The Central Role of Mapping Populations in Marker-Assisted Grape Breeding

A tale of three related mapping populations at Cornell AgriTech

Tim Martinson, Bruce Reisch and Rebecca Wiepz

Tim Martinson is senior viticulture extension associate, and **Bruce Reisch** is professor of horticulture, plant breeding and genetics based at Cornell AgriTech in Geneva, NY. **Rebecca Wiepz** is superintendent of the University of Wisconsin-Madison's Peninsular Agricultural Research Station.

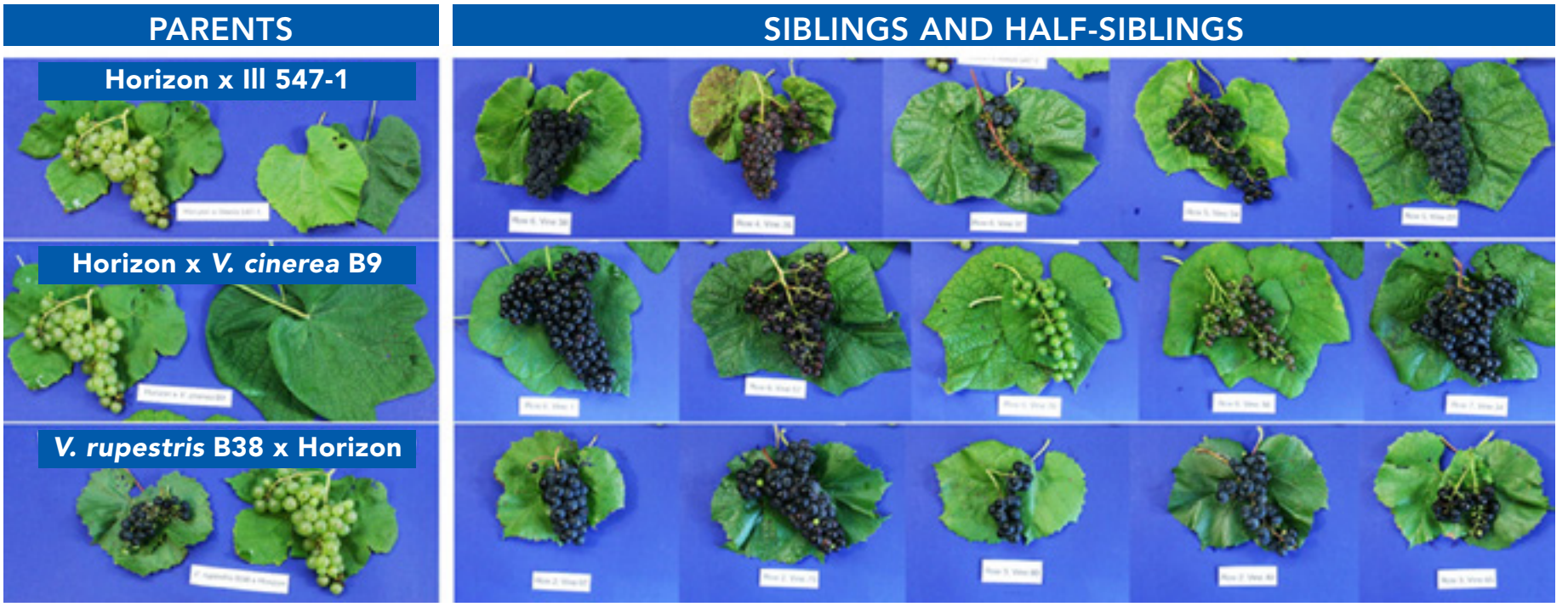

FIGURE 1: Parents and selected progeny from three mapping populations. Illinois 547-1 is the offspring of *V. rupestris* x *V. cinerea* and originated in the early 1960s at the University of Illinois. Horizon is a commercial cultivar released by Cornell University's grape breeding program in 1982. Illinois 547-1 and *V. cinerea* B9 are both male vines that do not produce fruit. The *V. rupestris* B38 parent is a female vine.

