PRACTICAL Winery&Vineyard

Grapevine Berry Shrivelling, Water Loss and Cell Death

Increasing challenge for growers in the context of climate change

Alain Deloire, Suzy Rogiers, Katja Šuklje, Guillaume Antalick, Zeyu Xiao, Anne Pellegrino

Alan Deloire is a professor at Montpellier University, L'Institut Agro (SupAgro), France.

Suzy Rogiers is a senior researcher at NWGIC, Department of Primary Industries-NSW, Australia.

Katja Šuklje is an associate professor, Agricultural Institute of Slovenia, Slovenia.

Guillaume Antalick is an associate professor at University of Nova Gorica, Vipava, Slovenia.

Zeyu Xiao is a post-doctoral fellow at the ARC Training Centre for Innovative Wine Production, National Wine and Grape Industry Centre, Charles Sturt University, Australia.

Anne Pellegrino is an associate professor at Montpellier University, L'Institut Agro (SupAgro), France.

BERRY SHRIVEL IS A PHENOMENON of weight loss in grapevine berries that can occur during various developmental stages, either before veraison, as early as bloom (affecting the ovaries) or after veraison. Both red and white varieties (Cabernet Sauvignon, Zweigelt, Barbera, Grenache, Semillon, Sauvignon Blanc, Shiraz and other cultivars) were shown to be prone to berry shrivelling.[6] Four types of berry shrivelling are reported in the literature[7]:

- Sunburn either before or after veraison, resulting in poor color development in red varieties on severe occasions;
- Late season fruit dehydration, characterized by berry mesocarp cell death and water loss that lead to an increase in total soluble solids concentration;
- Bunch stem necrosis characterized by necrotic rachis tissue that affect bunch tips, shoulders or even the entire clusters. Bunch necrosis can occur any time, from bloom until after veraison, with different impacts on grape composition;[1,6]
- Sugar accumulation disorder, resulting in soft, irregular-shaped berries with low fresh weight, reduced anthocyanin biosynthesis and sugar accumulation.

The most common type of shrivelling in warmer climates is fruit dehydration. Although fruit dehydration is variable between seasons and sites, it seems to be accelerated by higher temperatures, high vapor pressure deficit (VPD), water constraints and/or stress and excessive bunch sun exposure. Fruit dehydration can be attributed to dehydration and loss of berry cell vitality that result in losses of yield, quality and profitability.[20] H.C. Chou et al. (2018) reported that fruit dehydration caused a 30 to 70 percent lower fresh berry weight in Shiraz.[6]

Key Points

- Berry shrivel is an important phenomenon that occurs when grape berries lose more water through transpiration and xylem back-flow to the plant than is imported through the phloem during late ripening.[9,17] Berry shrivel can have a significant economic impact, reducing yield by more than 25 percent with consequences on berry composition and the resulting wine quality. Its occurrence is expected to increase due to predicted climate change, shifting grape development and ripening into warmer periods. The severity can be accelerated by heat waves and drought events.
- Single berry analyses are important to understand berry development (heterogeneity, asynchronous development) and the mechanisms involved in berry secondary and primary metabolite biosynthesis. From a practical standpoint, in order to follow the course of ripening across a number of vines, one has to work with a population of berries and not a single berry. However, at any particular time point, each berry is at its own developmental stage with its own suite of compositional characteristics, and this individuality can be exploited to better understand the progression of ripening.

Most publications on berry development present results obtained on populations of berries, thus mixing various stages of berry development (FIGURE 1). Although time-consuming, measurements on single berries of parameters, such as total soluble solids, fresh mass (g), organic acids, histological and cell death/berry internal oxygen, are a pre-requisite to improve the understanding of berry metabolite accumulation and loss over the different phases of berry development. This article presents some original results related to fruit dehydration obtained by analyzing single berries from various clusters of Shiraz.

