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The effect of pre-budbreak cane girdling on the physical and phenological development of the inner and outer arm in *Vitis vinifera* L. 'Sauvignon blanc' inflorescence structures

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Summary

The development of inflorescence primordia (IP) into floral bearing structures is influenced by many environmental and genetic factors. We hypothesise that carbohydrate (CHO) availability at budbreak (BB) has a strong influence on IP development, especially during the initial stages of shoot growth when pre-formed IP emerge from dormant buds and may be dependent on reserve CHOs for further branching and development. Carbohydrate availability to developing grapevine buds (*Vitis vinifera* L. 'Sauvignon blanc') was manipulated by girdling canes two weeks before BB. Dates of flowering, flower number, berry number and grape berry soluble solids (SS) were measured for the inner and outer arm bunch components of basal and apical bunches separately. Restricting pre-BB CHOs resulted in the abortion of some pre-formed inflorescences and reduced branching of the inflorescences that did develop. In general, berry SS were greatest for the basal inner arm, followed by those of the apical bunch inner arm, then those of the basal bunch outer arm, then lastly by those of the apical bunch outer arm. However, this was influenced by the relative berry numbers between the inner and outer arm. Bunches with more similar berry numbers on the inner and outer arms had more synchronous flowering and uniform SS, where the differences in SS were largely a reflection of the timing of flowering of the various inflorescence components and may be an important source of variation in SS within a vine at harvest.

K e y w o r d s : Bunch architecture, inflorescence, inner and outer arm, grapevine phenology, 'Sauvignon blanc'.

Introduction

The initiation of a grape bunch begins as an group of uncommitted cells (an anlage) in the leaf axils of developing shoots in the growing season preceding the appearance of flowers and fruit (Pratt 1971, Srinivasan and Mullins 1981). The development of these anlagen into leaf, tendril or inflorescence primordia starts at leaf position one (basal leaf on the shoot) at about the time of flowering in the first season (Swanepoel and Archer 1988, Morrison 1991, MacGregor 2000, Trought 2012) and then progresses along the shoot in an acropetal fashion (Snyder 1933, Pratt 1971, Srinivasan and Mullins 1981, Morrison 1991). This results in the development of six to 10 pre-formed nodes in the bud by dormancy in late autumn (Buttrose 1974). Factors such as the position of the bud on the developing shoot, temperature, light during initiation and grapevine variety will influence the number of developing inflorescence primordia, each of which has a bract with two arms (inner and outer) in its axil (Vasconcelos *et al.* 2009). Depending on environmental conditions, a degree of branching of the inner and outer arms is observed before the onset of dormancy (Buttrose 1969 a and b, Sommer *et al.* 2000, Sanchez and Dokoozlian 2005, Watt *et al.* 2008). Development of inflorescence primordia has been shown to continue throughout winter dormancy, but is cultivar and climate specific (Jones *et al.* 2009). Further branching and differentiation of the inner and outer arms into floral structures resumes shortly before and after budbreak in season two, forming an inflorescence structure that has the potential to flower and develop berries (Swanepoel and Archer 1988, Morrison 1991, May 2000). Typically observed inflorescence structures are shown in Fig. 1. While a number of studies have been conducted to determine the influence of carbohydrate (CHO) availability on the development of inflorescence primordia, they typically alter carbohydrate availability by reducing the leaf area or introducing a girdle on a developing shoot before the onset of dormancy (Candolfi-Vasconcelos and Koblet 1990, Caspari *et al.* 1998, Bennett *et al.* 2005, Sanchez and Dokoozlian 2008, Smith and Holzapfel 2009, Vrsic *et al.* 2009). As a consequence, it is difficult to separate the confounding effects of restricting carbohydrate availability to the developing shoot in the spring from the potential disruption to inflorescence primordia development in season one. However, in general, earlier leaf removal results in a greater reduction in inflorescence number, flower number and berry number in season two (Bennett *et al.* 2005).

