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Grape breeders and geneticists have identified numerous DNA markers for powdery mildew (10 markers), and downy mildew (~20 markers) that offer the prospect of dramatically reducing the number of fungicide sprays required for standard *V. vinifera* cultivars. Most originate in wild *Vitis* spp. native to North America. Once identified, breeders can use the markers to select the right seedlings from conventional cross-breeding to retain before planting them in vineyard trials.

The fruit quality team in the *VitisGen2* project, led by Gavin Sacks, is working to characterize and identify genetic associations with several fruit quality attributes to discover DNA markers that will be useful to breeders striving for high grape quality.

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**Figure 1.** North American *Vitis* spp. coevolved with powdery mildew and downy mildew – and represent an important source of disease resistance. They also have different fruit composition attributes that impact flavor, aroma, and mouthfeel of wines.

**Key Concepts**

- North American *Vitis* spp. are major sources of disease-resistance genes that European *Vitis vinifera* cultivars don’t have.
- The five wild *Vitis* species (*V. labrusca*, *V. aestivalis*, *V. rupestris*, *V. cinerea*, and *V. riparia*) used most often in breeding lines have genetic fruit attributes that differ from *V. vinifera* cultivars, affecting taste, aroma, mouthfeel, and color.
- The *VitisGen2* project is measuring fruit quality attributes in several ‘mapping populations’ to identify DNA markers associated with malic acid accumulation and degradation, tannin content, and 12 aroma-related compounds.
- By linking fruit quality attributes to DNA markers, grape breeders will be able to screen seedlings for desirable fruit attributes and reject those that have undesirable flavor, mouthfeel, or aroma profiles.
How fruit composition of wild *Vitis* *spp.* differs from *V. vinifera*.

Wild *Vitis* *spp.* juice and wine characteristics vary significantly from traditional *V. vinifera* wine cultivars. We can break down the issues into four general categories:

- **Taste.** Wild *Vitis* are characterized by high malic acid and high titratable acidity (TA), which correlates with sourness. Despite their high TA, wild *Vitis* often have high pH (>3.6) due to their high mineral content (especially K+) which increases the chances of microbial instability in finished wines.

- **Aroma.** Large-berried grapes such as *V. labrusca* hybrids can have undesirable “foxy” aromas. Small-berried grapes (such as *V. rupestris* and *V. aestivalis*) have vegetal, ‘herbaceous’ aromas.

- **Mouthfeel.** Wild grapes and their hybrids have lower amounts of tannins and lower tannin extractability.

- **Color.** The color of wines made from wild *Vitis* *spp.* and hybrids can be unstable, and wines can have a ‘blueish’ tint.

How sugar/acid relationships vary in *Vitis* *spp.* and interspecific hybrids.

Winemakers and growers track sugars (soluble solids) and acidity during the ripening process. In commercial cultivars, malic and tartaric acid accumulate before veraison. After veraison, sugars accumulate and malic acid degrades up until harvest. The degradation of malic acid, along with berry expansion, results in a dramatic decrease in TA from veraison (~30 g/L as tartaric acid equivalents) to harvest (5-9 g/L). But if you examine wild *Vitis*, the relationships between soluble solids, titratable acidity, and juice pH during the ripening process differ from patterns seen with *V. vinifera*. In particular the pattern of malic acid accumulation and degradation is different.

In *V. vinifera* grapes, malic acid accumulates in berries through veraison, then declines during the ripening phase (Figure 2). At harvest, tartaric acid tends to predominate.

![Figure 2](image)

**Figure 2.** Malic acid accumulation and degradation patterns in *V. riparia*, *V. cinerea* and interspecific hybrids compared to the typical pattern in *V. vinifera* cultivars.

North American *Vitis* *spp.* don’t behave the same way (Figure 2). In wild *V. riparia* and *V. cinerea*, malic acid accumulates through veraison, but then remains constant on a per-berry basis after veraison. The net result is that wild *cinerea* and *riparia* grapes can end up at maturity with both high sugars and high acid (~20 g/l TA). Hybrids tend to have an intermediate response between *V. vinifera* and *V. riparia*.

High Acidity and High pH.

This lack of malic acid degradation presents challenges to winemakers. Although pH and TA are inversely related, the relationship is imperfect – many interspecific hybrid cultivars can have both high levels of acids and high levels of minerals (especially K+) which results in both high pH and high TA in harvested fruit (Figure 3). This can lead to both high acidity (sourness) and high pH (>3.6 increases the risk of microbial stability) – and makes it challenging to reduce acidity in the winery to palatable levels without it resulting in excessively high pH at bottling.

