THE INFLUENCE OF TWO METHODS OF CROP REMOVAL AT DIFFERENT LEAF AREAS ON MATURATION OF SAUVIGNON BLANC (*VITIS VINIFERA* L.)

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Abstract

Aim : The research aims to determine if removing all bunches from alternate shoots had the same effect on berry maturity parameters of Sauvignon blanc as removing alternating bunches – apical versus basal – from each shoot.

Methods and results : Shortly after fruit set, 50 % crop was removed from four-cane vertical shoot positioned (VSP) pruned vines using the two different methods. At the same time, all the shoots were trimmed to six or 12 main leaves. Soluble solids (°Brix), pH, titratable acidity and berry weight were measured weekly from pre-veraison to harvest. Leaf area and yield were also measured at harvest. There were no differences in fruit composition between the two methods of crop removal. However, reducing leaf number per shoot from 12 to six leaves delayed veraison, reduced soluble solids accumulation and reduced berry weight with no additional effect from the thinning treatments.

Conclusions : The thinning methods produced no differences in berry maturity parameters of Sauvignon blanc, indicating that carbohydrates can be readily translocated from shoots with no bunches to those with bunches.

Significance and impact of the study : Carbohydrate translocation can occur at the whole-vine level where shoots behave as an integrated system and not as individual shoot units, especially under source-limited conditions.

Key words : carbohydrates, crop removal, maturation, Sauvignon blanc, translocation

Résumé

Objectif : L'objectif de cette étude est de déterminer si la suppression totale des grappes d'un rameau sur deux a le même effet sur les paramètres de maturité des baies de Sauvignon blanc que la suppression des grappes en alternance – apical par rapport à basal – sur chaque rameau.

Méthodes et résultats : Après la nouaison, 50 % du total des grappes portées par le pied sont supprimées suivant les deux méthodes. Les rameaux sont taillés à une longueur de six ou de 12 feuilles principales. Les sucres solubles (°Brix), le pH, l'acidité titrable et le poids des baies sont mesurés une fois par semaine à partir de la pré-véraison jusqu'aux vendanges. La surface foliaire et le rendement sont également mesurés à la vendange. Les paramètres de maturité mesurés ne présentent aucune différence entre les deux méthodes de suppression des grappes. Une diminution du nombre de feuilles de 12 à six par rameau retarde la véraison, réduit l'accumulation des sucres solubles ainsi que le poids des baies sans toutefois avoir d'effet supplémentaire par rapport à la suppression des grappes.

Conclusions : Les différentes méthodes de suppression des grappes n'ont pas de conséquence sur les paramètres de maturité de Sauvignon blanc, ce qui indique que les glucides peuvent être facilement transférés des rameaux sans grappes à ceux avec grappes.

Signification et impact de l'étude : Le transfert des glucides dans la vigne se produit au niveau global de la plante où les rameaux représentent un système intégré et non des unités individuelles, en particulier lorsque la source de glucides est limitée.

Mots clés : glucides, éclaircissage, maturité, Sauvignon blanc, transfert

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INTRODUCTION

Crop thinning, either mechanically or by hand, is a common practice in viticulture to help control target yields and to remove damaged or diseased bunches. Removal of crop can influence different maturity parameters at harvest. Early crop thinning – post bloom/at fruit set – has increased soluble solids in berries (Reynolds *et al.,* 1994; Ollat and Gaudillère, 1998; Guidoni *et al.,* 2002; Petrie and Clingeleffer, 2006; Gatti *et al.* 2012). However, in other cases, crop thinning post bloom had little effect on soluble solids at harvest (Keller *et al.,* 2005; Nuzzo and Matthews 2006). Little difference in titratable acidity or pH has been found in a range of crop removal studies using different cultivars or methods of selecting bunches for removal (Reynolds *et al.* 1994, Keller *et al.* 2005).

Ways to reduce bunch count by approximately 50 % through differential selection (apical versus basal) of bunches, assuming two bunches (which is not always the case) are present on each shoot, include:

1- Removal of apical bunches;

2- Removal of basal bunches;

3- Removal of all bunches on alternating shoots whereby one shoot has all bunches present and the subsequent shoot no bunches;

4- Removal of alternating bunches from each shoot whereby the first shoot has the apical bunch removed, the second shoot has the basal bunch removed, and so on;

5- Any combination of the above (or different spatial locations for shoot choice for 3 and 4).