PROGRESS IN UNDERSTANDING grapevine genetics was historically hampered by the fact that grapes are perennial crops, take three years to become established and are expensive to maintain. Moreover, genetic tools to extract information about how the traits were inherited were limited, cumbersome and expensive. While corn breeders have had access to inbred lines, they were able to generate large mapping populations to track inheritance of specific traits and could evaluate performance in one year. Grape breeders have historically relied mostly on the observation of traits, without much understanding of the underlying genetics of traits, such as disease resistance.[1]

That has all changed. New, robust and inexpensive DNA sequencing technology has provided grape breeders and genetics researchers with powerful new tools for understanding the genetics behind grapevine traits. Researchers and breeders now have access to detailed genetic maps with from 1,000 to 2,000 DNA markers that serve as "mileposts" for zeroing in on specific regions of the 19 chromosomes and work not only with *Vitis vinifera*, but also with North American and Asian *Vitis* species.

With these tools, grape breeders have been able to identify DNA markers for specific traits and make their selection process dramatically more efficient by testing vines at the seedling stage. Instead of waiting three years to see which progeny have powdery mildew resistance, for example, they now know which seedlings have the appropriate genes, retain those and discard the remainder. This process, called marker-assisted selection, is stocking the grape breeding pipeline with vines that have known traits, which makes the selection process faster, more efficient and less costly.

AMERICAN AGCREDIT
MONEY FOR AGRICULTURE

SUPPORT WHEN YOU NEED IT

During these challenging times, we remain your steadfast financial partner—offering services, resources and expertise to keep your business running while lessening your risks. Your partner through what ifs and what's next.

Call 800.800.4865 today or visit AgLoan.com

The Central Role of Mapping Populations

The key to identifying markers is the use of mapping populations. Breeders are able to discover DNA markers by crossing two dissimilar parents, planting 100 to 1,000 sibling vines in a single block and collecting data that measure how they vary. Unlike a commercial vineyard with thousands of identical plants of one variety, the mapping population consists of genetically different plants with unique combinations of traits from each parent.

The researchers can then use two detailed pieces of information—DNA markers and vine characteristics (phenotypes) scored in the lab or field—to look for marker-trait associations. For example, if some of the vines have no powdery mildew while others are heavily infested, the DNA information, along with the phenotype observed in the field, will be highly correlated with a small region of DNA, typically on one chromosome.

The DNA profiles of each individual vine serve as a reference table that can be matched up to specific vine characteristics observed in the field. The current DNA rhAmpSeq platform[2] developed by the *Vitis*Gen2 project can produce a map with about 2,000 robust markers that work across *Vitis* species and cover 99 percent of the grapevine "core genome." Moreover, researchers can bulk samples from up to hundreds of unique vines in one sequencing reaction and "barcode" with unique DNA base sequences, all at a cost of about $10 per vine sampled (FIGURE 2).