![Figure 3](image)

**Figure 3.** (Top) Relationship between TA and pH in a wide range of hybrid and vinifera cultivars across several years. Note that Vignoles (hybrid) and Riesling have a two-fold difference in TA, but similar juice pH values. (Bottom) Interspecific hybrids tend to have higher TA levels at a given pH than do vinifera cultivars. (data from Veraison to Harvest, Gerling & Martinson, eds).

Tannins and Mouthfeel.

Condensed tannins are an important contributor to mouthfeel, particularly in red wines. These condensed tannins are polymers (long chains) of flavanols (Figure 4), a class of polyphenol molecules, and are found in the skins and seeds of grapes. Condensed tannins bind with proteins in saliva, decreasing their ‘lubricating’ ability and leading to the sensation of ‘astringency’ or drying in the mouth.

![Figure 4](image)

**Figure 4.** Condensed tannins are an important contributor to mouthfeel, particularly in red wines. These condensed tannins are polymers (long chains) of flavanols, a class of polyphenol molecules, and are found in the skins and seeds of grapes.
In contrast to red *vinifera* cultivars, wines produced from traditional interspecific hybrids (Figure 5) have little or no condensed tannins – leading to the perception that as a group they are ‘thin’, and have very little ‘body’. To find out why, the Sacks lab undertook a study to measure proteins and tannins in a wide variety of interspecifics and wild *Vitis* spp. They found that the fruit tended to have somewhat lower – but overlapping – concentrations of tannins than *V. vinifera* cultivars, but the resulting wines had little or no tannins. (Figure 6). What could account for this difference?

Simply put, the higher protein content in wild *Vitis* spp. and hybrids binds tannins and removes them from juice and wine. Even if high amounts of exogenous tannins are added during winemaking, most of it gets bound to soluble proteins and removed from the wine. Some of these proteins are referred to as pathogenesis-related proteins (PRPs), because early studies in other plants...
observed that they increased following exposure to plant diseases. However, PRPs can also increase and decrease in response to other stresses. Ongoing work between Sacks and collaborators is investigating if removal of PRP throughout the winemaking process can increase final tannin concentrations.

“Foxy” and vegetal aromas.

Many odorant compounds contribute to overall wine aroma – and may be present in wines at miniscule (nanogram per L, ng/L) to higher (mg/L) levels. Odorants known to contribute include methyl anthranilate and o-aminoacetophenone – both associated with Labrusca-type grapes such as Concord and Niagara. Small-berried Vitis spp. tend to have compounds associated with vegetal flavors, such as methoxypyrazines and hexanol. Although these compounds can also be found in vinifera, their concentrations are much higher in wild Vitis.

How the VitisGen2 project is identifying associations between genes and flavors.

Traditionally, wine grape breeding programs have relied upon tastings and sensory ratings to evaluate new grape selections. In our program at Cornell, tasting panels are asked to rate wine quality and flavor attributes from research wines made annually. Specifically, they are asked to rate the aroma, flavors, and prevalence of ‘hybrid characters’, as well as ‘pleasantness’ in the wines. The VitisGen2 project is taking a different approach that involves linking observed traits (such as low TA) with DNA markers, so that future grape breeders can use the markers to screen new breeding selections.

The process starts out with a mapping population (Figure 9) of 100-300 vines. Each of the vines in a mapping population are siblings resulting from a cross between two distinct parents. Each sibling has a unique combination of parental genes (50% from each parent) – so researchers can ‘fingerprint’ each vine with many DNA markers. The VitisGen2 project has identified 2000 different DNA snippets (markers) that provide coverage of 99% of the 19 grape chromosomes that all Vitis species (except muscadine grapes) have in common.

The fruit quality team collects fruit samples during ripening and at harvest from each of the mapping population’s vines and tests them for a number of fruit quality attributes (Figure 10) including:

- Malic acid degradation and accumulation
- Total tannins (extracted in 70% acetone)
- Extractable tannin (extracted in 15% ethanol)
- 12 different volatile compounds (extracted by gas chromatography)

Finally, the fruit quality analytical results from each vine in the mapping population are compared to the existing genetic map (Figure 11) to discover genetic markers associated with fruit characteristics such as malic acid accumulation and degradation, total and extractable tannins, and odor-active concentrations of 12 aroma compounds. To date the fruit quality team has processed over 1500 samples from seven mapping populations associated with the VitisGen2 project.
VitisGen2 researchers have already identified over 70 genetic markers related to disease resistance and fruit characteristics. These DNA markers have provided grape breeders with new tools to evaluate vine characteristics at the seedling stage and make the breeding process more efficient. Discovery and use of fruit quality DNA markers is still at the early stages. As more fruit quality markers become available, breeders will be able to make smarter selections, and get more high-quality vines into the breeding pipeline.

References


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