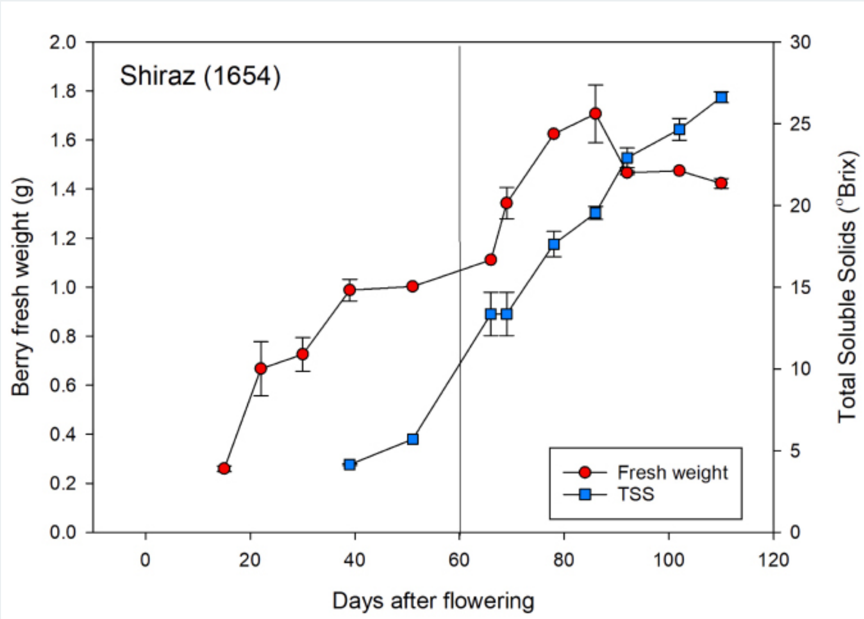

FIGURE 1. Changes in berry fresh weight and total soluble solids over time in Shiraz grown in a hot climate. Loss in fresh weight typically occurs at around 80-90 days after flowering. Due to the loss in berry water content, the existing sugars concentrate and further increase total soluble solids.

We have learned that:

1. There is no direct relationship between berry volume and its sugar content.
2. In a controlled environment, single berry sugar accumulation from the onset of veraison lasts 26 to 30 days, which will be the reference when comparing interaction between genotypes and environment by measuring the dynamic of berry sugar accumulation on a population of fruits to assess the tempo of ripening.[14]
3. It allows delineation of two "new" sub-stages, during ripening, from the onset of veraison onwards (berry softening), which are pre- and post-plateau for berry sugar accumulation.[16]
4. Harvest dates are determined according to the plateau of berry sugar accumulation.[2,16]

Single berries start loading sugar from the onset of veraison (berry softening) until the sugar per berry reaches a plateau about 26 days later.[2,14] The plateau of berry sugar loading generally occurs at around 18o to 20° Brix), irrespective of the cultivar. In parallel to sugar accumulation (but not necessarily in a linear relationship), the volume of the fruit increases through the loading of water.

To assess the real plateau of berry sugar accumulation (irrespective of the cultivar), the results have to be calculated on a per berry basis (mg of sugar per berry), not to be confused between berry sugar accumulation and berry sugar concentration (°Brix). Then the plateau is reached when berry sugar accumulation stops and the Brix value is associated with this plateau.[2,14,16]

From the plateau of berry sugar accumulation (second phase of berry ripening), the increase in total soluble solids is mainly due to berry water loss.[11,12] At this stage, the berry's water budget is no longer balanced between water loading (because xylem and phloem transport between the berries and the plant is less active than in previous developmental stages), and berry water loss by transpiration continues. The flow of water from berry to the vine (back-flow) may also occur; therefore the berry may be prone to shrivelling in some cultivars, such as Shiraz.[19]

The direction of the xylem hydrostatic pressure gradient between the fruit and the plant is critical to the occurrence of back-flow, and it has been reported in several species, including kiwifruit. Hydraulic conductance measurements for flow out of the berry was greater for Shiraz than Chardonnay, and it was calculated that this could account for about 7 percent of berry volume loss per day, equivalent to rates of berry weight loss.[17]

The occurrence and conditions leading to back-flow have not been fully described, but hydrostatic pressure gradients in the xylem are determined by genotype X environment interactions. An increase in apoplastic solutes may lead to an increase in apoplastic osmotic pressure, and this may encourage water to exit from the phloem so that the phloem sink pressure declines.[9] Water flow from the berry may offer an avenue for the discharge of excess phloem-derived water so that solute accumulation may continue, especially during conditions where berry transpiration is limited under a low vapor pressure deficit.

