PHLOEM ANATOMY TRAITS PREDICT MAXIMUM SUGAR ACCUMULATION RATES

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Abstract:

Context and purpose of the study

Heat and water stress can accelerate berry sugar accumulation and lead to excessive sugar-to-acid ratios at harvest, producing bland, overly-alcoholic wines. Selecting grapevines for slower sugar accumulation could help maintain wine quality under future, hotter conditions, but these efforts have been stymied by our limited understanding of the traits determining sugar accumulation rates. Here, we measured traits characterizing the structure and anatomy of the sugar transport system – the phloem – in 16 winegrape cultivars and tested for relationships with sugar accumulation rates and cultivar climate classifications. We expected hot-climate cultivars to delay sugar accumulation through traits that increase resistance to phloem transport.

Material and methods

We measured mature vines of 8 hot-climate (red: Syrah, Montepulciano, Mourvèdre, Tempranillo, Zinfandel, and Anglianico; white: Fiano and Verdelho, 7 warm-climate (red: Barbera, Cabernet Sauvignon, Merlot, Carignane, and Nebbiolo; white: Chardonnay and Sauvignon Blanc), and 1 temperate-climate (white: Riesling) cultivars growing in an experimental vineyard block on the UC Davis campus (N = 3 – 4 vines/cultivar). We measured berry total soluble solids (TSS) every 2 - 3 weeks from Jun – Sep 2020 and calculated the maximum sugar accumulation rate for each cultivar as the maximum slope of the relationship between TSS and growing degree days (GDD). We sampled leaves and berries in Sep 2020 and used light microscopy to measure total and mean phloem area area and scanning electron microscopy to measure sieve plate porosity and sieve element area in the leaf midvein, petiole, and berry pedicel.

Results

The maximum sugar accumulation rate was significantly correlated with the total phloem sieve element area in the pedicel (r^2 = 0.25, p = 0.046, N = 16) and petiole (r^2 = 0.48, p = 0.004, N = 15). Maximum rates of sugar accumulation were faster in the cultivars with more phloem area. The total phloem area in the pedicel and the petiole was significantly smaller, and sugar accumulation was slower, in the hot-climate than the warm-climate red cultivars (ANOVA, $p < 0.05$). Mean sieve element area and sieve plate porosity were not significantly different between the climate groups or correlated with sugar accumulation rate ($p > 0.05$). These findings show that heat-adapted cultivars may avoid excessive sugar accumulation through phloem traits that reduce the capacity for sugar transport. Future work should test whether reduced phloem area also contributes to a water-saving strategy, by impeding sugar export from the leaves and activating sugar-induced signalling for stomatal closure. These findings also suggest a potential application for petiole phloem area in screening for rates of sugar accumulation, since petioles could be sampled years before vines are mature enough to produce fruit.

Keywords: Sugar accumulation, phloem, plant anatomy, climate adaptation, viticulture

1. Introduction

Wine quality reflects the balance between sweetness and acidity, which is strongly impacted by the ratio of berry sugar to acid content at harvest (Conde *et al.,* 2007). Berries with excessive sugar-to-acid ratios produce overly alcoholic, "flabby" wines. Higher temperatures generally accelerate sugar accumulation and acid degradation, causing the berries to reach optimal sugar-to-acid ratios before flavor and aroma compounds have fully developed (van Leeuwen & Destrac-Irvine 2017). Thus, identifying mechanisms to reduce the rate of sugar accumulation is a key strategy to mitigate the effects of climate change on wine quality (Duchene *et al.,* 2012).