Initial shoot development in the spring depends on the stored carbohydrate reserves in roots, shoots and the trunk of the vine (Zapata *et al.* 2004, Greer and Sicard 2009, Eltom *et al.* 2013). To date, little work has been reported on the effects of varying the amount of available endogenous CHO pre-BB *via* cane girdling on inflorescence de-

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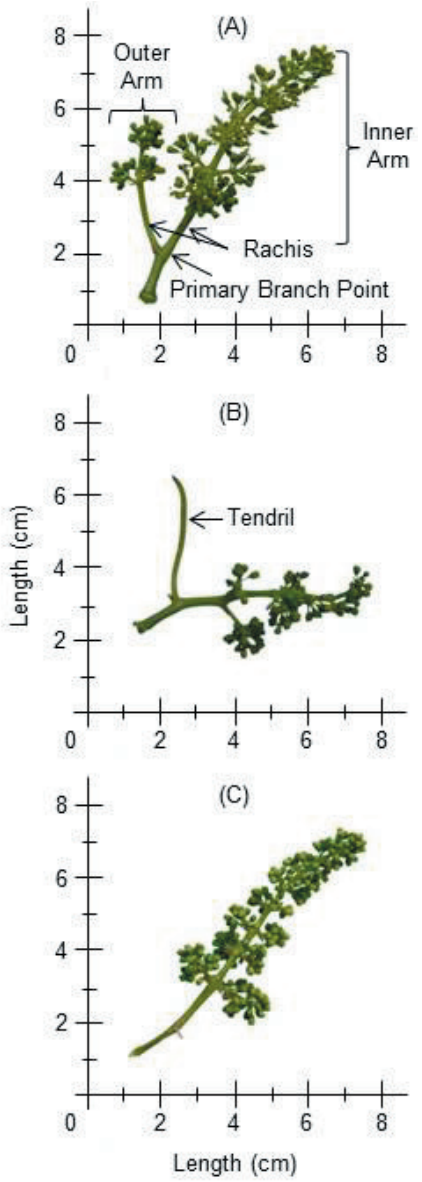

Fig. 1: Identification of the structure at the primary branch point of the grapevine rachis. Structures were photographed during flowering. The type of structures observed in our study were: (**A**) an outer arm, which in turn can be branched; (**B**) a tendril, which in turn can be branched; (**C**) no structure observed at the primary branch point.

velopment and/or bunch architecture. However, girdling pre-BB not only blocks the transport of sugars from source to sink (Glad *et al.* 1992), but also has the inherit limitation of blocking all the compounds in the phloem sap that could influence inflorescence primordia (IP) development, such as auxin, amino acids, mRNA and other proteins and phytohormones (Glad *et al.* 1992, Kehr 2006, Robert and Friml 2009).

Our study focused on separating the confounding effects of limiting CHOs during season one by allowing unaltered growth of the compound bud to occur during season one, and then limiting CHO availability in season two *via* pre-budbreak cane girdling. An analysis of bunch number, the types of structures observed at the primary branch point of the rachis during flowering for all bunches, the timing of flowering for all bunch components, the numbers of flower caps and berries per inflorescence and bunch structure, and berry soluble solids (SS) were all recorded to help to quantify the physical and phenological differences between bunch components and bunch positions on the shoot.

Material and Methods

Definition of terms and structure identification: We defined any structure at the primary branch point of the main rachis that had flower(s) present as an outer arm (Fig. 1). The inflorescences/bunches were compared separately, giving four bunch components on a shoot (basal/apical bunches: inner/outer arms).

Plant Material: Forty eight randomly selected three-cane pruned *Vitis vinifera* L. 'Sauvignon blanc' vines (University of California Davis Mass Select, SO4 rootstock), located on a commercial vineyard, Marlborough, New Zealand (-41.48° latitude, 173.95° longitude), were used for this study. Vines were pruned during winter dormancy (August 2010) to retain 10 nodes per cane and monitored over a single growing season from 2010 to 2011. The rows were planted in a north west-south east orientation, and vines were spaced 1.8 m along and 2.7 m between rows. Three canes were lightly wrapped on fruiting wires, 0.9 and 1.1 m above the ground (two on the lower and one on the upper wire). Foliage wires were used to keep shoots in a vertical position and vines were trimmed (other than treatment shoots) two or three times during the season, at a height of 2.0 m from the ground and 0.5 m between the vertical faces of the canopy. Pest and disease management was achieved following Sustainable Winegrowing New Zealand guidelines (http://www.nzwine.com/swnz/).