Therefore, berry sugar concentration is an equation between the total amount of sugar that has been deposited into the berry (mg/berry) and berry volume.

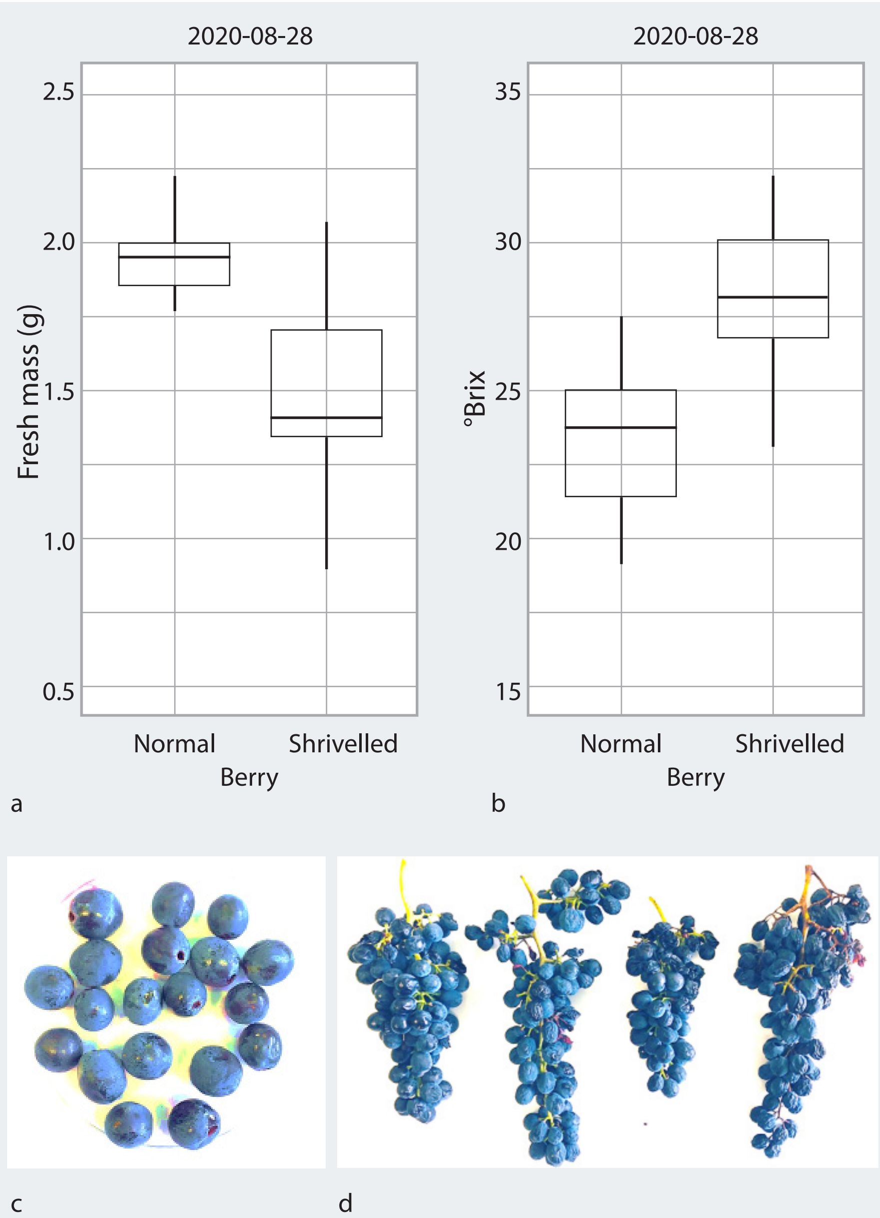

FIGURE 2: Boxplot representing the berry fresh mass (a) and °Brix value (sugar concentration) (b) of the same individual normal berries (c) and shrivelled berries (d). This illustrates the overall berry weight loss and °Brix increment for shrivelled berries compared with normal berries.