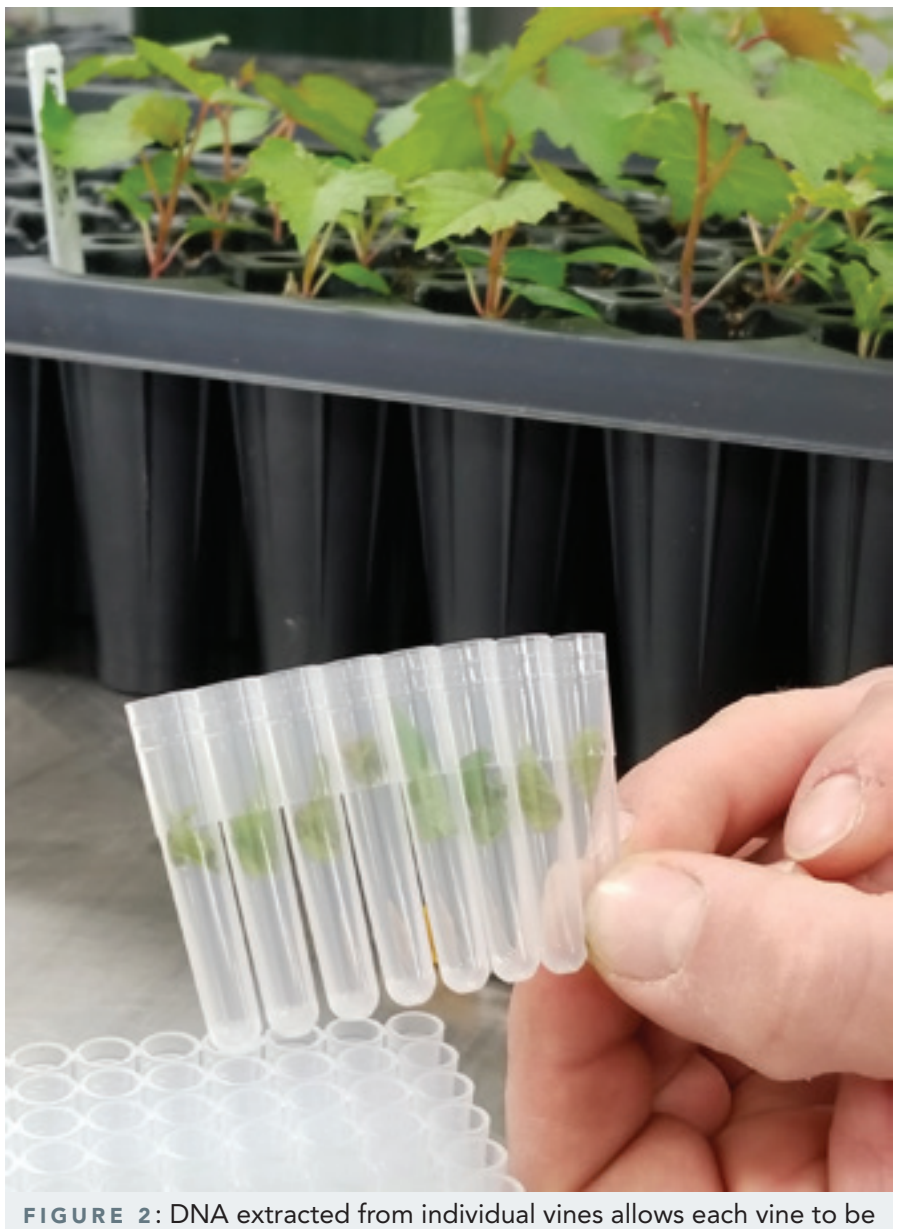

FIGURE 2: DNA extracted from individual vines allows each vine to be "fingerprinted" with approximately 2,000 DNA markers that cover 99 percent of the grape genome.

This is a huge contrast to earlier techniques to extract DNA information, which were expensive, time-consuming, laborious and had limited resolution. For example, an early map produced with Simple Sequence Repeats (SSR) markers required ~50 separate "PCR amplification" reactions (4 SSR primers per reaction) to produce a map with 120 markers (6-10 on each chromosome).[3]

Now, we have genetic maps with up to 2,000 markers from hundreds of vines in one single reaction. This dramatic reduction in cost and complexity has made mapping populations more valuable because the amount of information that can be extracted on a variety of traits is both much more detailed and less expensive to acquire. It's a better and cheaper road map for grapevine genetics.

The *Vitis*Gen2 project is a nationwide effort funded by the USDA's Specialty Crop Research Initiative to discover DNA markers for marker-assisted selection. The project has established and maintains 16 mapping populations of wine and table grape accessions from breeding programs at the USDA laboratory in Parlier, California; the University of Minnesota; Missouri State University; the USDA Grape Germplasm Research Unit in New York; and Cornell University. These mapping populations constitute a great and continuing resource for identifying useful DNA markers and studying inheritance of traits.

