TO WHAT EXTENT DOES VINE BALANCE ACTUALLY DRIVE FRUIT COMPOSITION?

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Abstract:
Context and purpose of the study - Vine balance is a concept describing the relationship between carbon assimilation (usually estimated using a measure of vine vigour, e.g. pruning weight) and its utilisation for fruit production (usually estimated using harvest yield). Manipulating vine balance through leaf area or crop load adjustments affects the proportion of the vine's total carbohydrate production required to mature the fruit. It is commonly considered that composition of the berry, and resulting wine, is strongly affected by vine balance.
Material and methods – Field manipulations of vine balance were replicated in three contrasting viticultural regions of Australia, Hilltops, Murray Valley and Langhorne Creek, over three seasons. The manipulations were early defoliation (pre-capfall), late defoliation (pre-véraison) and 50% crop removal (pre-véraison). Fruit were sampled prior to a treatment being applied and then at approximately two-week intervals until harvest, where small lot wines were made from each field replicate. The fruit samples were analysed for maturity, basic composition and the expression of key genes that regulate anthocyanin and tannin formation. In addition, the effect of defoliation was simulated, without changing bunch environment, by enclosing whole vines in chambers and supplying them with low CO_2 air to reduce photosynthesis.
Results – Changing vine balance consistently altered the rate of ripening, but did not correlate with treatment effects on fruit composition, where they occurred. Late defoliation extended the maturation period, but reduced total anthocyanin content. Crop removal shortened the maturation period, but had little effect on the fruit. Interestingly, early defoliation had no clear effect on vine balance, but resulted in both increased anthocyanin and increased tannin content. The chamber experiment also extended the maturation period, but had no effect on the relationship between sugar and anthocyanins. Overall, there was no conclusive evidence that the changes in vine balance achieved had any significant effect on fruit or wine composition when fruit were harvested at the same sugar ripenesss.
Keywords: maturation rate, vine balance, *Vitis vinifera*, wine composition.

1. Introduction

Theimportance of balancing the vegetative growth with the yield of vines to produce fruit of optimal quality is widely acknowledged and significant resources are often utilized to achieve such a balance. However, the concept of *vine balance* is an empirical one and defies a clear definition. What most definitions do have in common, is an acknowledgement of the role of the canopy in providing carbon for the ripening of fruit and the volume of fruit in generating a demand for that carbon. Vine balance is, therefore, a source-sink relationship.
For a given climate or environment, carbon fixation by the vine depends on the photosynthetic capacity of- and light absorption by- the canopy. Consequently, indices of vine balance typically include canopy size, or a surrogate of it, as well as fruit mass. As both canopy size and yield can be measured directly, a definition based on these factors can be measured objectively. For the purposes of this study we are defining vine balance as the ratio between crop load (kg $vine^{-1}$) and canopy area (m^2 $vine^{-1}$); this also has the advantage of being a fairly widely used viticultural index, if not as common as the Ravaz index.
Decades of research have demonstrated that the rate of sugar accumulation in the berry and the development of the secondary metabolites, such as anthocyanins, flavonols and condensed tannins, are highly correlated (e.g. Pirie and Mullins, 1977; Larronde et al 1998). Clearly, manipulating vine balance,

thereby altering the rate of carbohydrate supply to the fruit, has the potential to alter berry composition. Answering the question as to whether the accumulation of sugar and these other metabolites can be separated is not so straightforward.
Further, the potential influence of fruit exposure and canopy microclimate on berry composition is also widely recognized. The concentration and type of phenolic compounds in berry skins, for example, can be influenced by both temperature and light, with temperatures above 35°C detrimental to anthocyanin production and light affecting both the type of anthocyanins and accumulation of flavonols (Spayd et al., 2002, Downey et al., 2004). Superimposed on any effect of carbohydrate supply due to an in-field manipulation of vine balance is, therefore, an effect of the fruit microclimate, which may vary according to specific management practices or differences in climate between localities.
This study combined replicated field manipulations of vine balance across three different viticultural regions with whole vine chambers that reduced vine carbon uptake through scrubbing of CO_2 from the air, to manipulate vine balance independently of fruit environment. This approach allowed direct (carbon availability to the crop) and indirect (e.g. changes in fruit light environment) effects of vine balance management to be separated and the relative importance of those effects investigated.