The impact of heat on sugar accumulation reflects the physiology of the sugar transport tissue (phloem). The phloem is loaded with sugar in the source tissues (e.g., photosynthesizing leaves) to create a concentration gradient that draws in water from the adjacent water transport tissue (xylem). This water influx creates a pressure gradient that pushes sugar sap through the phloem transport cells (sieve elements) towards sugar-consuming sinks (e.g., ripening berries) (Thompson & Holbrook 2003). Heat increases transpiration and dehydrates the leaves, increasing the concentration gradient in the leaf cells for water uptake from the xylem (Sevanto 2018). Thus, under hot conditions, the phloem must be loaded with higher sugar concentrations to outcompete the leaf cells for xylem water, sending more concentrated sugar sap to the berries.

Phloem anatomy is expected to impact the rate of sugar transport by determining the hydraulic resistance to sap movement. Traits that increase the total conductive area for sugar transport (e.g., a higher density of sieve elements) or reduce the hydraulic resistance of individual sieve elements (e.g., wider sieve elements and more porous sieve plates, which are the perforated end wall structures vertically separating individual elements) are hypothesized to increase sugar transport rates (Jensen *et al.,* 2016; Stanfield & Bartlett 2022). However, despite the direct, mechanistic impact of phloem biology on sugar transport, phloem anatomy has never been tested as a driver of grape cultivar diversity in sugar accumulation rates. Here, we measured phloem anatomy for 16 winegrape cultivars growing in a common garden to test the hypotheses that: 1) phloem anatomy is significantly correlated with the maximum capacity for sugar transport (i.e., maximum sugar accumulation rates) and 2) heat-adapted cultivars (i.e., cultivars primarily grown in hot regions) exhibit phloem traits that reduce maximum sugar accumulation rates to avoid excessive sugar-to-acid ratios (i.e., smaller total cross-sectional phloem areas, narrower sieve elements, and less porous sieve plates).

2. Material and methods

Plant material – Phloem anatomy and sugar accumulation were measured for 16 winegrape cultivars (11 red and 5 white) growing in an experimental vineyard on the University of California, Davis campus (38.53°N, -121.75°W) (*N* = 3 – 4 vines/cultivar). 8 are classified as hot-climate cultivars (red: Syrah, Montepulciano, Mourvèdre, Tempranillo, Zinfandel, and Anglianico; white: Fiano and Verdelho), 7 are classified as warm-climate cultivars (red: Barbera, Cabernet Sauvignon, Merlot, Carignane, and Nebbiolo; white: Chardonnay and Sauvignon Blanc), and 1 is classified as temperate-climate (white: Riesling). Classifications follow Anderson & Nelgen (2020), and are based on growing season temperatures in the regions where each cultivar is most often planted. Davis is a hot growing region, with temperatures ranging from 12 – 41°C over the study period (Jun - Oct 2020). Vines were mature (> 5yo), trained to a bilateral spur-pruned VSP system, grafted onto the same rootstock (420A), and drip irrigated to replace approximately 60% of evapotranspiration.

Measurements – To measure anatomy, two leaves and berries per vine were sampled in early September and stored in formalin acetic acid (FAA) until sectioning. Half of the samples for each vine were measured with light microscopy and the other half with electron microscopy. For light microscopy, the berry pedicel and a 1 cm segment of the leaf petiole and lamina were paraffin-embedded, sectioned with a rotary microtome, stained with a 1% aniline blue and 1% safranin solution, and visualized and photographed with a brightfield microscope (Leica DMB4000B). Sections were manually measured for mean and total cross-sectional phloem area. Electron microscopy samples were processed with standard digestion and mounting steps, visualized with a field emission scanning electron microscope (ThermoFisher Quattro ESEM), and manually measured for mean sieve element area and sieve plate porosity (the ratio of sieve plate pore area to total sieve plate area). Berry sugar content was measured for each vine as total soluble solid (TSS) content every 2 – 3 weeks from Jun – Sep 2020.

Statistical analysis – ANOVAs were used to test for differences in mean and total phloem cross-sectional area, mean sieve element area, and sieve plate porosity between warm- and hot-climate cultivars. Maximum sugar accumulation rates for each cultivar were calculated as the maximum slope of the relationship between TSS and growing degree days (GDD). GDD were calculated from temperatures recorded by the Davis campus climate station. Linear regression was used to test for correlations between the phloem anatomy traits and maximum sugar accumulation rates.