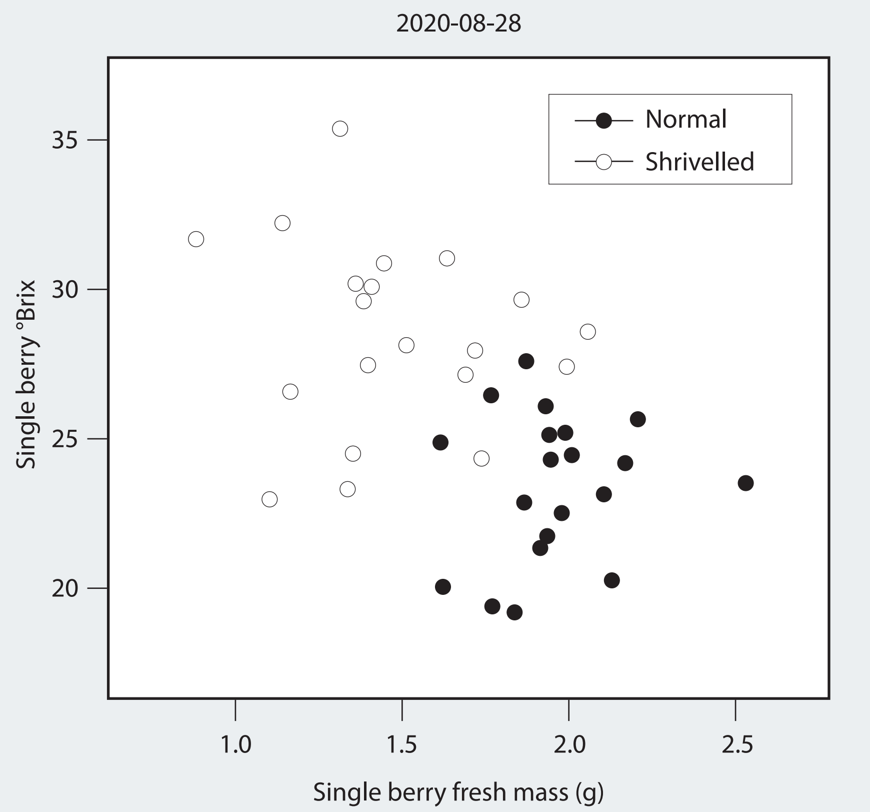

FIGURE 3: The relationship between single berry fresh mass and °Brix value (sugar concentration) of the same individual berry is significant, but not very tight, for normal berries and shrivelled berries. This is illustrating: i) the complex interaction between berry sugar content and berry volume; ii) for a specific sampling date, berries are not at the same developmental-ripening level for these parameters.

A recent study at the Montpellier l'Institut Agro vineyard on irrigated, vertically shoot-positioned Shiraz vines compared normal to shrivelled berries, all sampled from the same clusters on the same date. It nicely illustrated the overall shrivelled berry fresh mass loss, while the °Brix increases, when compared to normal berries (FIGURE 2). The results reveal a significant but weak relationship between individual berry fresh mass and its °Brix, both for normal and shrivelled berries (FIGURE 3), which confirm recent published results.[17]

Berry sugar concentration is an equation between the volume of the fruit and its sugar content. This means that determining ripening only using °Brix (sugar concentration) is meaningless if you wish to understand the origin of a specific sugar concentration (volume or berry sugar content).[13]

Studies have shown that high temperature and deficit irrigation can exacerbate the extent of late ripening berry cell death and dehydration, which can be ameliorated with shading.[2,4,5]

It was demonstrated that hypoxia (low concentration of oxygen [O_2]) in the berry mesocarp may be related to the onset of cell death.[20] Cell death in the berry mesocarp can be visually assessed by using a fluorescent stain (FIGURE 4). It usually begins in the middle of the mesocarp and spreads outwards towards the skin.

A decline of O_2 was observed from the berry skin towards the center of the mesocarp. Parts of the mesocarp showed near zero O_2 during late ripening. Interestingly, the pattern of the O_2 gradient was closely related to that of the berry mesocarp cell death. Lenticels on the berry pedicels can act as gas exchange pathways for the berries. By blocking lenticels on the pedicels with silicone gel (i.e., blocking the main gas exchange pathway of the berries), internal O_2 decreased within a few days, and more cell death was observed in post-veraison Chardonnay berries.[21]