Naylor (2001) showed that bunch position influences soluble solids at harvest where small differences were measured between apical and basal bunches (apical bunches reached target soluble solids 1-3 days later than basal bunches). Therefore, method 1) may appear to advance maturation through preferential removal of less advanced bunches, while method 2) selects the opposite. Because apical bunches are also generally smaller than basal bunches, with fewer berries, it could also be hypothesised that, with regard to % yield removal (by weight), method 1) is likely to remove less than 50 % of the crop by weight, and result in an increase in the average number of berries per bunch (for example see Morris *et al.* 1987), method 2) is likely to remove more than 50 % and methods 3) and 4) are likely to remove 50 % of the yield. Methods 3) and 4) test different underlying assumptions with regard to carbohydrate assimilation as to whether carbohydrate translocation occurs between shoots and to what degree.

Labelling experiments and thinning experiments have indicated that fruit growth and composition were influenced at the whole-vine level and the effect of crop load was more important than the location of bunches (sinks), relative to source leaves. Hale and Weaver (1962) conducted labelling experiments which indicated that transport of photosynthates basipetally from labelled leaves to the parent vine can occur from flowering onwards. However, when a bunch was present on the same shoot as the labelled leaf, this was the preferred sink. Meynhardt and Malan (1963) showed through labelling that carbohydrates were translocated from leaves on a fruitless (harvested) stem to an opposite stem bearing fruit where the stems were substantially far apart (12.5 feet). Fournioux (1997) found sugars were translocated from three non-fruit-bearing shoots with leaves to three fruit-bearing shoots without leaves to the same level as vines with three shoots with leaves and bunches. Intrigliolo *et al.* (2009) also tested whether between-shoot translocation may occur: vines were either left with high crop or thinned to low crop; within each treatment, two shoots were treated conversely (two shoots were thinned on the high crop unthinned vines, two shoots were unthinned on the low crop thinned vines). For berry growth parameters and soluble solids, there were no differences between the high crop unthinned shoots and the high crop thinned shoots on the same vine, and likewise for the low crop unthinned shoots and the low crop thinned shoots. The only difference was that measured berry growth parameters on all shoots on the unthinned vines had lower values than the berry growth parameters on the shoots on the thinned vines.

Whole-vine carbohydrate allocation would be necessary for thinning method 3) to achieve similar soluble solids at harvest for all bunches to that of thinning method 4). None of the previous studies has investigated this principle across a whole vine, rather testing a select number of defoliated shoots, or on harvested shoots; it is therefore unclear whether there would still be some preferential carbohydrate supply to bunches on the same shoot and adjacent leaves under scenario 3). Source size (trimming shoots to different leaf numbers) may also create differences between the thinning treatments in terms of available photosynthates for translocation, so the impact of this also needs to be considered in combination with the thinning methods.

The objective of this study was therefore to examine whether the method of removing bunches by alternating shoots (method 3) versus alternating bunches (method 4) resulted in differences in berry maturity parameters for Sauvignon blanc. The hypothesis was that there would be no difference in the time of veraison, yield or maturity parameters between crop thinning by alternate shoots and crop thinning by alternate bunches. This study did not test directly the two different assimilate hypotheses, but any differences in outcome between the methods would support one or the other assimilate mechanism.

MATERIALS AND METHODS

1. Experimental site and design

The experiment was conducted in a commercial vineyard in the Wairau valley, Marlborough, New Zealand (41°32'S, 173°51'E) in the 2010-2011 season on Sauvignon blanc (clone MS, rootstock Richter 110), which was planted in 1997. Rows were orientated +15° from north, in a north to south direction; vines were planted 1.8 m apart in the row and the row spacing was 3.0 m.

2 leaf number (12 leaves or six leaves) x 2 thinning methodology ('alternate shoots' or 'alternate bunches') completely randomised design was used with four replicates per treatment and single vines as the experimental unit. 'Alternate shoots' consisted of the removal of all bunches on alternating shoots, where one shoot had all bunches present and the subsequent shoot no bunches. 'Alternate bunches' was achieved by removing alternating bunches from each shoot: the first shoot had the apical bunch removed, the second shoot had the basal bunch removed and so on.