2. Material and methods

Field trials

Three trial sites were established in commercial Shiraz vineyards prior the start of the 2013/14 growing season: Cleggetts, Langhorne Creek, SA; Deakin Estate, Sunraysia, VIC and Barwang, Hilltops, NSW. In SA, vines were on their own roots, planted at 2 m by 3.2 m spacing, trained to a bilateral cordon and spur-pruned. In VIC, vines were on Schwarzman rootstock, planted at 2.44 m by 3 m spacing, trained to a double, bilateral cordon and mechanically-hedge pruned with hand clean-up. In NSW, vines were on own roots, planted at 2.1 m x 3.3 m spacing, trained to a bilateral cordon and spur pruned.
Climate at each site was monitored using matched weather stations placed adjacent to the trial blocks (Measurement Engineering Australia, Magill, SA, Australia).
Treatments - Four management regimes were used to manipulate vine balance (ratio of yield to canopy size) and applied as randomized complete blocks, with 45 vines per replicate at SA and NSW and 60 vines per replicate at VIC (including treated buffer vines):

- T1: control – all vines in T1 replicates received the standard management practice for that site.
- T2: early defoliation – all fully expanded leaves (approximately the first eight leaves) were removed from each vine, pre-anthesis, at E-L stage 19 (Coombe 1995).
- T3: crop thinning – all bunches were counted on at least eight vines per site, averaged and half this number of bunches removed from each vine, pre-veraison, at E-L stage 32.
- T4: late defoliation – all vines were mechanically hedged, pre-veraison, at E-L stage 32. The hedging cut the foliage at the edge of the fruiting zone, with approximately half the canopy removed.

The treatments were first applied during the 2013/14 growing season and re-applied to the same vines in the 2014/15 and 2015/16 growing seasons.

Field sampling and measurements - Vine balance was assessed as the ratio between peak canopy size and harvest yield. Canopy size was estimated from leaf area index, measured using a LiCor Li2200 or Li2000 Plant Canopy Analyser (LiCor Nebraska, US) on at least two occasions from late December to early February in each season. Harvest yield was measured directly by weighing the hand harvested fruit from multiple vines per replicate (number of vines varied by site and season).
Samples for fruit/juice composition were taken fortnightly from veraison to harvest in all seasons. At each site, four entire bunches per replicate were collected and the berries removed from the rachis before being split into four randomised 50 berry sub-samples. Samples were stored on ice and used within 24 hours (juice assessment), or immediately frozen in liquid nitrogen and stored at -40°C until use (fruit composition).
Harvest date was defined as the point where juice total soluble solids (TSS), measured using a refractometer, reached 24°Brix and was assessed individually for each treatment at each site. Fruit taken for harvest yield estimates was sent by refrigerated transport for standardised wine making at the NWGIC in Wagga Wagga. The wines were made in stainless steel variable capacity fermenters with an

initial SO_2addition and standard adjustments of acidity and yeast assimilable nitrogen. One wine was produced per field replicate from each site in each season.

Chamber experiment design

An experimental system was developed at the NWGIC in Wagga Wagga to investigate the impact of carbon supply on berry ripening and composition. This utilized mature potted grapevines, which were cordon trained to a comparable canopy size to field grown vines. The system consisted of six transparent whole vine chambers, which enclosed the canopy, with three chambers fitted with sodalime based scrubbers to reduce the CO_2 concentration of the supply air to approximately 200 ppm. The remaining three chambers were supplied with air at ambient CO_2 concentrations (400 ppm), generating a two-fold difference in canopy photosynthesis between treatments. The aim of this system was to vary carbon supply to the fruit (i.e. vary vine balance) independently of light and temperature effects and to be able impose and reverse these changes to vine balance at different times during berry development. For the results presented here, the system was utilised during the 2014-15 season to reduce CO_2 availability for 27 days from veraison, with a scrubbing period from January 4th to January 30th.

During the course of the CO_2 scrubbing, berries were sampled on eight occasions to monitor fruit composition response to differing carbon supply. Two further sets of samples were taken following the return of the 200 ppm treatment to ambient, and a final set at harvest. At each sample date, 50 berries were collected and weighed, and then separated into skin, juice and seed fractions. These were frozen in liquid N and stored at -80 °C for subsequent analysis. At 24 °Brix, the fruit was harvested and yield parameters recorded.

3. Results and discussion

The climate during the experimental work followed long-term trends, with mean January temperature (MJT) highest at the VIC site and lowest at the SA site; with 2014 having the hottest MJT and 2015 the lowest(Table 1). Site differences were similar season to season, irrespective of the absolute MJT. However, MJT was not necessarily indicative of other climate factors, such as the diurnal temperature range or seasonal rainfall and these also impacted reference evapotranspiration (ET_o) (data not shown).

