Is the Shiraz berry the biggest loser?

The double-edged sword of cell death in winegrapes

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CELL DEATH IN the berry occurs in most winegrape varieties late in development. It correlates with the onset of berry shrinkage in Shiraz. There is also a correlation across varieties between the extent of cell death and berry shrinkage. However, this does not mean that cell death causes shrinkage, since flow of water into and out of berries is also involved and this differs markedly between varieties. Cell death is also linked to flavour development. Thus, cell death may be a double-edged sword: on the one hand, it is potentially contributing to berry water loss, which concentrates sugar and may lead to high alcohol levels in wine; on the other hand, it is contributing to flavour development. The challenge will be to uncouple cell death from berry shrinkage where they are correlated and develop methods to rapidly monitor cell death in the vineyard.

A few years ago a significant and hitherto unrecognised stage in winegrape development was discovered by Dr Joanne Tilbrook during her PhD studies within the CRC Viticulture at the University of Adelaide (Tilbrook and Tyerman 2008). The phenomena is characterised by reduced vitality of the large mesocarp cells within the berry. This 'cell death' starts 90-100 days after flowering, coinciding with maximum berry weight, additional softening and the beginning of weight reduction in Shiraz berries. This event is characterised by vital stains that indicate a loss of membrane integrity, and is therefore defined as the beginning of mesocarp cell death. In the accompanying figure the dark areas indicate non-living regions in a berry cross section. The figure compares Chardonnay, Shiraz and Sultana where cell death is evident in Chardonnay and Shiraz but not in Sultana.

The onset of cell death has been confirmed by two other independent reports, one published in the same year by Ken Shackel's group at UC Davis, US, where they emphasised that loss of vitality began well after veraison and therefore did not correspond with sugar accumulation (Krasnow *et al.*, 2008). In the second independent report, Steve Tyerman's group at the University of Adelaide investigated the phenomena in relation to berry shrinkage, common to Shiraz in Australia, since it was reasoned that if membrane breakdown occurred then this would prevent the osmotic pull of the high sugar concentration in the berry counteracting the water tensions generated in the vine, particularly under water stress. Thus, cell death would allow the vine to pull water out of the berry. Dr Suzy Rogiers' group at the NWGC examined the phenomena in relation to berry splitting (Clarke *et al.*, 2010). Here the osmotic consequences of membrane breakdown were also considered but in the 'opposite' context to shrinkage, rather in relation to the occurrence of berry splitting. Since the osmotic pull of high sugar in the cells would not be able to draw water into the berry, then splitting would be slower or not occur at all. They found, as expected, that splitting did not occur after the onset of cell death.

In Adelaide, we compared varieties that were less prone to berry shrinkage, such as Chardonnay and Sultana, and found surprising differences in both the onset of cell death and hydraulic features of the berries (Tilbrook and Tyerman 2009). Sultana showed no sign of cell death and had very little weight loss, while both Chardonnay and Shiraz showed substantial cell death (see Figure 1). So, if cell death is related to shrinkage, why is it that Chardonnay is not prone to berry shrinkage? The answer lies in differences in the plumbing of the berries.

Using special micro-flow systems, Jo Tilbrook and Johannes Scharwies have been able to measure the flow rates into and out of detached berries during development. When cell death starts in Chardonnay, this variety essentially shuts off flow through the xylem conduits from the berry, thus reducing flow from the berry back to the vine. In contrast, Shiraz seems not to be able to shut off the connection back to the vine (Tilbrook and Tyerman 2009). Further studies by Johannes Scharwies for his Masters Degree at the University of Adelaide have demonstrated this in very high detail and compared other varieties such as Grenache. He has also shown that transpiration (water evaporation from the berry) does not differ between varieties. Our hypothesis is that Shiraz tends to lose weight because of a mismatch in timing of cell death, its location in the berry, and control of the internal berry hydraulics. It remains to be discovered how the sugar import system (the phloem) is linked with these processes, since when sugar is translocated to the berry, there is a significant flux of water that accompanies it.

