A proposed method for monitoring grapevine berry sugar and fresh mass evolution
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Proposal for a protocol for measuring berries sugar loading and the evolution of berry fresh mass

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This working document proposes a method for monitoring the evolution of sugar loading in berries and the tracking of berry volume (figure 1).

- The evolution of berry volume provides information about the vine's water status and dynamics of accumulation and water loss in the berries. These measurements are useful for anticipating berry shriveling and assessing the effectiveness of irrigation (note: after the sugar loading plateau, the berries irreversibly lose water; Shahood *et al*., 2020; Deloire *et al.,* 2021; Savoi *et al.,* 2021).
- Monitoring sugar accumulation in the berries can be used to determine the harvest date (Antalick *et al*., 2021) or as a physiological indicator of vine and grape functioning (Deloire, 2013; Wang *et al*., 2003).

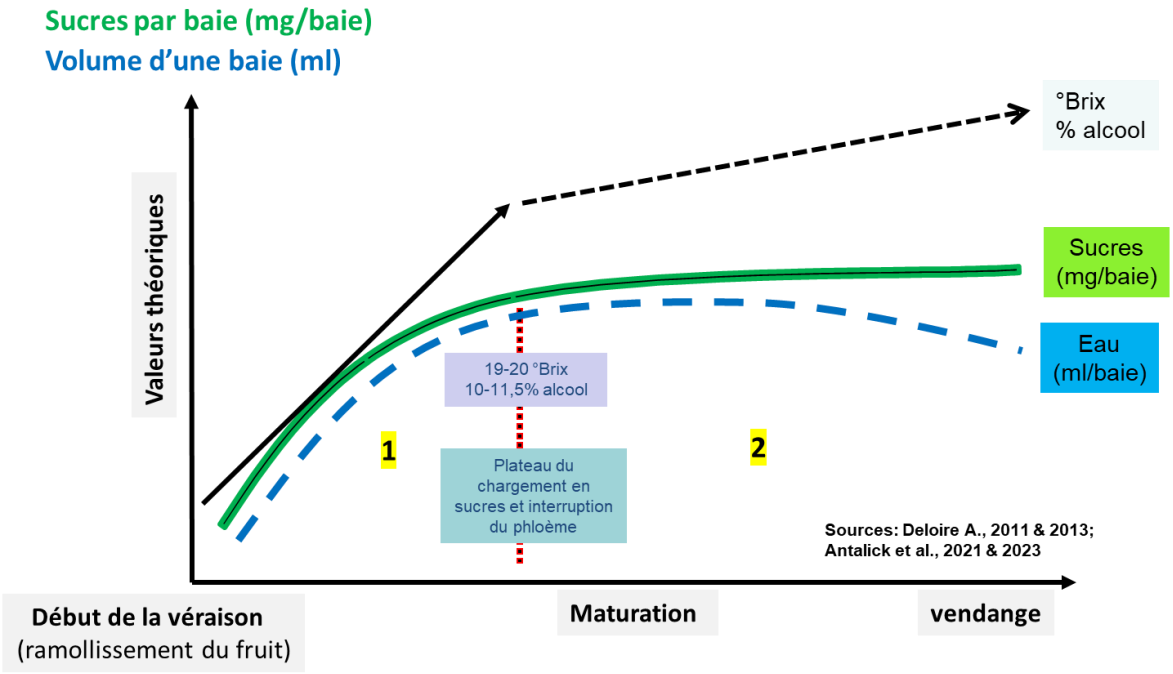

Figure 1: Evolution of berry fresh weight and sugar loading (mg/berry) from veraison (onset of ripening) until harvest. These analyses provide information on: 1) the rate of sugar accumulation in the berries (slope of the curve; mg/berry/day); 2) the duration of loading (generally around 28-30 days from the onset of veraison (berry softening) to the plateau); 3) the sugar concentration (°Brix or probable alcohol %) at the plateau (usually between 19 and 20 °Brix depending mainly on the terroir (soil x climate) and the variety); 4) the fresh weight or volume of berries at the plateau; 5) the rate of water accumulation pre plateau and the rate of water loss and sugar concentration (g/l) from the plateau to harvest.

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For Berry Sugar Loading (BSL : mg/berry)), it is necessary to perform sampling kinetic based on sugar concentration values (expressed in °Brix in this document), rather than fixed dates.

The suggested sampling kinetics of berries (or clusters) are aligned with the following sugar concentration values:

- 6-8 °Brix (°Brix values corresponding to green and soft berries, indicating the beginning of veraison) (figure 2).
- 12-15 °Brix (°Brix values corresponding to mid-veraison, with generally 80% of soft berries and 50% of colored berries on bunches).
- 18-20 °Brix (°Brix values corresponding to the theoretical plateau of berry sugar loading: 100% soft and red berries for red grape varieties) (Shahood *et al*., 2020).
- 22-24 °Brix (°Brix values corresponding to the post-plateau stage of berry sugar loading.
- °Brix values beyond 24 correspond generally to over ripe grapes.