The three sites were chosen to represent a range of climates and a range of vineyard management strategies and it was anticipated that this would result in different canopy sizes and yields between the sites. Averaged across the three seasons of measurements this was indeed the case in the control plots (Table 2), with a near two-fold range in canopy size (8-14 m^2) and a near four-fold range in yield (5.3-19 kg $vine^{-1}$). As a result, the calculated vine balance (yield per unit canopy area) at the VIC site was double that of the NSW site, which was 10% higher than that of the SA site.

3.1.*Treatment effects on vine balance and its components*

The T2 treatment was based on the work of Poni et al. (2013), who found that early-defoliation resulted in a 30-50% decrease in yield with little or no effect on canopy size, thereby reducing the yield:canopy ratio. However, this work was carried on vertical shoot positioned vines (VSP) with in-season canopy adjustment, whereas our work was carried out on spur pruned vines that were allowed to 'sprawl'. To the best of our knowledge this is the first time early-defoliation has been applied to vines managed in this way. The results of our work produced only a 8-13% reduction in yield, but also caused a 10-25% reduction in peak canopy size (Table 2). Consequently, the effect on yield:canopy ratio wasmuch less than observed by Poni et al. (2013) andwas very small, not consistent between sites and not statistically significant.

Of the management strategies utilised, a mechanical form of the T3 treatment is, perhaps, the most likely be implemented widely in Australian viticulture (Petrie and Clingeleffer, 2006). Our crop removal treatment produced a 30% and 45% reduction in yield, with no effect on canopy size (Table 2). Consequently, the yield:canopy ratio was reduced.

Summer pruning, equivalent to the T4 treatment used here, has been trialled in Europe as a mechanism to slow maturation (Stoll et al. 2010). Limited trials have previously been attempted in Australia, again on VSP managed vines (Whiting 2012; Savarino et al. 2013). As with the T2 treatment, to the best of our knowledge, the experiments presented here are the first attempt to implement this management strategy on 'sprawl' vines, noting that small adjustments to canopy size during the season is common practices in many vineyards, both cool and warm climate. The treatment reduced canopy size during the maturation period by 30-40% (Table 2) and had no effect on harvest yield at the SA and NSW sites, with

a marginal effect at the VIC site (p=0.101) due to accidental removal of some bunches during the treatment implementation. Therefore, the yield:canopy ratio wasincreased at all sites.

3.2.*The impact on maturation rate of changing vine balance*

Altering vine balance alters the source:sink relationship in the vine. Treatments that increase this ratio (e.g. T4) would generate a greater demand on the canopy for photosynthate than controls and treatments that reduce this ratio (e.g. T3) would reduce the demand on the canopy. In the former case, the additional carbohydrate could be supplied by an increase in photosynthetic rates, depletion of reserves (Smith and Holzapfel, 2009), a longer maturation period, or a combination of these. In the latter case, the additional carbohydrate available could result in a down-regulation of photosynthetic rates, an increased allocation to reserves or a shorter maturation period.

Although maturation period (number of days between veraison and harvest) was not accurately determined, the effect of altering vine balance on maturation period could be inferred from the number of days between the harvest date of a treatment and control because all treatments were harvested at a nominal total soluble solid content (TSS) of 24°Brix(Figure 1). As might be expected, reducing the yield:canopy ratio (T3) advanced the harvest date and increasing the yield:canopy ratio (T4) delayed that date. The canopy structure of the T2 treated vines was affected as well as the canopy size, with greater porosity (data not shown) that may have increased photosynthetic efficiency of the canopy as a whole and resulted in a small advancement of harvest despite the lack of an effect on yield:canopy ratio.

3.3.*Effect of altering vine balance on wine composition*

In general, T2 had a very limited effect on fruit composition (assessed as total anthocyanins and total tannins by UV-vis spectrophotometry) at harvest and T3 had no effect, whereas T4 significantly reduced anthocyanins (data not shown). When the wine was assessed for the same components, one month after bottling, the pattern of treatment effects was similar, but not identical (Figure 2). For example, the effect of T2 was greater, with a significant increase in wine colour density (WCD) and wine total tannin content across the three sites and limited effect of T3, with a small increase in WCD at two sites and an increase in tannins at one. The T4 treatment resulted in decreased WCD at all sites and decreased tannins at two sites (excluding NSW). Reduced fruit total anthocyanin concentration and WCD have been previously reported for late defoliation treatments (Whiting 2012); possibly due to the effect of increased bunch exposure to light and high temperature during the anthocyanin accumulation period.