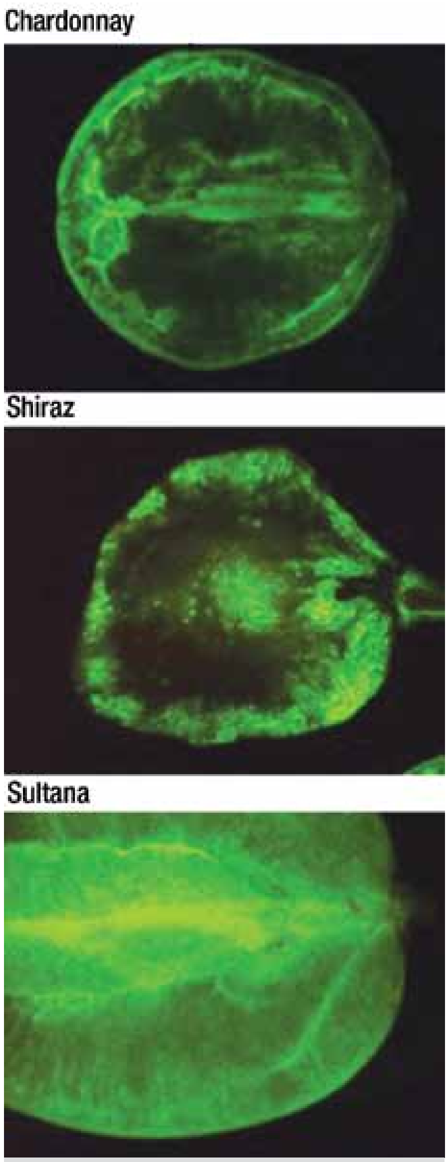

Figure 1. Comparison between varieties of cell vitality in berries late in ripening. Vital stained images of longitudinal sections of berries from Chardonnay (141 DAF), Shiraz (127 DAF) and Sultana (132 DAF). DAF = days after flowering. Each sample was taken from the field on the same day and stained with an isosmotic solution (= juice °Brix) of fluorescein diacetate (FDA). The images were recorded with a fluorescence dissecting microscope all at the same magnification. The intense green signal indicates vital cells. Further details in Tilbrook and Tyerman (2008). Images courtesy of Dr Joanne Tilbrook.

An examination of many varieties by Dr Sigfredo Fuentes and the team at the University of Adelaide showed there was a significant correlation between the degree of cell death and berry shrinkage (Fuentes *et al.*, 2010). Three groups of varieties could be separated, with most of the winegrapes showing some degree of cell death and berry shrinkage late in ripening, while table grapes and some varieties used for both table grape and wine production showed very little cell death and shrinkage. Speculating on this point, it seems likely that when humankind first began to select grapes for winemaking, they preferred those varieties that yielded juice more easily. Cell death and membrane breakdown basically creates a bag, the berry skin, containing the juice. On the other hand, those varieties that remained crisp and turgid with living cells have been selected for table grapes. These are much harder to extract juice from.

In terms of the impact on berry flavour development, it is likely that the breakdown of cell membranes will allow various compounds and enzymes, which normally would be separated, to mix, producing new secondary metabolites. We hypothesised that cell death in the berry may explain the phenomena of 'engustment' originally coined by Bryan Coombe and Mike McCarthy (Coombe and McCarthy 1997) to indicate the sudden change in flavour profile that can be detected in some varieties near harvest.