The Surprising Contribution of "Illinois 547-1"

Three related mapping populations established by Bruce Reisch's grape breeding program at Cornell AgriTech illustrate the central role of these plantings (and associated detailed DNA maps) in the discovery of DNA markers.

The story starts with an accession called "Illinois 547-1." A University of Illinois scientist named Herbert C. Barrett made extensive crosses of wild and *Vitis sp.* hybrids in the 1950s and 1960s. When the program was terminated in 1967, many of the materials Dr. Barrett had produced went to Cornell's breeding program, including a seed that later became the variety Traminette, which was released by Dr. Bruce Reisch and colleagues in 1996, and a cutting from a male vine called Illinois 547-1.

The Illinois 547-1 cutting resulted from a cross between two wild species: *Vitis cinerea* and *Vitis rupestris*. It grew quietly in a somewhat neglected vineyard at Cornell AgriTech for several years until Reisch noticed that it rarely got powdery or downy mildew, even with minimal fungicide sprays.

"The single vine of Illinois 547-1 just stood out like a sore thumb," said Reisch. "When you viewed it in the summer, it was a mass of foliage without disease, and in the winter, you couldn't help noticing its tremendous trunk, unlike any other in the vineyard" (**FIGURE 3**).

FIGURE 3: Illinois 547-1, a male offspring of two wild species, *V. rupestris* and *V. cinerea*, proved to be a source of powdery and downy mildew resistance. Note the large canopy and enormous trunk of this vine planted in a "no spray" block at Cornell AgriTech.

Mapping Population #1: Horizon x Illinois 547-1

The observed features of Illinois 547-1 inspired Reisch in 1988 to cross it with Horizon, a well-adapted, high-yielding commercial variety with fair disease resistance and low fruit acidity. The initial population of 100 vines (which was expanded in 1996 to 344) was used in studies to identify disease resistance loci and loci influencing acidity and fruit quality.

This mapping population quickly led to the identification of a major powdery mildew locus, later named REN2 (Resistance *Erysiphe necator* 2). By 2000, Reisch's research group had used "randomly amplified polymorphic DNA" (RAPD) markers to identify the REN2 marker on chromosome 14 and demonstrated that it could be used to identify resistant seedlings.[4] They also were able to determine that the REN2 marker originated in the *Vitis cinerea* grandparent.

This was one of the earliest demonstrations that marker-assisted selection would be feasible in grapevine breeding. Yet adoption was slowed by inefficient DNA extraction techniques and the amount of lab work required to detect the presence of REN2 markers.

Illinois 547-1 also exhibited resistance to downy mildew, but locating a single major locus proved to be more elusive. It was thought that the resistance might have been associated with several minor resistance genes, or what geneticists call a "quantitative" trait.

Mapping Populations #2 and #3: Horizon x *V. cinerea* and *V. rupestris* x Horizon

In 2008 and 2009, Reisch established two new mapping populations by crossing Horizon with both the parents of Illinois 547-1. The Horizon x *V. cinerea* B9 (mapping population #2) and *V. rupestris* B38 x Horizon crosses (mapping population #3) were made with the expectation that they would reveal more directly the traits inherited from each of the wild parents of Illinois 547-1. Since reaching maturity in 2012, they have been useful in evaluating and mapping a variety of traits:

FIGURE 4: Variation in timing of bloom in three mapping populations.
4A: Top (The Parents): Clusters of mapping population parents Horizon, Illinois 547-1 (male flowers), *V. rupestris* B38 (female vine) and *V. cinerea* B9 on July 6, 2020.
4B: Bottom (The Progeny): Five representative progeny from each of the three mapping populations. Note that *V. rupestris* x Horizon siblings (left) have more developed berries, following an early bloom. Horizon x *V. cinerea* (right) siblings range from "still in bloom" to post-bloom "shatter." Horizon x Illinois 547-1 siblings show an intermediate range of development from early to late.