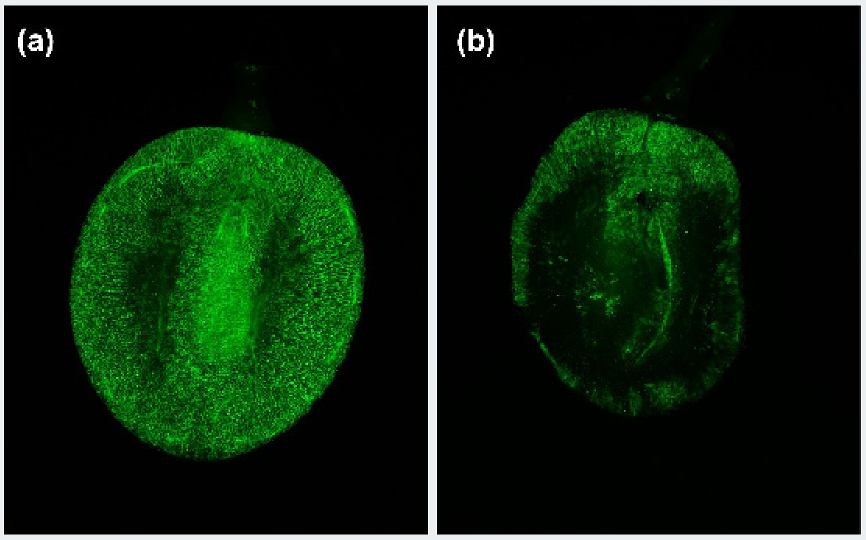

FIGURE 4: Fluorescent images of the berry cut surface (longitudinal cut through berry central axis) stained with fluorescent diacetate (FDA). Intact vital cells show fluorescent green. (a) A turgid berry that shows healthy/intact cells within the mesocarp; (b) A shrivelled berry showing large dark areas that indicate cell death. Scale bars are 2 mm.

Characterizing lenticel morphology could be useful in future research to identify cultivars that are suitable for hot and dry conditions (i.e., to achieve better control of cell death and berry shrivel). In a subsequent study, water stress applied in the field decreased the O_2 level in Shiraz grapes and increased berry cell death. The decrease in oxygen concentrations inside Shiraz grapes was also found to be related to a decrease in tissue porosity (i.e., fewer miniature air pockets inside the grapes).[21]

Berry shrivelling has a profound effect on Shiraz grape and wine composition; however, modulations are shrivelling type dependent.[6] Wines made from grapes where 80 percent of the berries were affected by fruit dehydration resulted in significantly higher alcohol levels (more than 1 percent volume), increased chemical age and wine hue, and decreased color density. Chemical age measures the proportion of SO_2-stable wine pigment in the total red pigment at low pH.

Adding SO_2 to must or in very young red wine leads to a temporary loss of color due to the sulfonation of anthocyanin monomers. During wine aging, co-pigmentation reactions will occur between anthocyanins and polyphenolic compounds to form directly and indirectly linked anthocyanin-derived pigments. Those reactions prevent sulfonation and therefore help to stabilize red wine color. Consequently, the proportion of SO_2—stable pigment increases with wine aging, and it is used as an indicator correlated with wine age.

Some of the co-pigmentation reactions are favored with oxygenation. For a fixed age, variety, geographical area and winemaking techniques in our study, chemical age value is directly linked to the antioxidant capacity of the wine. Lower antioxidant capacity favors oxygen-related co-pigmentation reactions and a higher chemical age value. Those wines will also be subject to a faster shift of wine color from purple to orange as shown by the shift in wine hue.

Thus, fruit dehydration negatively affects wine antioxidant activities, accentuates color development in red wines and, in turn, shortens the aging potential of a wine. In addition, anthocyanins were lower in wines made from shrivelled grapes.[3,7]

The sensitivity of wines made from shrivelled berries to oxidation was confirmed by a higher concentration of compounds, such as gamma-nonalactone and massoia lactone involved in the expression of cooked fruit and prune aromas found in prematurely oxidized red wines (Premox). Some other markers of berry dehydration, which were not analyzed in the present studies, are also markers of Premox wine, such as methyl-2,4-nonanedione and even sotolon to a lesser extent.[1]

Fruit dehydration also decreased concentrations of some higher alcohol acetates and beta-damascenone (enhancer of fruity aroma) and favored the expression of dry fruit notes in grapes and wines through higher concentrations of compounds, such as gamma-nonalactone, massoia lactone, furaneol, homofuraneol, 3-methyl-2,4-nonanedione and (Z)-1,5-octandien-3-one.[1]