3.4.*Manipulation of vine carbohydrate availability independently of bunch environment*

The chamber system allowed carbohydrate availability within the vine to be manipulated independently of an environmental effect at the bunch level. Specifically, the system was used to replicate the effect of the T4 treatment through veraison, without increasing the sun exposure of the bunches; reducing whole vine photosynthesis by approximately half. This treatment (reduction in CO_2) reduced the *rate* of sugar accumulation in the berries and, consequently, the absolute amount of sugar in the berries (Figure 3a). Following the end of the CO_2 scrubbing period, juice sugar concentrations increased at a *rate* that matched the controls, but the absolute amount remained lower than the control fruit. The fruit did eventually reach the target TSS for harvest, but this was delayed by more than two weeks, relative to controls. To this extent, the effects of the CO_2 scrubbing treatment were indeed similar to those of the T4 treatment in the field.

In contrast to the field result, where fruit had greater light exposure and, presumably, a greater heat load (Haselgrove et al. 2000), the rate of anthocyanin accumulation matched the rate of sugar accumulation. Consequently, the ratio of sugar to anthocyanins was identical in the treatment and the controls (Figure 3b). The similarity in effect of the scrubbing treatment to T4 on sugar accumulation, but not anthocyanin accumulation suggests that the difference is due to some other factor in the field, with the effect of the late defoliation on the exposure of the fruit, and in particular temperature (Spayd et al. 2002) being the most obvious candidate.

4. Conclusions

These results suggest that maturation rate can be manipulated successfully through adjusting vine balance and that such manipulations are effective across a range of climates and viticultural management styles. However, there is little evidence of maturation rate or vine balance directly impacting fruit composition.

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Table 1 – Mean January temperature (MJT; °C) of the three field sites in each of the three seasons during which field measurements were made.

	2014	2015	2016
SA	21.2	19.5	20.4
VIC	25.5	23.4	24.7
NSW	23.5	21.0	22.2

Table 2 – Canopy size, harvest yield and their ratio (vine balance) averaged across three seasons of implementation of management treatments (T1-4), or for a single season (T5), at the three experimental sites, n=4.

		Canopy (m^2)	Yield (kg)	Vine Balance (kg m^{-2})
SA	**T1: Control**	9.7	5.5	0.58
	T2: Early Defoliation	7.3	4.8	0.67
	T3: Crop Thinning	9.7	3.1	0.31
	T4: Late Defoliation	5.9	5.7	0.99
VIC	**T1: Control**	13.9	19.1	1.35
	T2: Early Defoliation	12.8	16.2	1.28
	T3: Crop Thinning	16.0	13.7	0.84
	T4: Late Defoliation	9.6	16.3	1.64
NSW	**T1: Control**	8.0	5.3	0.66
	T2: Early Defoliation	6.4	4.9	0.77
	T3: Crop Thinning	7.7	3.3	0.43
	T4: Late Defoliation	5.5	5.0	0.91

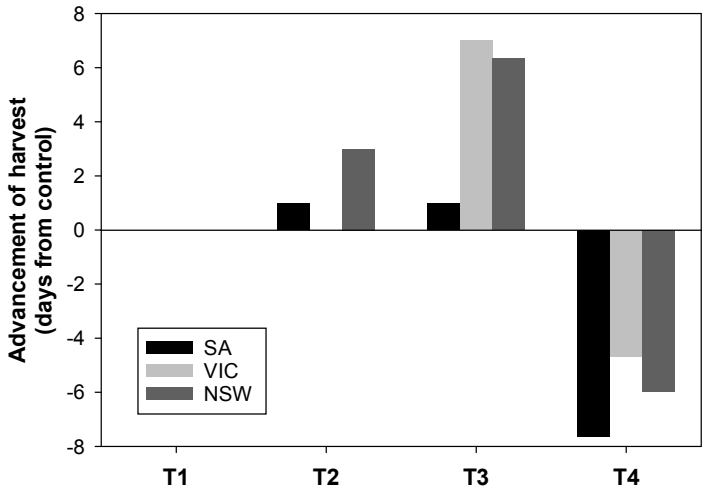

Figure 1: Days in advance (positive) of control or days delayed (negative) that fruit reached a TSS concentration of 24°Brix), averaged across three seasons of implementation of management treatments (T1-4), or for a single season (T5), at the three experimental sites, n=4.