DOWNY MILDEW RESISTANCE

Five minor downy mildew resistance quantitative trait loci (QTL) were identified from Horizon x *V. cinerea* and *V. rupestris* x Horizon populations.[5] These five, designated Rpv17 through Rpv21 (Rpv stands for "Resistance to *Plasmospora viticola*," the causal agent of downy mildew), provided partial resistance through three mechanisms: Hypersensitive response (cell death in response to infection), reduction in sporulation and leaf trichome suppression of *P. viticola*.

BLOOM PHENOLOGY

Vitis rupestris blooms early, and *V. cinerea* blooms late. The three Horizon x *V. rupestris*, *V. cinerea* and Illinois 547-1 mapping populations exhibit a wide range of bloom phenology (**FIGURES 4A AND 4B**). On July 6, siblings from the *V. rupestris* x Horizon population were at the "pea-sized" berry stage (having bloomed in mid-June) while the Horizon x *V. cinerea* population was still at the bloom to "bb-sized" berry stage. Horizon x Illinois 547-1 siblings ranged from unopened flowers to pea-sized berries, reflecting segregation of traits from the *V. cinerea* and *V. rupestris* grandparents.

Data collected by former Ph.D. student Al Kovaleski and student interns over two seasons tracked the progression of bloom in ~600 individual vines (**FIGURE 5**). True to their parentage, the *V. rupestris* x Horizon siblings bloomed early and rapidly while the Horizon x *V. cinerea* siblings bloomed late and over a more extended time. The Horizon x Illinois 547-1 siblings were intermediate.

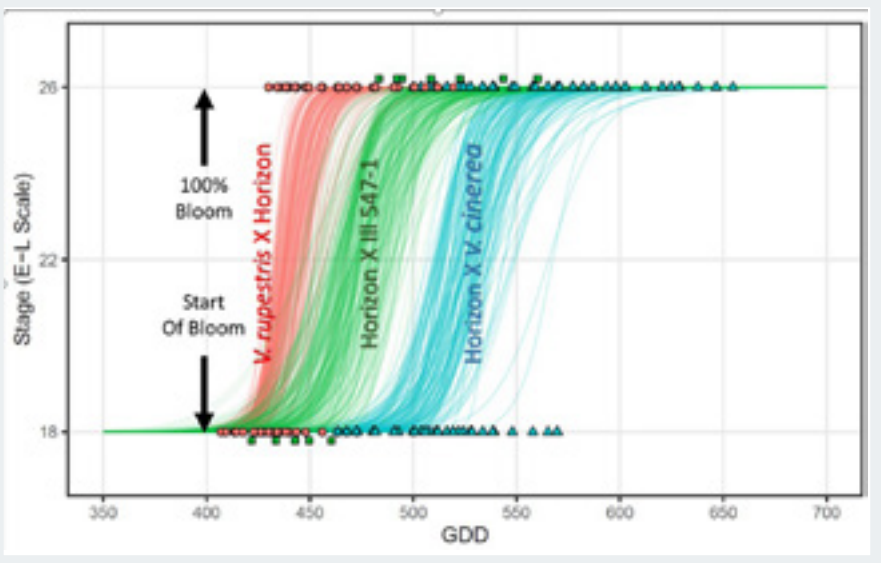

FIGURE 5: Bloom progression from the start of bloom (Eichhorn Lorenz stage 18) to 100 percent bloom (E-L stage 23) in hundreds of individual vines from the three mapping populations over two growing seasons.