2. Vine management

All vines were pruned to four canes with 12 nodes per cane in August 2010. The lower cane was 900 mm and the upper cane 1100 mm from the soil surface, respectively. The canopy was trained using vertical shoot positioning (VSP), with foliage wires used to maintain the canopy approximately 300 mm in width. Where two shoots arose from the same node, the primary shoot was retained and the other was removed pre-flowering from 19-24 November 2010. General vineyard management (fungicide spraying, irrigation, under-vine weed control) was undertaken in accordance with New Zealand Sustainable Winegrowing practices (Sustainable Winegrowing New Zealand, 2013). All vines were trimmed manually to 12 main leaves per shoot (approximately to the height of that of commercial practices) and six main leaves per shoot (50 % reduction in leaf number and therefore source limited compared to the 12-leaf treatment) by shoot topping and thinned on 7-8 January 2011, approximately 3.5 weeks after 50 % flowering and where berry development corresponded to stage 29-31 on the modified Eichhorn-Lorenz (E-L) scale (Coombe 1995). For thinning methods, it was assumed that there would be approximately two bunches per shoot. The average bunch number per shoot across all replicates was 1.71; therefore, in the case where one bunch was present on the shoot, it was removed and designated as a 'thinned' shoot for the 'alternate shoots' method, whereas it was unthinned for the 'alternate bunches' method. Percentage of crop removed was calculated as the bunch count after thinning/ bunch count pre thinning x 100. Lateral shoots were removed at the time of treatment application and fortnightly thereafter until harvest to maintain a fixed number of main leaves on all replicates.

3. Phenology and berry composition measurements

A 30-berry sample was collected twice a week from each vine from pre-veraison until harvest. Total berry weight for each 30-berry sample was measured. Veraison was assessed weekly for each replicate by scoring each berry as hard or soft simply by gently pressing on it.

For veraison, a logistic curve (equation 1) was fitted (Genstat 12, VSN International Ltd., Hemel Hempstead, United Kingdom) to the softness data for each individual replicate:

$$y = \frac{100}{\left(1 + e^{\left(-\frac{(x-m)}{b}\right)}\right)} \qquad (1)$$

where the value 100 corresponds to the maximum score for veraison, *b* corresponds to rate constant, *m* is the inflection point on the curve (corresponding to a Day Of Year, DOY), and *x* is the DOY of the measurement. 10 % and 50 % values were interpolated from individual curve fits to each replicate. The duration of veraison was also assessed as the number of days to go from 10 % to 90 % softness.

Berry samples were then crushed by hand and coarsely filtered. Berry juice was analysed for 1) soluble solids (°Brix) determined by refractometry with an Atago Pocket PAL-1 refractometer (Atago Co., Ltd, Japan), 2) berry soluble solids content (mg per berry) estimated by multiplying the % soluble solids by the average berry weight (g) and dividing by 100, 3) titratable acidity by endpoint titration (tartaric acid equivalents in g/L) using 0.1 M NaOH to pH 8.4 20°C with a Mettler Toledo DL 50 Graphix titrator (Mettler Toledo GmbH, Analytical, Switzerland) and 4) pH with a Metrohm 744 pH meter (Metrohm AG, Switzerland). At harvest, all vines were hand harvested and yield and bunch counts per vine were recorded.

4. Leaf area estimation

All leaves of the southern half of each vine (both upper and lower canes) were removed. The fresh weight of the whole leaf sample and the fresh weight of a 100-leaf subsample were weighed. The leaf area of the subsample was measured using a LiCOR 3100 leaf area meter (LI-COR, Inc., Lincoln, Nebraska, USA). The total leaf area defoliated (m^2) (LA) was then calculated from the correlation between leaf fresh weight and leaf area and adjusted to give a value for LA in m^2/m row.

5. Statistical analysis and graphics

Results were analysed by two-way (leaf number x thinning method) ANOVA using Genstat 12 (VSN International Ltd., Hemel Hempstead, United Kingdom) for each maturity, veraison and yield parameter. Mean separations were determined by Fisher's least significant difference (LSD) at the 5 % level of significance. Mean plots presented in Figures were plotted using Sigmaplot 12 (Systat Software, Inc., USA).