3. Results and discussion

3.1. *Total phloem cross-sectional areas in the petioles and pedicels were positively correlated with maximum berry sugar accumulation rates*

Maximum sugar accumulation rates were significantly correlated with the total cross-sectional phloem area in the berry pedicels ($r^2 = 0.25$, $p = 0.046$, $N = 16$) and leaf petioles ($r^2 = 0.48$, $p = 0.004$, $N = 15$), but not the leaf midrib ($p > 0.05$, $N = 15$) (Fig. 1). Maximum sugar accumulation rates were faster in the cultivars with more phloem area. However, maximum sugar accumulation rates were not correlated with mean sieve element areas or sieve plate porosity (both $p > 0.05$, $N = 15$). These findings suggest that faster-ripening grape cultivars have increased the capacity for sugar transport by increasing sieve element density, rather than decreasing the hydraulic resistance of individual sieve elements to phloem transport. Consistent with these findings, previous work has found that a larger phloem area is associated with faster sugar transport across pumpkin cultivars, and that using hormone treatments or knock-out mutations to increase phloem area also increased sugar concentrations for grape and tomato, respectively (Savage *et al.,* 2015; Murcia *et al.,* 2016; Nam *et al.,* 2022). These findings suggest a potential application for petiole phloem area in screening plant material from breeding programs for sugar accumulation rates, since petioles could be sampled years before vines are mature enough for fruit production. However, sugar accumulation rates are also strongly determined by mechanisms endogenous to the berries, including phloem unloading, sugar metabolism, and the hormonal regulation of sink strength (Li *et al.,* 2021). More work is needed to understand how phloem anatomy is related to sink activity to determine whether altering phloem area is an effective strategy to change ripening rates and sugar/acid ratios at harvest.

3.2. *Cultivars associated with hotter growing regions exhibited phloem anatomy traits that would be expected to reduce sugar accumulation rates*

Maximum sugar accumulation rates and total phloem cross-sectional areas in the pedicels and petioles were significantly smaller in the hot-climate than warm-climate red cultivars (ANOVA, $p < 0.05$) (Fig. 2). Conversely, total phloem area in the midvein and mean sieve element area and sieve plate porosity for all three organs were not significantly different between climate groups ($p > 0.05$). These findings suggest that cultivars adapted for winemaking in hot regions avoid excessive sugar contents at harvest by reducing sieve element density, rather than altering the properties of individual conduits. This phloem anatomy could also be part of a water-saving stomatal strategy for hot-climate cultivars. Cultivars associated with hotter, drier growing regions exhibited lower maximum stomatal conductances, which could reduce transpiration and allow vines to consume soil water resources more slowly over the growing season (Bartlett & Sinclair 2021). Stomatal opening is closely regulated by leaf sugar concentrations, with high concentrations indicating that the sugar supply exceeds demand and signaling for the stomata to close (Koblet *et al.,* 1996; Kelly *et al.,* 2013). Thus, a phloem anatomy that reduces the rate of sugar export from the leaves could increase sugar-induced signaling for stomatal closure and promote water savings under hot conditions. Future work is needed to test whether manipulating sieve element formation and phloem area could both extend the ripening period to adapt grapevine phenology to hotter conditions and reduce vine gas exchange and irrigation demand.

4. Conclusions

Phloem anatomy significantly predicted cultivar diversity in berry sugar accumulation rates. Heat-adapted cultivars exhibited less cross-sectional phloem area and lower maximum sugar accumulation rates, suggesting that reducing sieve element formation in the petioles or pedicles through breeding or genetic engineering could slow sugar accumulation and extend the ripening period.