MALIC ACID ACCUMULATION AND DEGRADATION

Fruit from wild *Vitis spp.* often has high malic acid concentrations at harvest. Burzynski-Chang et al.[6] found that the pattern of pre-veraison malate accumulation and post-veraison malate degradation typical of *V. vinifera* cultivars did not occur in wild *Vitis spp.*, which tend to retain malic acid during ripening. Post-doc Dr. Noam Reshef and Dr. Gavin Sacks monitored both pre-veraison and post-veraison malic acid concentrations in the Horizon x Illinois 547-1 mapping population and concluded that variations in both accumulation and degradation affect final malic acid. They also found a QTL on Chromosome 7 associated with accumulation that also was associated with final malic acid levels across multiple harvests (FIGURE 6).

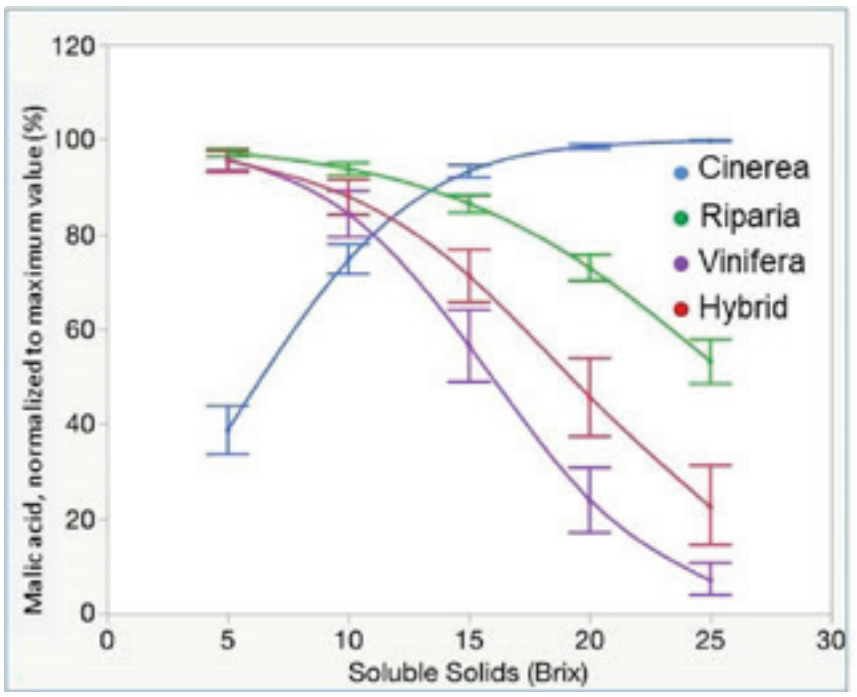

FIGURE 6: Patterns of post-veraison malic acid metabolism in *V. vinifera*, *V. riparia*, interspecific hybrid and *V. cinerea*. Note that *V. cinerea* continued accumulating malic acid after veraison (redrawn from Burzynski-Chang et al. 2020).

SEX DETERMINATION

Wild grapevine species are dioecious–either male or female–unlike cultivated vines, which are hermaphroditic and capable of self-pollination. The Horizon x Illinois 547-1 mapping population provided one of the earliest markers for sex determination (on what was then known as Chromosome 14, now known as Chromosome 2).[7] Recent research by the *Vitis*Gen2 project has further pinned down specific markers that control sex determination. Note that this will be useful in breeding programs with wild parents and will enable breeders to discard male- or female-only vines before planting them out in the field for further evaluation.

PHOMOPSIS RESISTANCE MARKERS DISCOVERED IN ONE DAY

Researchers were not specifically looking for resistance to phomopsis cane and leafspot disease (caused by *Diaporthe ampelina*, formerly known as *Phomopsis viticola*), but it was identified because of the initiative of Steve Luce, technician with Dr. Reisch's program.