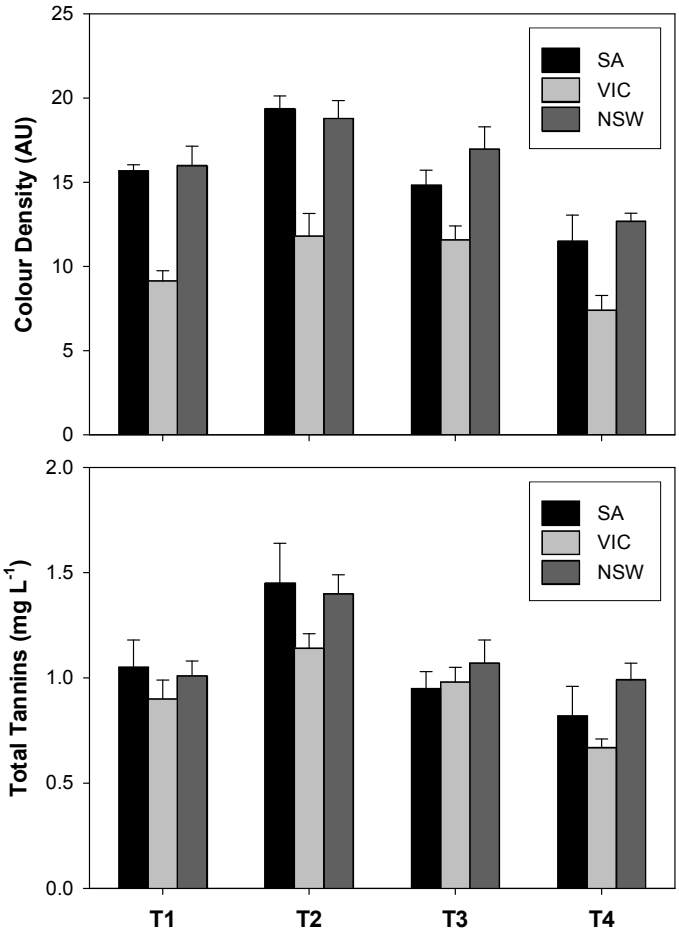

Figure 2: Wine colour density (top) and wine total tannins (bottom) one month after bottling, averaged across three vintages, where the fruit used were from vines subjected to a range of management strategies (T1-T4).

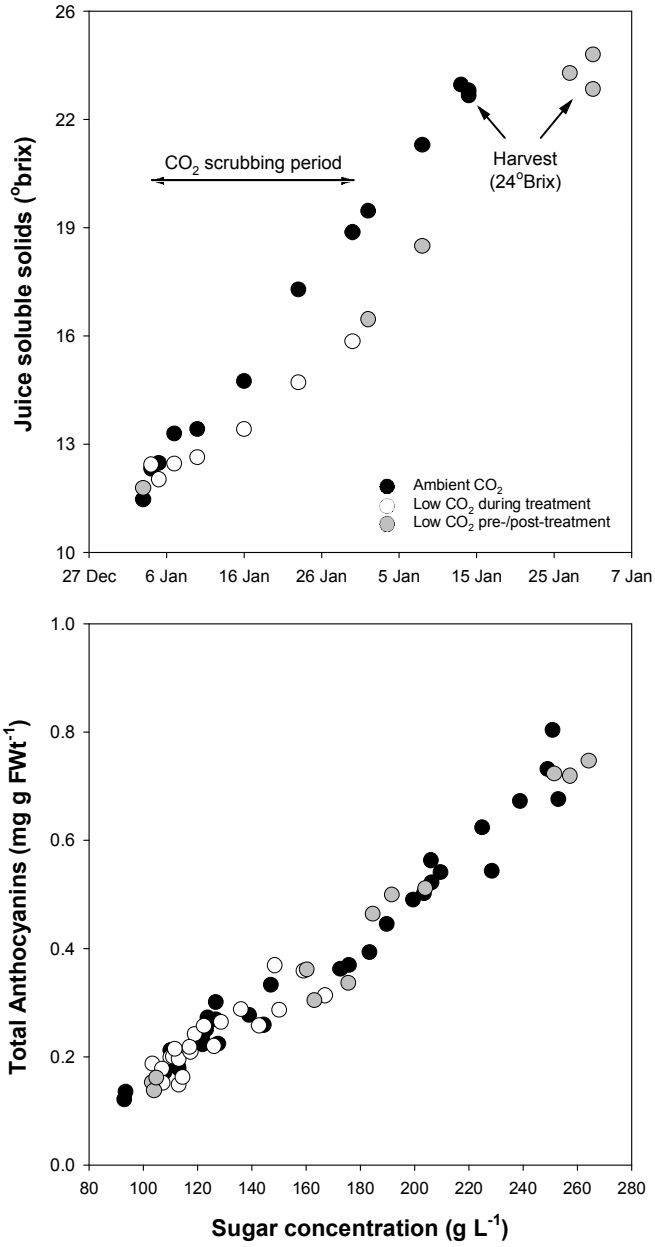

Figure 3: Juice total soluble solids (top) and anthocyanin concentration (bottom) of fruit from vines grown in ambient (blue) or reduced (red) atmospheric CO_2 concentrations for 26 days.