5. Acknowledgments

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6. Literature cited

Bartlett M., Sinclair G., 2021. Temperature and evaporative demand drive variation in stomatal and hydraulic traits across grape cultivars. J Exp. Bot. 72, 1995-2009.

Conde C., Silva P., Fontes N., Dias A., Tavares R., Sousa M., Agasse A., Delrot S., Geros H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food, 1-22.

Duchene E., Dumas V., Jaegli N., Meridinoglu D. 2012. Deciphering the ability of different grapevine genotypes to accumulate sugar in berries. Aust. J. Grape Wine Res. 18, 319-328.

Jensen K., Berg-Sorensen K., Bruus H., Holbrook N., Liesche J., Schulz A., Zwieniecki M., Bohr T., 2016. Sapflow and sugar transport in plants. Rev. Mod. Phys. 88.

Kelly G., Moshelion M., David-Schwartz R., Halperin O., Wallach R., Attia Z., Belausov E., Granot D., 2013. Hexokinase mediates stomatal closure. Plant J. 75, 977-988.

Koblet W., Carmo Candolfi-Vasconcelos M., Keller M., 1996. Effects of training system, canopy management practices, crop load, and rootstock on grape photosynthesis. Acta Hortic. 427, 133-140.

Nam Y., Gupta, A., Name H., Lee S., Cho H., Park, C., Park, S., Park, S., Hwang I., 2022. JULGI-mediated increment in phloem transport capacity relates to fruit yield in tomato. Plant Biotech J 20, 1533-1545.

Li Y., Forney C., Bondada B., Leng F., Xie Z., 2021. The molecular regulation of carbon sink strength in grapevine (*Vitis vinifera l.*) Front. Plant. Sci 11.

Murcia G., Pontin M., Reinoso H., Baraldi R., Bertazza G., Gomez-Talquena S., Bottini R., Piccoli P., 2016. ABA and GA3 increase carbon allocation in different organs of grapevine plants by inducing accumulation of non-structural carbohydrates in leaves, enhancement of phloem area and expression of sugar transporters. Phys. Plant. 156, 323-337.

Savage J., Haines D., Holbrook N., 2015. The making of giant pumpkins: how selective breeding changed the phloem of Cucurbita maxima from source to sink. Plant Cell Environ 38, 1453-1682.

Sevanto S., 2018. Drought impacts on phloem transport. Current Opin. Plant Bio. 43, 76-81.

Stanfield R., Bartlett M., 2022. Coordination between phloem loading and structure maintains carbon transport under drought. Front Plant Sci 13, 1-20.

Thompson M., Holbrook N., 2003. Scaling phloem transport: water potential equilibrium and osmoregulatory flow. Plant Cell Environ 26, 1561-1577.

van Leeuwen C., Destrac-Irvine A., 2017. Modified grape composition under climate change conditions requires adaptations in the vineyard. OENO One 51, 147-154.