While pruning, Luce noticed that about half of the vines in the mapping population had severe phomopsis lesions on canes, and half had none or only mild lesions. He rated phomopsis severity on each vine and brought the data to a lab meeting. The same day, Ph.D. student Paola Barba was able to use Luce's data, along with her existing genetic maps, to identify two strong resistance genes on chromosome 7 (later named Rda1) and chromosome 15 (Rda2).[8]

This final example illustrates the power of having detailed genetic markers for each of the ~500 individual vines in the three mapping populations. The process of linking traits observed in the field to specific genetic markers—that until recently would have involved years of effort—*took place in a single day* (FIGURE 7).

Conclusion

THANKS TO ILLINOIS 547-1

To date, the male vine with the large trunk and disease-free foliage originating in Illinois in the 1960s has resulted in the discovery of DNA markers associated with resistance to three major fungal diseases, sex determination, malic acid accumulation and degradation, and timing of flowering. Illinois 547-1 has contributed a lot to our understanding of genetics behind these traits, and the resulting markers are being incorporated into advanced breeding lines that will result in improved, more disease-resistant varieties.

THREE OF THE 16 *VITIS*GEN2 MAPPING POPULATIONS

The three mapping populations at Cornell AgriTech provide a snapshot of the enormous resource that mapping populations provide to grape breeding programs. However, they are just one part of the nationwide *Vitis*Gen projects (*Vitis*Gen1 and *Vitis*Gen2), which have provided funding to support 16 mapping populations in California, Minnesota, Missouri, South Dakota and New York. This USDA support has changed the nature of ongoing conventional breeding programs.