Fruit dehydration significantly affected wine sensory characteristics. Wines made from grapes affected by fruit dehydration resulted in an increased perception of stewed fruit and were more alcoholic and astringent.[1]

It should be noted that late maturity is not always accompanied with berry shrivelling and high concentrations of chemical markers related to dried fruit aromas. Some of these compounds are also chemical markers of Premox wines, confirming that berry dehydration tends to accelerate wine color and aromatic evolution, limits wine potential aging and is detrimental to varietal and terroir typicality.

Conclusion and Perspectives

The occurrence of cell death in the mesocarp of grape berries appears to be associated with low oxygen levels in the berry. The projected increase in temperatures with climate change may worsen hypoxia by increasing respiration of both the mesocarp cells and the seeds.

The additional impacts of heat stress and drought on other metabolic processes in the berry will likely not only reduce yield through berry weight loss but also alter berry composition and wine sensory profiles.

What do growers have in the toolbox to limit or avoid post-veraison berry water loss and ultimately shrivelling?

- Protect the fruit zone with leaves to limit berry transpiration and improve the bunch microclimate in terms of vapor pressure deficit. Overly vigorous vines with large canopies may experience more water stress under severe heat if root uptake is insufficient to meet vine water demand, however.
- Apply a mild water constraint from berry-set to veraison to force the vine and its bunches to adapt to a water constraint through the season (smaller berries are likely to undergo less total weight loss because there is less cell expansion prior to the onset of weight loss.)[10,11] That said, larger berries at the maximum weight will still likely be larger if harvested soon after the onset of shrivel.
- Protect the vine with irrigation before a heat wave to limit berry water back-flow, keeping in mind that post-sugar accumulation, irrigation might not curtail berry water loss. (However, it has been shown that rain, during the shrivelling phase, may decelerate the rate of weight loss by direct water absorption through the skins.)
- This is a practice that is largely applied in vineyards in warm and hot wine regions, such as New South Wales (Griffith) in Australia. Preparing the vine for high transpiration levels makes sense and could avoid berry water back-flow. More research is needed to understand this phenomenon. From a vineyard perspective, it is working in various situations. All the practical knowledge has not been published.
- Protect vines by shading using adapted nets or sunscreen application (such as kaolin), trellis crossarms and proper vine row orientation. WBM

This text was edited from the original February 2021 publication by the *International Viticulture & Enology Society Technical Reviews* with permission of the publisher.