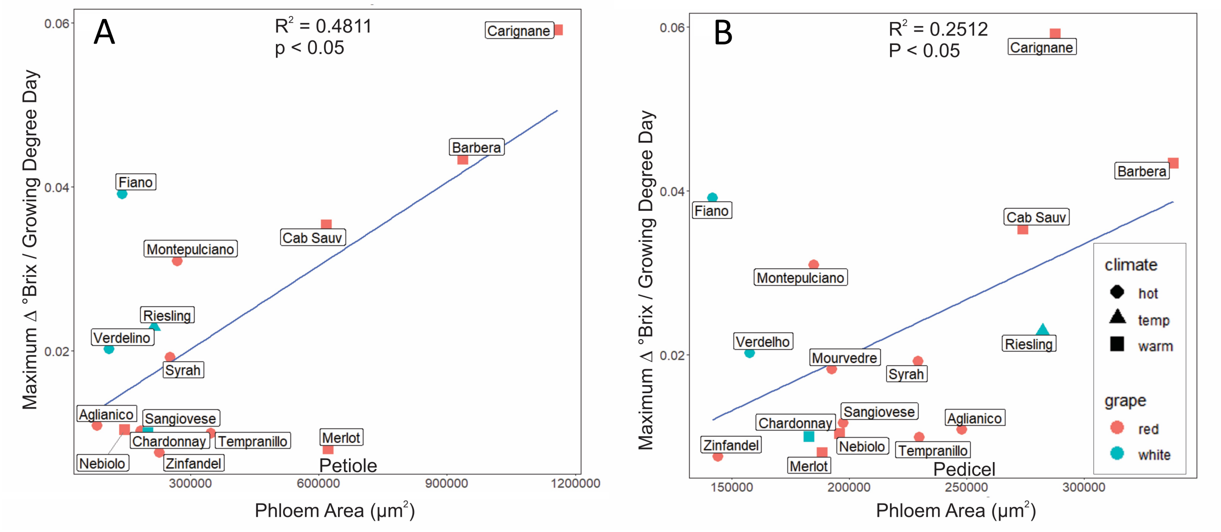

Figure 1: Relationships between the maximum rate of sugar accumulation in the berries (i.e., maximum slope between total soluble solids content and growing degree days) and total cross-sectional phloem area in the leaf petioles **(A)** and berry pedicels **(B)**. Each point is the average of 3 – 4 plants per cultivar. Point color indicates berry color (red or white) and shape indicates climate grouping (temperate-, warm-, or hot-climate).

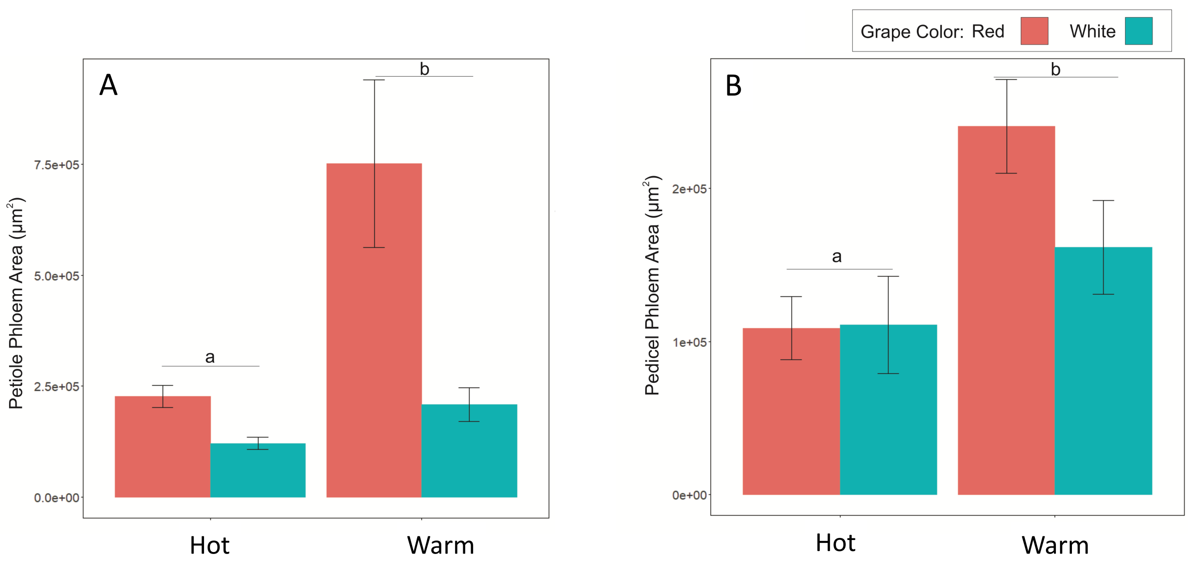

Figure 2: Differences in total cross-sectional phloem area in the petioles **(A)** and pedicels **(B)** between berry color and climate groups.