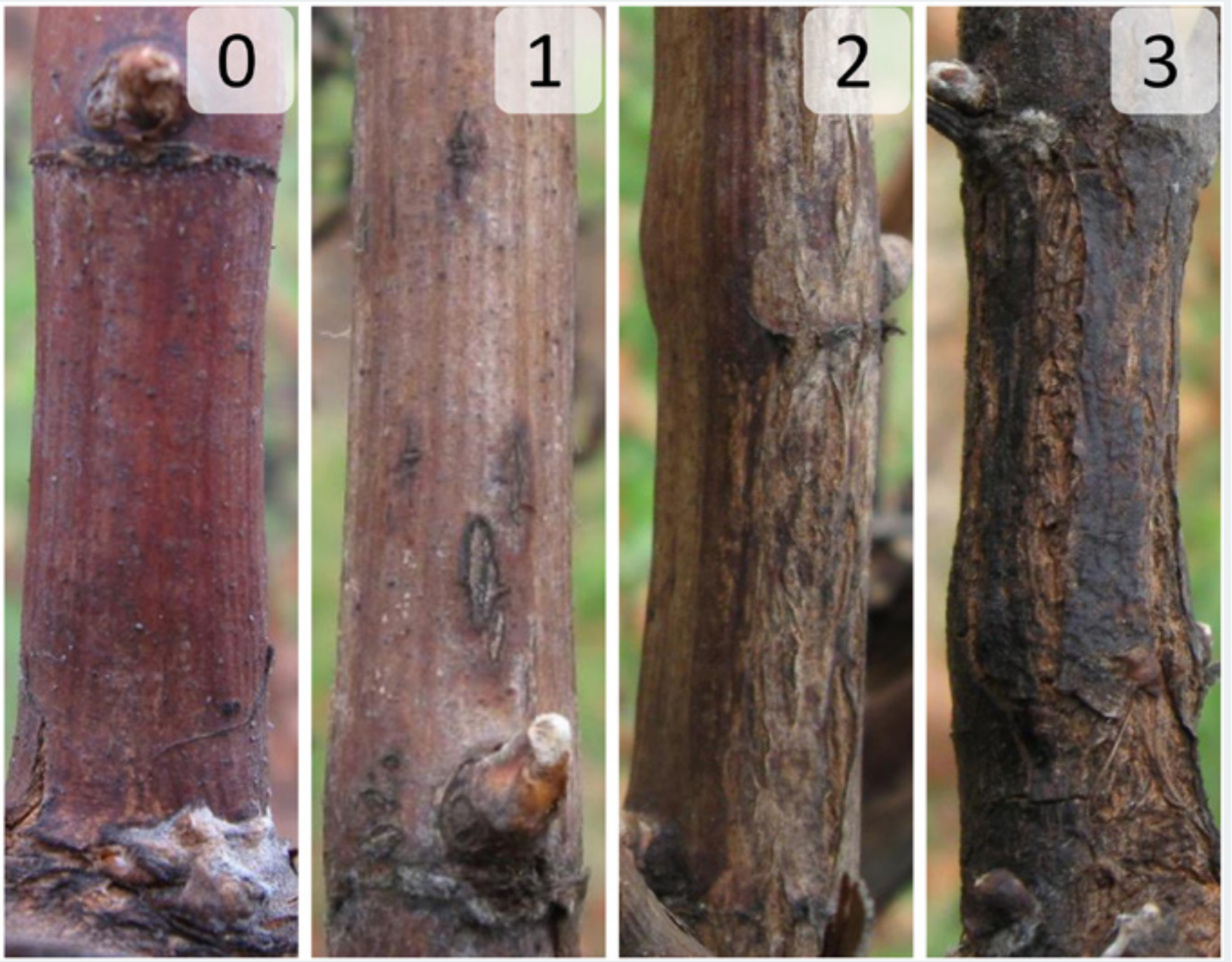

FIGURE 7: Different severity of Phomopsis disease on overwintering canes discovered during dormant pruning (Barba et al. 2018).

Historically, grape breeders' goals were simply to cycle through each year's crosses to identify the rare vines with commercial potential and discard those that didn't measure up. It was hard to justify the added expense of maintaining "extra" vines in a dedicated mapping population. Funding from the USDA has been a game-changer. It has allowed breeders to maintain larger populations of vines over several years to discover the genetics behind the traits and share DNA marker information with each other.

As a result, DNA markers that track over 25 new traits, each with four to 12 DNA markers, are now available to breeding programs, allowing them to stock the pipeline more efficiently with vines that are more disease-resistant, resilient to climate change and have better fruit quality. **WBM**

Acknowledgement

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

References

1. Martinson, T. Grape Breeders No Longer Flying Blind. *Wines & Vines*. March 2018.
2. Martinson, T., Sun, Q., Zou, C. and L. Cadle-Davidson. Grape Breeders Search for Reliable DNA Markers. *Wine Business Monthly*, December 2019.
3. Garris, A., Clark, L., Owens, C., McKay, S., Luby, J., Mathiason, K. and A. Fennell, 2009. Mapping of Photoperiod-induced Growth Cessation in the Wild Grape *Vitis riparia*. *J. Amer. Soc. Hort. Sci.*, 134:261-272.
4. Dalbó, M.A., Ye., G.N., Weeden, N.F., Wilcox, W.F. and B.I. Reisch. 2001. "Marker-assisted selection for powdery mildew resistance in grapes." *J. Amer. Soc. Hort. Sci.* 126(1): 83-89.
5. Divilov, K., Barba, P., Cadle-Davidson, L. and B.I. Reisch. 2018. "Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines." *Theoretical and Applied Genetics* 131(5): 1133-1143.
6. Burzynski-Chang, E.A., Brown, E.J., Reshef, N. and G.L Sacks. 2020. "Malate content in wild Vitis spp. demonstrates a range of behaviors during berry maturation." *Am. J. of Enol. Vitic.* 71(1): 80-87.
7. Dalbó, M., Ye, G., Weeden, N., Steinkellner, H., Sefc, K. and B. Reisch. A gene controlling sex in grapevines placed on a molecular marker-based genetic map. *Genome* 2000, 43(2): 333-340.
8. Barba, P., Lillis, J., Luce, R.S., Travadon, R., Osier, M. et al. 2018. "Two dominant loci determine resistance to *Phomopsis* cane lesions in F1 families of hybrid grapevines." *Theoretical and Applied Genetics* 131(5): 1173-1189.