In this context, it is interesting that certain enzymes that are involved in breakdown of lipid (components of membranes), the lipoxygenases (LOX; linoleate oxygen oxidoreductases) are present in the berry at the latter stages of ripening. The LOX enzymes incorporate molecular oxygen into polyunsaturated fatty acids. This reaction produces fatty acid hydroperoxides that are subsequently converted into a group of biologically active compounds called oxylipins. Several of these secondary metabolites include C6 and C9 aldehydes and alcohols that contribute to characteristic flavours of fruits, vegetables and green leaves. The C6 and C9 compounds can be converted to esters, which normally have a fruity scent. The involvement of the LOX pathway in the biosynthesis of fruit aroma compounds has been reported in other fruits such as kiwi fruit, tomato, olive and strawberry (Zhang *et al.*, 2009). Our colleagues in New Zealand (Dr Chris Winefield's group at Lincoln University) have described the LOX enzymes of Sauvignon Blanc berries (Podolyan *et al.*, 2010), while Charlotte Jordans at the University of Adelaide has recently identified a potential candidate gene that may be associated with membrane breakdown in Shiraz. A paper from this research will soon be published detailing the association between cell death and berry sensory properties (e.g. Lohitnavy *et al.*, 2010) by comparing contrasting industry categories of fruit quality. The role of cell death in flavour development in the berry and subsequent effects on wine quality warrants further study.

Finally, it has not escaped the attention of the Adelaide group that cell death in winegrapes may be exacerbated by high temperatures and climate change scenarios. Heatwaves are predicted to become more frequent with global warming as a consequence of continued increase in our emissions of carbon dioxide into the atmosphere. Earlier ripening also means grapes are subjected to warmer temperatures at harvest (double warming impact) (Webb *et al.* 2007). In collaboration with Dr Victor Sadras (SARDI), using his vine heating systems, Marcos Bonada has undertaken a Masters supervised by Dr Sigfredo Fuentes to examine how high temperatures may trigger cell death in Chardonnay and Shiraz. His studies have revealed some very interesting differences between varieties which will soon be published. Berry weight loss will concentrate sugars and lead to higher alcohol wines if they are fermented to dryness. So cell death, if triggered by high temperatures, would add another dimension to impacts of climate change that may lead to higher sugar and higher alcohol, which has health and taxation implications for consumers and producers. Watch this space for further updates.

We hypothesise that cell death late in winegrape ripening is significant for berry water relations and flavour development, and is likely to be exacerbated by higher temperatures. But how can winegrape growers use this information in decision-making for harvest dates and berry quality? Unfortunately the techniques to show cell death are time-consuming, and require specialist microscopes and imaging facilities. To bring the discovery in reach of the grower, we are currently working with new postgraduate student projects within the Vineyard of The Future project at the University of Adelaide to find fast and convenient field-based techniques that can be used to measure berry cell death. One method is to use Near Infrared Reflectance Spectroscopy and Hyperspectral techniques. Dr Roberta De Bei (University of Adelaide researcher funded by the Grape and Wine Research and Development Corporation) has been investigating these techniques recently (De Bei *et al.*, 2010) and is currently working on this in collaboration with the group of Dr Javier Tardaquila in University of Logrono, Spain. There are also electrical techniques that can be used to investigate cell membrane breakdown and these have been used in other fruits. These will be trialled by postgraduate student Luciano Ricardo Caravia Bayer, a Chilean government -sponsored PhD student at the University of Adelaide in the coming season.

To conclude, although Shiraz might be the 'biggest loser' in terms of weight loss, the potential link to flavour development indicates that cell death in grape berries is a double-edged sword. On one hand, there is a link with weight loss and therefore yield reduction and higher sugar concentration, which means higher alcohol. On the other hand, cell death is also likely to be linked to positive flavour components and even to varietal flavours. Although we have found a correlation between cell death and berry shrinkage across varieties, this does not mean that cell death is causative of berry shrinkage. In fact we know that cell death can occur in Chardonnay, but it does not show substantial shrinkage. It is possible that cell death and berry shrinkage could be uncoupled using either irrigation techniques (such as PRD), canopy management techniques and/or techniques to control evaporative demand in the canopy's microclimate. We need to continue research on this topic both to discover what triggers the onset of cell death and to uncover rapid methods of measuring it in the field for decisionmaking in optimising harvest timing.

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