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VITICULTURE & ENOLOGY
UNIVERSITY OF CALIFORNIA DAVIS

Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes

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In: American Journal of Enology and Viticulture. 59(3):235-247. 2008

• In this article, which was awarded the 2009 Best Paper in Viticulture by the American Society for Enology and Viticulture, the authors designed an elegant experiment to learn about the independent effects of temperature and sunlight –very difficult to tease apart in the vineyard– on the phenolic profiles of Merlot grapes.

• There are 3 main groups of flavonoids in grapes: flavonols, anthocyanins, and flavanols. 1) *Flavonols* are the "sunscreen" of grape berries and are important for color enhancement – they are often bound to glucose to form glycosides; 2) *anthocyanins* are colored monomers responsible in large part for the color of young wines; and 3) *flavanols* are the "building bricks" of tannins, also known as proanthocyanidins, and they are important to stabilize color, protect the wine against oxidation, and contribute to a wine's taste and mouthfeel. The current authors studied the two first.

• Through the use of ingenious practices, such as shoot positioning over the clusters, air blowers right below the clusters, and thermostats to regulate air temperature, the authors were able to subject Merlot clusters to the following 10 treatments consisting of various combinations of temperatures and solar exposures:

"SUN" = **exposed to direct solar radiation on the east aspect of the canopy (rows ran N-S) + ambient temperature**
"SHADE" = **shaded from direct solar radiation on the east aspect of the canopy + ambient temperature**
"WEST-SUN" = **exposed to direct solar direction on the west aspect of the canopy + ambient temperature**
"SUN+1"= **like "sun" but treated with hot air [1]**
"SUN+2"= **like "sun" but treated with even hotter air [2]**
"SHADE+1"= **like "shade" but treated with hot air**
"SUN-1"= **like "sun" but treated with cold air**
"SUN-2"= **like "sun" but treated with even colder air**
"SUN-BLOWER"= **like "sun" but treated with ambient-temperature air (to test for effects of blowing air)**
"SHADE-BLOWER"= **like "shade" but treated with ambient-temperature air (to test for effects of blowing air)**

[1] In "+1" and "-1", clusters were heated to the average temperature of sun clusters, or cooled to the average temperature of shaded clusters, respectively

[2] In "+2" and "-2", the magnitude of temperature change at which the clusters were heated or cooled, respectively, was doubled.

• The authors harvested the fruit from the different treatments at 24 °Brix, peeled the skins off the berry samples, and extracted and measured individual anthocyanins and flavonol-glycosides. (They actually sampled and analyzed berries in various locations on the cluster separately, but did not find a difference). Given that the authors used skin discs of known surface area for the extraction, the results (expressed in $\mu g/cm^3$) are independent of berry size. They also measured Brix, TA and pH from whole berries.

• Before we take a look at the results, let's go over the award-winning experimental design, and why the authors chose to compare these treatments and not others. In various treatments (*Sun-2, Sun-1, Sun, Sun+1, Sun+2*), the clusters were all sunlit, but the temperature was gradually increased. These treatments, therefore, allow the authors to learn about the effects of *temperature* on berry composition. In contrast, in various pairs of treatments (*Sun* and *Shade+1*, or *Shade* and *Sun-1*), the clusters had the same temperature, but solar radiation was different. Therefore, any differences in berry composition between these treatments are considered to be due to differences in *solar radiation*. Let's now see the results.

• **Effect on juice composition: Brix, TA, pH.** Treatments had no effect on berry size or berry Brix. In contrast, berries exposed to higher temperatures had lower TA (5.9 g/l in *Sun+2* vs. 7.6 in *Sun-2*) and higher pH (3.76 in *Sun+2* vs. 3.58 in *Sun-2*).

• **Effect on berry skin composition: anthocaynins and flavonols.**
1) Effect of temperature. As berry temperatures increased, total anthocyanins in the skin decreased. Increased berry temperatures also caused a shift in the anthocyanin populations (the proportion of acylated vs. non-acylated anthocyanins increased, and the proportion of trihydroxylated vs. dihydroxylted anthocyanins also increased, even though the practical implication of these changes is not yet understood). When the authors compared the composition of berries sun-exposed on the east (*Sun*) versus the west (*West-Sun*), they found that the west-exposed berries had the lowest total anthocyanins (as well as lower levels of all individual types of anthocyanins). These "west-berries" had been exposed to temperatures above 35^{o}C for 3 times longer than "east-berries", and to temperatures above 40^{o}C for 5 times longer than "east-berries". Thus, anthocyanin accumulation appears to respond negatively to prolonged high temperature extremes.

• **2) Effect of solar radiation.** In agreement with previous results, berries from clusters exposed to solar radiation (i.e. *Sun*) had more than twice the amount of flavonol-glycosides (such as quercetin) than berries from shaded clusters (i.e. *Shade+1*). Solar radiation also caused certain shifts in the individual anthocyanin populations.

• **3) Interaction of temperature and solar radiation**. Under heavy shading, higher berry temperature led to lower levels of the most abundant anthocyanin type: malvidin-glucoside. However, under exposure to direct solar radiation, high berry temperature normally had no influence on malvidin-glucoside (except when temperatures were extremely high, like on the west side). Thus, **low incident solar radiation alone appeared not to compromise total anthocyanin accumulation. Instead, it was the combination of low light <u>and</u> high berry temperature that decreased total anthocyanins.**

Some highlights from the authors' discussion:
_ anthocyanin accumulation in Merlot grapes appears to be determined by a synergistic combination of solar radiation and berry temperature more complex than previously thought;
_ temperature, particularly daytime temperature during ripening, appears to be the overriding environmental determinant of anthocyanin development;
_ it is still unknown whether anthocyanin accumulation responds to the integration of various temperature parameters (specific temperature extremes, specific durations to these temperature extremes, the combination of the two, also called "thermal time"), or if one overrides the others;
_ likewise, it is still unknown at which critical stage of development excessive temperatures might have the most negative impact. A previous study indicated that a key period might be *the fortnight (14 days) following the first signs of veraison*.

Congratulations to the authors!

Author: Bibiana Guerra, Editor: Kay Bogart. This summary series funded by J. Lohr Vineyards & Wines