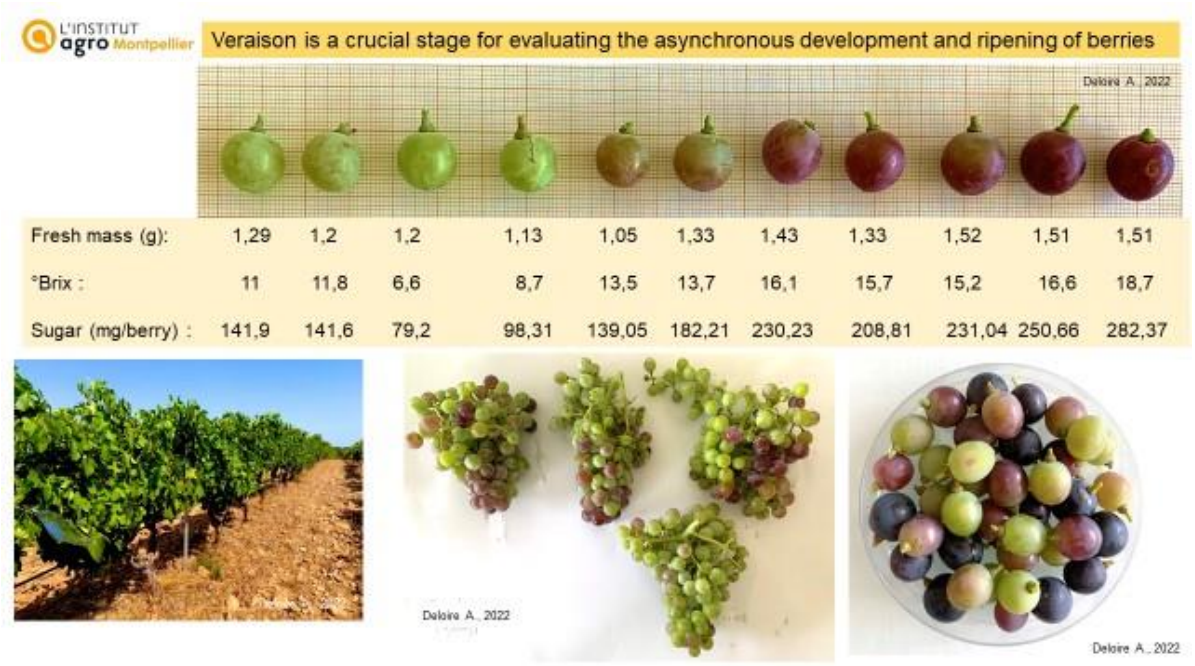

Figure 2 : Example of variability in berry fresh masses (g), °Brix values (sugar concentration), sugar per berry (mg), and coloration (anthocyanin biosynthesis) within and between Grenache clusters for a specific date during veraison. This is illustrating the asynchronous development of berries within a bunch.

This protocol requires weekly visits to the vineyard to measure the °Brix values of a few berries (per berry) to determine whether further sampling of berry to reach maximum sugar loading is necessary (note: this step involves conducting weekly sampling, considering that the sugar accumulation rate in berries depends on the grape variety, environment, and cultivation practices).

Sampling should start at the very beginning of veraison, at average °Brix values of 6-8.

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Between 18 and 22 °Brix, it may be necessary to visit the vineyard twice a week, as the sugar loading plateau can be reached rapidly within 2 or 3 days from 18 °Brix.

Simultaneous measurements of fresh weight and °Brix of the berries are necessary to calculate the quantity of sugar per berry (mg/berry) using the formula below.

Sampling: The quantity of berries or clusters to be sampled (including potential repetitions) also depends on the number of available vines in the experiment and required additional analyses.

In practice, a sample of 200 to 400 berries or about 10-12 clusters is usually taken in the vineyard. However, this sample size can be increased and adjusted on a zone scale for heterogeneous vineyards or reduced if the objective is to evaluate the development of berries on a limited number of plants in the vineyard. It is possible to work on a per berry basis for research purposes (Shahood *et al.,* 2020; Bigard *et al*., 2019).

Berry-by-berry analyses can also be performed, considering each berry as an individual. Berry-by-berry analyses are mainly conducted for research purposes to understand vine functioning mechanisms and assess the heterogeneity of their development during veraison and harvest. In this regard, the Dyostem® tool developed by Vivelys (https://www.vivelys.com/) is very useful for automating the measurement of volumes in a population of 200 berries, assessing their homogeneity/heterogeneity, and taking only a few seconds for measurement.

In any case, the number of berries sampled should not exceed 10% of the vine's maximum fruit load to avoid altering the development of the remaining berries. A vineyard sampling method has been proposed by Oger *et al*., 2021, and additional information on sampling is available in the book by Carbonneau *et al.*, 2020.

Equipment: a) A refractometer and a precision balance (1g) for measuring the fresh weight of the berries. A precision balance of 0,1g will be required to measure the fresh mass of single berry. The calculation of sugar quantity per berry is given by the equations below (McCarthy and Coombe, 1999).

The proposed formula to calculate the quantity of sugar per berry (mg/berry) is:
Sugar (solutes) per berry (mg) = (berry fresh mass (g) x (°Brix/100)) x1000

b) **Scissors**: the berry should be cut at the pedicel level rather than being pulled off. If the clusters are compact, it is preferable to sample entire clusters in the vineyard. However, for maturity monitoring with less sampling constraint in the vineyard, the berries can be separated from the clusters (sampled) without the pedicel (paying attention to avoid excessive juice loss).

Real-time volume and sugar accumulation curves of the fruit enable prompt decision-making, for example:

- Managing water supply through irrigation.
- Anticipating harvest at the beginning of water loss or berry shrivelling, especially after the sugar loading plateau (to limit yield losses and fruit concentration).
- Anticipating harvest dates based on desired aromatic profiles of wines (e.g., fresh fruit or ripe fruit; Antalick *et al*., 2021).

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- Evaluating vine functioning. Reduction or inhibition of photosynthesis due to thermal and/or water stresses is reflected in real-time by a decreased rate of sugar loading and reduction in berry volume (Wang *et al*., 2003).
- Assessing the heterogeneity of maturation dynamics within a population of berries. This developmental heterogeneity can have consequences on the aromatic profile of wines.

The **Dyostem®** tool allows real-time monitoring of volumes in a population of 200 berries.

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