References

1. Allamy, L., Darriet, P. and A. Pons, 2018 Molecular interpretation of dried-fruit aromas in Merlot and Cabernet-Sauvignon musts and young wines: Impact of over-ripening. *Food Chemistry*, 266, 245-253. *https://doi.org/10.1016/j.foodchem.2018.06.022*.
2. Antalick, G., Šuklje, K., Blackman, J.W. Schmidtke, L.M. and A. Deloire., 2021 Performing sequential harvests based on berry sugar accumulation (mg/berry) to obtain specific wine sensory profiles, *OENO One* 2021, 2, 131-146, DOI:10.20870/oeno-one.2021.55.2.4527.
3. Bonada, M., Sadras, V., Moran, M. and S. Fuentes, 2013 Elevated temperature and water stress accelerate mesocarp cell death and shrivelling, and decouple sensory traits in Shiraz berries. *Irrigation Science* 31, 1317-1331.
4. Bonada, M., Sadras, V.O. and S. Fuentes, 2013 Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. *Aust. J. Grape & Wine Research* 19, 87-94.
5. Caravia, L., Collins, C., Petrie, P.R. and S.D. Tyerman, 2016 Application of shade treatments during Shiraz berry ripening to reduce the impact of high temperature. *Aust. J. Grape & Wine Research* 22, 422–437.
6. Chou, H.C., Šuklje, K., Antalick, G., Schmidtke, L.M. and J.W. Blackman. 2018 Late-season Shiraz berry dehydration that alters composition and sensory traits of wine. J. of Ag. & *Food Chemistry*. 66(29), 7750-7757. doi: 10.1021/acs.jafc.8b01646.
7. Fang, Y., Meng, J., Zhang, A., Liu, J., Xu, T., Yu, W., Chen, S., Li, H., Zhang, Z. and H. Wang, 2011 Influence of shrivelling on berry composition and antioxidant activity of Cabernet Sauvignon grapes from Shanxi vineyards. *J. Sci. Food Agric*. 91 (4), 749-757.
8. Krasnow, M.N., Matthews, M.A., Smith, R.J., Benz, J., Weber, E. and K.A. Shackel, 2010 Distinctive symptoms differentiate four common types of berry shrivel disorder in grape. *California Agriculture*, 64(3), 155-159. doi: 10.3733/ca.v064n03p155.
9. Patrick, J.W. 1997 Phloem Unloading: sieve element unloading and post-sieve element transport. *Annual Review of Plant Physiology & Plant Molecular Biology* 48, 191-222.
10. Rogiers, S.Y., J.M. Hatfield and M. Keller, 2004a. Irrigation, nitrogen, and rootstock effects on volume loss of berries from potted *Vitis vinifera* L. cv. Shiraz vines. *Vitis* 43:1-6.
11. Rogiers, S.Y., J.M. Hatfield, V.G. Jaudzems, R.G. White and M. Keller, 2004b. Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and preharvest weight loss. *Am. J. Enol. & Viti*. 55:121-127.
12. Rogiers, S.Y., Greer, D.H., Hatfield, J.M., Orchard, B.A. and M. Keller, 2006 Solute transport into Shiraz berries during development and late-ripening shrinkage. *Am. J. of Enol. & Vit*. 57, 73-80.
13. Schimidtke, L.M., G. Antalick, K. Šuklje, J.W. Blackman, J. Boccard and A. Deloire. 2020 Cultivar, site or harvest date: the gordian knot of wine terroir. *Metabolomics* (2020) 16:52, *https://doi.org/10.1007/s11306-020-01673-3*
14. Shahood, R., Torregrosa, L., Savoi, S. and C. Romieu, 2020 First quantitative assessment of growth, sugar accumulation and malate breakdown in a single ripening berry, *OENO One*, 4, 1077-1092, DOI:10.20870/oeno-one.2020.54.4.3787.
15. Šuklje, K., Zhang, X., Antalick, G., Clark, A.C., Deloire, A. and L.M. Schmidtke, 2016 Berry Shrivelling Significantly Alters Shiraz (*Vitis vinifera* L.) Grape and Wine Chemical Composition, *J. Agric. Food Chem*., 64, 870-880, DOI:10.1021/acs.jafc.5b05158.
16. Šuklje, K., G. Antalick, C. Meeks, J.W. Blackman, A. Deloire and L.M. Schmidtke, 2017 Grapes to wine: the nexus between berry ripening, composition and wine style. *https://doi.org/10.17660/ActaHortic.2017.1188*.
17. Tilbrook, J. and S.D. Tyerman. 2009 Hydraulic connection of grape berries to the vine: varietal differences in water conductance into and out of berries, and potential for back-flow. *Functional Plant Biology*, 36(6), 541-550.
18. Triolo, R., Roby, J.P., Plaia, A., Hilbert, G., Buscemi, S., Di Lorenzo, R. and C. van Leeuwen, 2018 Hierarchy of factors impacting grape berry mass: separation of direct and indirect effects on major berry metabolites. *Am. J. of Enol. & Vit.*, 69 (2), 103-112.
19. Tyerman, S.D., J. Tilbrook, C. Pardo, L. Kotula, W. Sullivan and E. Steudle, 2004 Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. *Aust. J. Grape Wine Res*. 10:170-181.
20. Xiao, Z., Liao, S., Rogiers, S.Y., Sadras, V.O. and S.D. Tyerman, 2018a. Effect of water stress and elevated temperature on hypoxia and cell death in the mesocarp of Shiraz berries, *Aust. J. of Grape & Wine Research* 24, 487-497, *https://doi.org/10.1111/ajgw.12363*.
21. Xiao, Z., Rogiers, S.Y., Sadras, V.O. and S.D. Tyerman, 2018b. Hypoxia in grape berries: the role of seed respiration and lenticels on the berry pedicel and the possible link to cell death. *J. Exp Bot* 69, 2071-2083.