in this case, powdery mildew or downy mildew resistance) somehow and associate the trait with specific markers on the genetic map.

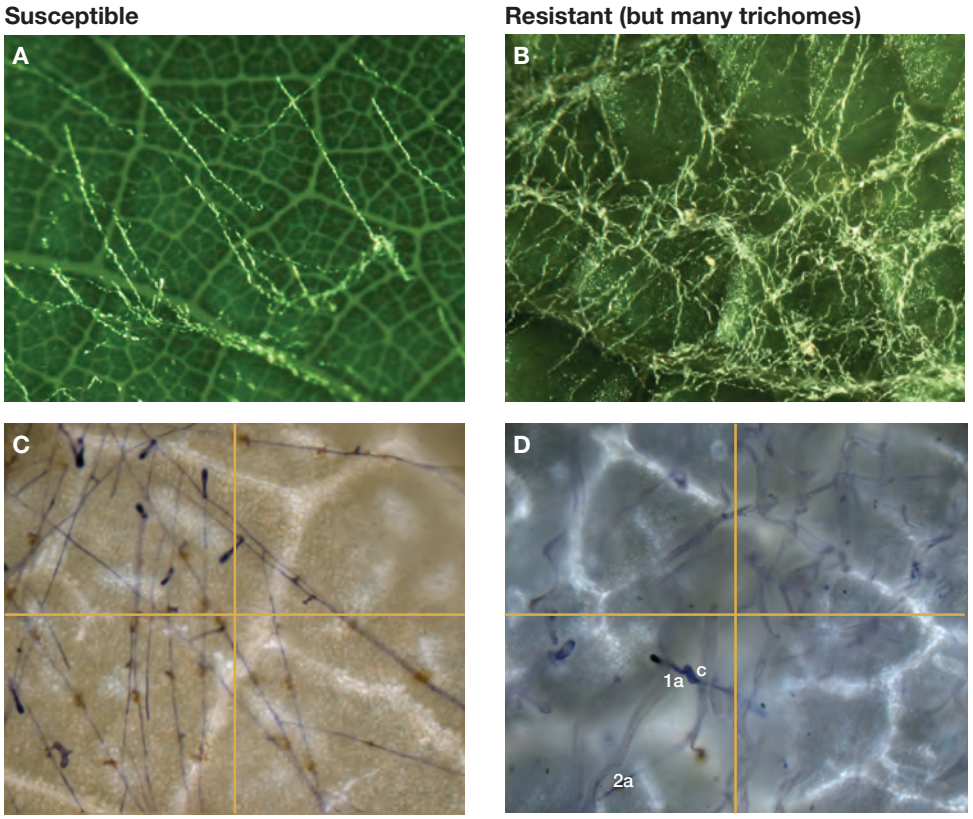
From then on, it's a matter of statistics. Researchers use statistical methods, most commonly a technique called quantitative trait loci (QTL) analysis, to associate phenotypes with DNA markers. These markers — once validated — can be used by grape breeders for marker-assisted selection of the best vines.

## Phenotyping

Obtaining the DNA markers from the 200 progeny — once inconceivably difficult — is now easy and routine. The real bottleneck in the process is measuring the desired traits and how they vary (i.e., phenotyping).

Consider powdery mildew, for example. The challenge in phenotyping powdery mildew resistance is that field populations present notoriously variable environmental conditions, even within a small planting. To address this

FIGURE 2:

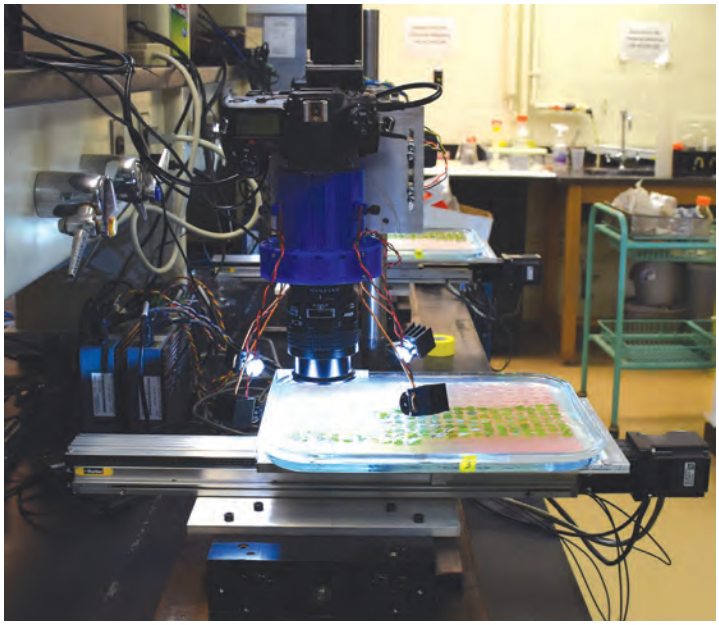



A leaf disk sample susceptible to powdery mildew (A,C) compared to a resistant sample (B,D)