RESULTS

1. The influence of thinning method and leaf removal on maturation parameters : soluble solids concentration and content, average berry weight, titratable acidity and pH

At all times pre-harvest and at harvest there was no difference in soluble solids concentration between the two thinning methods ('alternate bunches' versus 'alternate shoots') at a given leaf number (Figure 1). However, six-leaf treatments had lower soluble solids concentration at all time points compared with the 12-leaf treatments, irrespective of the crop thinning method ($P<0.001$).

Increased leaf number (12 leaves) resulted in a heavier average berry weight ($P<0.001$) at each time point (Figure 2), with the only exception observed on DOY 96 (at harvest) where there was no difference in berry weight between the six-leaf 'alternate shoot' thinning treatment and the 12-leaf 'alternate bunch' thinning treatment. The 12-leaf 'alternate shoot' thinning treatment also had an average berry weight heavier than the 12-leaf 'alternate bunch' thinning treatment on one occasion, DOY 48 (Figure 2).

Berry soluble solids content (g per berry) was not different between the two thinning methods but greater for 12-leaf treatments compared with six-leaf treatments at all time points, confirming the trends observed in soluble solids concentration and berry weight (Figure 3).

There were few differences in titratable acidity among any treatments at any time points. The only differences observed were : on DOY 33, the titratable acidity in berries from six-leaf 'alternate bunches' was less than that of berries from 12-leaf treatments ($P<0.05$) with no differences between all other mean comparisons, the same differences were observed for six-leaf 'alternate shoots' on DOY 76 ($P<0.05$), and on DOY 62, six-leaf treatments had higher titratable

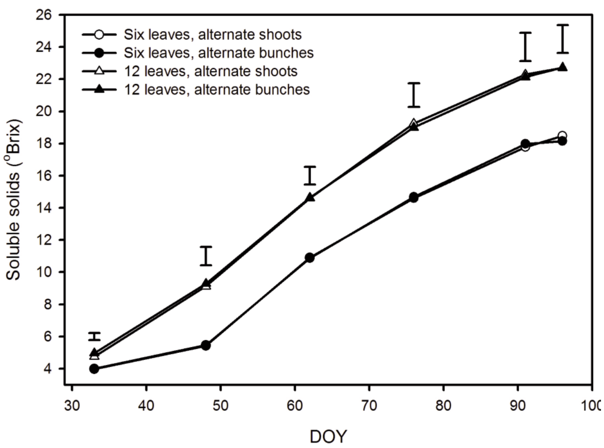

Figure 1. Soluble solids accumulation for different leaf number and thinning methods. Shoot trimming and thinning were undertaken on Day Of the Year (DOY) 7-8. Vertical bars at each time point represent values for Fisher's unprotected LSD ($P<0.05$).

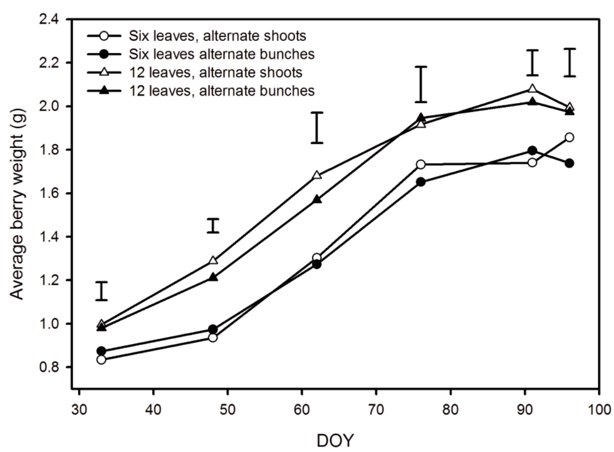

Figure 2. Change in average berry weight (g) for different leaf number and thinning methods. Shoot trimming and thinning were undertaken on Day Of the Year (DOY) 7-8. Vertical bars at each time point represent values for Fisher's unprotected LSD ($P<0.05$).

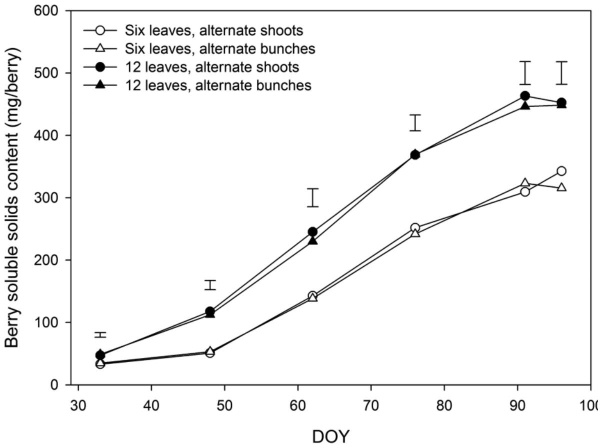

Figure 3. Change in berry soluble solids content (g per berry) for different leaf number and thinning methods.

Shoot trimming and thinning were undertaken on Day Of the Year (DOY) 7-8. Vertical bars at each time point represent values for Fisher's unprotected LSD (*P*<0.05).

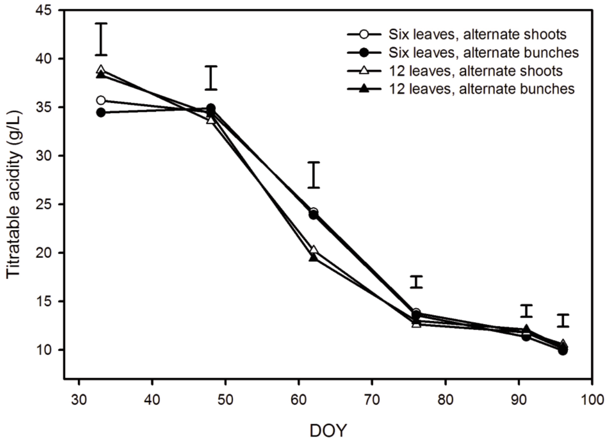

Figure 4. Change in titratable acidity (g/L) for different leaf number and thinning methods.

Shoot trimming and thinning were undertaken on Day Of the Year (DOY) 7-8. Vertical bars at each time point represent values for Fisher's unprotected LSD (*P*<0.05).

acidity compared with 12-leaf treatments (*P*<0.001) (Figure 4).

Six-leaf treatments had slightly higher pH values than 12-leaf treatments on all DOYs except DOY 76 where no differences were observed (*P*<0.05), and DOY 62 where the pH was lower for six-leaf treatments (*P*<0.05). There was some overlap of the six-leaf 'alternate shoots' treatment with both 12-leaf treatments (DOY 33, 48 , 91, 96) and on DOY 91, the six-leaf 'alternate bunches' treatment had a higher pH than all other treatments (*P*<0.05) (Figure 5).

2. The influence of thinning method and leaf removal on yield components and leaf area

Thinning by 'alternate shoots' removed a greater % of crop than thinning by the 'alternate bunches' method (*P*=0.042). Main effects of thinning (*P*=0.035) and leaf number (*P*=0.043) influenced bunch counts (accounting for 22 and 25 % of the total sum of squares, respectively) ; however, harvest yields remained the same irrespective of thinning method or leaf number (Table 1). The differences in the Leaf Area to Fruit Weight (LA:FW) ratio were a result of difference in leaf area only (main effect, *P*<0.001) (Table 1) with the main effect of leaf area accounting for 67 % of the total sum of squares.

3. The influence of thinning method and leaf removal on veraison

The method of thinning ('alternate bunches' versus 'alternate shoots') did not change the time of veraison (assessed at the start, 10 %, and midpoint, 50 %), nor

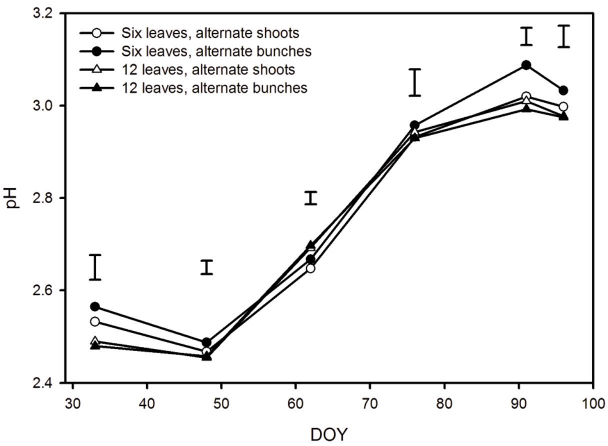

Figure 5. Change in pH for different leaf number and thinning methods.