AMERICAN PHYTOPATHOLOGICAL SOCIETY, 2016 DOI: 10.1094/PHYTO-02-16-0080-FI







Firgure 3: Automated powdery mildew phenotyping setup uses image analysis and inoculated leaf disks to rate powdery mildew resistance.

problem within the VitisGen2 project at Cornell AgriTech, the powdery mildew team has developed highly controlled laboratory assays to assess powdery mildew resistance. This is how we do it:

**Rating resistance in the field.** The traditional approach is to allow natural infection of vines (grown with no fungicides) and then to subjectively estimate the severity of disease infections on each vine, typically by using a 4- or 5-point rating scale. However, field ratings are subject to many uncontrolled variables that affect how accurate and precise results are.

Powdery mildew inoculum, though ubiquitous, is not evenly distributed throughout the vineyard, or even within vines, and field populations of the pathogen have their own genetic variability. It almost goes without saying that temperature, solar radiation and rainfall vary from season to season and moment to moment, affecting disease severity and the lighting conditions needed to record ratings accurately.

**Rating resistance in the laboratory.** Laboratory-based phenotyping offers scientists many advantages over field-based disease ratings, including the ability to control the environment, the genetics of the pathogen and the quantity of pathogen spores that land on the leaf. In our laboratory, we use leaf disks and inoculate them with a suspension of spores. After incubation, we then remove the green chlorophyll and stain the fungus dark to count the number of fungal hyphae that intersect with a microscope grid (Figure 2).

This standard procedure eliminates much of the environmental "noise" in field evaluations, removes human bias and subjectivity, and allows us to replicate tests of each vine on several disks. By doing so, we can identify more moderate forms of resistance that would be difficult or impossible to detect in the field.

**Automating the process.** Examining and scoring leaf disks is still a time-consuming, tedious task. With 200 siblings to evaluate, tissue from four shoots and duplicate leaf disks for each shoot, each evaluation involves examining 1,600 leaf disks, which requires three to 12 weeks of staff time spent on physically exhausting and repetitive microscopy. This is one reason why we've developed a robotic evaluation tool that uses live sample imaging to quantify the hyphae in each sample. Our automated process can capture ratings for the same 1,600 leaf disks in one day, delivering results 20- to 80-fold faster than manual microscopy, and with much less pain (Figure 3).

This imaging of living samples and increase in efficiency means we can rate the individual leaf

disks several times or use different strains of powdery mildew spores to test each one of the individual samples. These multiple "snapshots" allow us to characterize how robust the observed resistance is with better accuracy.

### A eureka moment! Phomopsis resistance identified

The ability to use inexpensive sequencing techniques to identify thousands of markers and associate them with desired traits has already resulted in four newly released varieties in France with two powdery mildew and two downy mildew genes (see "ls Europe Starting to Embrace Hybrid Wine Grapes?" *Wines & Vines,* August 2018).

It is interesting to note that information from one of these mapping populations also resulted in other surprising results beyond the VitisGen2 project's focus on powdery mildew.

Technician Steve Luce was pruning a mapping population at Cornell AgriTech for Bruce Reisch, professor of plant breeding and genetics in Cornell's department of horticulture, and noticed that another disease — phomopsis cane and leaf spot — varied in intensity. He recorded which siblings in the mapping population had severe disease symptoms and which had no symptoms. Because the project already had a marker-based map of the vineyard, graduate student Paola Barba was able to use his observations to identify two strong DNA loci for phomopsis resistance on the same day, literally within a few hours of receiving the data. This unanticipated, rapid and useful result further demonstrates the power of having the detailed genetic information available.

To date, the VitisGen project has identified more than 70 new marker-trait associations not only for disease resistance, but also for fruit-quality attributes such as anthocyanin modifications, skin color, sugar and acid content. Inexpensive DNA sequencing techniques, establishment of 12 mapping populations, and field/laboratory phenotyping are the resources that made it possible.

The payoff of these new markers will be better tools for breeders and more high-quality, disease-resistant varieties in the pipeline for current and future generations of grapegrowers.

This research was supported by the USDA-NIFA Specialty Crop Research Initiative (Award No. 2017-51181-26829).

Dr. Tim Martinson is senior extension associate at Cornell University. Dr. Lance Cadle-Davidson is a research plant pathologist with the USDA Agricultural Research Service's Grape Genetics Research Unit, based at Cornell AgriTech.

**To see references and acknowledgements for this article, go to winesandvines.com and search under Magazine › Features › December 2018.**