Shoot trimming and thinning were undertaken on Day Of the Year (DOY) 7-8. Vertical bars at each time point represent values for Fisher's unprotected LSD (*P*<0.05).

its duration or rate of development at a given leaf number (either six or 12 leaves) (Table 2). The only difference detected was veraison (10 % or 50 %) was significantly later for six-leaf treatments compared to the 12-leaf treatments (*P*<0.05) (Table 2).

DISCUSSION

Crop removal acted at the whole-vine level for the experimental system tested here : there was no difference in the soluble solids concentration or content (Figures 1 and 3) as a result of carbohydrate supply for the 'alternate bunch' versus 'alternate shoot' removal methods, even when the source was

Table 1. Mean harvest values for % crop removed, grape bunch number, yield and Leaf Area to Fruit Weight (LA:FW) ratio.

Treatment	% crop removed	Bunch number	Yield (kg/vine)	LA:FW (m^2/kg)
Six leaves, alternate shoots	43.1^{ab}	36.3^{ab}	5.42^{a}	0.45^{a}
Six leaves, alternate bunches	40.9^{a}	40.5^{b}	5.63^{a}	0.45^{a}
12 leaves, alternate shoots	47.6^{b}	30.5^{a}	5.31^{a}	0.98^{b}
12 leaves, alternate bunches	40.8^{a}	36.5^{ab}	5.49^{a}	0.81^{b}
LSD	6.07	6.65	1.73	0.28

Means within columns followed by different letters differ significantly from one another (Fisher's unprotected LSD test, $P<0.05$).

Table 2. Time and duration of veraison for different leaf number and thinning methods for Sauvignon blanc.

Treatment	10 % veraison (DOY)	50 % veraison (DOY)	Duration (days from 10 % - 90 % veraison)	*b*
Six leaves, alternate shoots	52.0^{c}	56.1^{b}	8.1^{a}	1.85^{a}
Six leaves, alternate bunches	47.6^{bc}	54.0^{b}	12.8^{a}	2.92^{a}
12 leaves, alternate shoots	42.5^{ab}	46.1^{a}	7.17^{a}	1.61^{a}
12 leaves, alternate bunches	38.7^{a}	45.4^{a}	13.3^{a}	3.03^{a}
LSD	6.78	4.89	9.95	2.27

Mean Day Of the Year (DOY from 1 January) for 10 % and 50 % veraison and duration (the time to go from 10 % to 90 % veraison) were interpolated from individual logistic curve fits of each replicate plot where ; *b* is the measure of the rate of development; means within columns followed by different letters differ significantly from one another (Fisher's unprotected LSD test, $P<0.05$).

reduced to six leaves per shoot. The results are supported by those of Fournioux (1997) and Intrigliolo *et al.* (2009) and suggest that thinning by either methodology and subsequent sampling from both apical and basal bunches at harvest should produce equivalent results in soluble solids. A possible preference of source supply from adjacent leaves was not tested directly in this experiment, but the results suggested that even at a reduced source of six main leaves per shoot, the whole vine was able to compensate and maintain a constant soluble solids concentration and content irrespective of the source of carbohydrates. In other studies, Intrigliolo *et al.* (2009) did not test whether there was a potential threshold source size or activity below which adjacent shoots would not be able to compensate for the lack of source present on a shoot with bunches. Trimming to six leaves per shoot represents a severely reduced canopy, and it is unlikely that industry trimming practices would go beyond this. Fournioux (1997) tested a similar lower limit of six leaves per shoot: "three shoots each with one bunch and no leaves" that were adjacent to "three shoots each with six leaves and no bunches" had the same level of sugar and acidity as "three shoots each with six leaves and one bunch". This indicates that adjacent shoots were able to compensate for fully defoliated shoots, but it remains unclear whether under even more source-limited conditions there could be a preferential carbohydrate supply mechanism.

All other tested berry parameters – berry weight, pH and titratable acidity (Figures 2, 4 and 5) – were not affected by the thinning methods, which indicates that not only carbohydrate translocation was possible but maintenance of berry composition for other parameters was not affected by the relative location of leaves (source) to sinks (bunches). The only difference detected was in titratable acidity on DOY 62, where six-leaf treatments had higher concentrations than 12-leaf treatments (Figure 4). Likewise, pH was higher in six-leaf treatments at most time points (Figure 5), suggesting that small differences due to leaf number may be detected during the ripening phase for both parameters, with berries from lower leaf number shoots having slightly higher titratable acidity and pH values.

Therefore, acidity was uncoupled from soluble solids accumulation by the different trimming treatments; reducing the leaf area reduced soluble solids concentration on any given day but titratable acidity was unaffected. This result warrants further investigation in the presence of full crop where these differences may potentially be bigger (due to even greater source limitation on soluble solids accumulation).

The differences observed between the six-leaf and 12-leaf treatments need to be investigated in the future in more detail to determine the influence of LA:FW ratio on phenological development and maturation, especially given that differences were observed between the two leaf numbers with crop thinning (reduced sink demand); it is yet to be determined how big these differences may be in the presence of full crop. Initial work on potted vines has indicated that reduced source via shoot trimming post bloom (Poni and Giachino, 2000) or leaf removal (Ollat and Gaudillère, 1998; Petrie *et al.,* 2000) can delay the onset of maturity/veraison, but this has not been tested on field VSP vines. Stoll *et al.* (2011) reported that trimming in the field reduced soluble solids in berries. Such trimming treatments need to be considered in the presence of crop removal as well.

In our experiment, laterals were removed to maintain a fixed leaf area on the vines from trimming to harvest. However, the absence of laterals presents two limitations: 1) this is not a very practical method in the vineyard where shoot trimming is predominantly mechanised and 2) laterals may compensate for some of the source limitation effects. Research by Bernizzoni *et al.* (2011) showed that there was a marked increase in main and lateral leaf area when vines had 40 % of shoots removed approximately 72 days pre-veraison. The result was a non-significant difference in total vine leaf area at harvest. Koblet (1969) found that leaves on laterals start to export assimilates at 40 % of the full leaf size and laterals have been shown to compensate for leaf area changes in other studies (Poni and Giachino, 2000). It was also found that removal of laterals four weeks post-flowering reduced sugar in Müller-Thurgau (Koblet and Perret 1971). Therefore, future studies investigating the relative contribution of laterals would be of interest to reflect more commercial conditions. Furthermore, six leaves per shoot represents a strong source limitation that can be used to understand the impact of trimming in future studies but may not represent a trim height that is currently used in practice.

1. Practical implications of different methods of crop thinning

The relative advantages and disadvantages of keeping both apical and basal bunches need to be considered in practice when carrying out any crop thinning. The two methodologies tested here meant that equivalent quantities of both apical and basal bunches were removed. Thinning by the ‘alternate shoots’ method has the practical advantages that 1) it does not require a choice between apical and basal bunch removal, 2) it is easy to instruct to those thinning vines and 3) it easily controls the % removal (for example one in two shoots thinned for 50 %, one in four shoots thinned for 25 %). It thus provides more control in experimentation over the percentage and positions of bunches present, ultimately removing a representative distribution of bunches from the vine.

In terms of mechanising crop removal, it may be easier to target thinning to a set height within the canopy, and this could potentially lead to selective removal of apical or basal bunches, depending on the targeted height. Therefore, these factors require further investigation in terms of a better understanding of the underlying physiology driving differences in grape and wine quality, and the practical applications of such findings.

CONCLUSION

Reducing crop by removing all bunches from alternate shoots or removing alternating bunches from each shoot had the same effect on maturity parameters with no differences between treatments. This supports the hypothesis that carbohydrate supply from source leaves to bunches (sinks) acts at the whole-vine rather than at an individual shoot level, even under source-limiting conditions. Crop thinning through the removal of bunches from alternate shoots presents a more controlled practice to manipulate source-sink ratios for experimental purposes, when avoiding selection of different bunch positions is desirable. This method also offers a practical solution for better control of percentage bunch removal.

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