Treatments were grouped into 12 blocks along a row of vines, each block consisting of two vines, with treatments randomly applied within the blocks. The selected vines were restricted to the middle two vines of a bay (of four) to prevent any effect of the proximity of posts used to support the wires on the developing shoot. The same girdling treatment was applied to both of the bottom two canes on a vine, for a total of 24 replicates per treatment. Canes were girdled 20, 10, or 5 cm from the terminal node and a non-girdled treatment was used as the Control treatment. A razor blade was used to make two cuts 4-6 mm apart around the cane, severing the phloem. Tweezers were then used to remove the periderm between the cuts. The resulting girdles were monitored throughout the growing season and any callus that formed was removed. The terminal bud was retained and shoot growth was measured at regular intervals during the growing season. All buds up to 20 cm from the terminal bud were excised to ensure that no other shoots were present to compete with the shoots that grew from the experimental buds.

Flower cap collection and flowering progression: One week before flowering commenced, fine mesh bags were placed over entire inflorescence structures, and were removed one week after

flowering was complete. Flowering progression of the inner and outer arm (where present) of each inflorescence was monitored three times a week during flowering. Once flowering was completed and the bags were removed, the flower caps were counted to estimate the number of flowers per inflorescence.

Grape bunch collection, grape measurements: Bunches were harvested shortly before the commercial harvest date in the second week of March 2011. All treatment bunches were processed in the same manner. Bunches were separated into inner and outer components (if present) and weighed. All berries were removed from their rachis, counted, and weighed. Soluble solids (°Brix) were determined by crushing all the berries of a bunch component in a plastic bag. The juice was sieved to remove the majority of particulates, and 0.5 mL was analysed using a digital refractometer (Pocket Refractometer, PAL-1, ATAGO, Auckland, New Zealand) to give a reading of °Brix. Total bunch soluble solids content ($g \cdot shoot^{-1}$) was determined by adding the soluble solids content (°Brix*component weight (g) / 100) values for the basal and apical bunch components on a shoot.

Total bunch SS was calculated using the regression of percentage inner arm berry number per bunch versus the difference in SS between the inner and outer arm. The relative proportions of berries for the inner and outer arm components were then multiplied by their respective °Brix values, and then added together.

Calculation of total shoot leaf area *versus* total bunch soluble solids content: Total leaf area (main and lateral shoots) was previously reported (Eltom *et al.* 2013), and used in this study to identify its relationship with total bunch soluble solids content ($g \cdot shoot^{-1}$) at harvest. Total main shoot leaf area *versus* the total bunch soluble solids content ($g \cdot shoot^{-1}$) was fitted in SigmaPlot V12.0 using a three-parameter exponential rise to maximum curve, y = y0 + a*(1-exp(-b*x)).

Statistical analysis: All regressions were carried out in GenStat Edition 12.1. One-way analysis of variance (ANOVA) tests were carried out within and between treatments to determine P-values and to check if the data were normally distributed. Fisher's Unprotected Least Significant Difference (LSD) test (at a significance level of $P = 0.05$) was used post hoc to separate treatment effects from one another.

Results

Structures observed at the primary branch point of the main rachis during flowering: The effect of girdling on bunch number and structure observed at the primary branch point of the rachis can be divided into two groups: the Control and 20-cm treatments as one; and the 10-cm and 5-cm treatments as the other (Tab. 1). The 10-cm and 5-cm treatments resulted in a reduced bunch number per shoot, by approximately 40 %, from that in the Control and 20-cm treatment group. There was a reduction in the presence of an outer arm in the basal bunch position of approximately 50 % and an increase in the presence of a tendril of approximately 40 % in the 10-cm and 5-cm treatments from the 176 Control and 20-cm treatments. The occurrence of no structure in the basal bunch position was statistically similar between all treatments. The presence of an outer arm decreased in apical bunches compared with basal bunches, and was statistically similar between all treatments, where the decreased presence of an outer arm in the Control and 20-cm treatments was far greater than the decreased presence in the 10-cm and 5-cm treatments. The presence of a tendril increased in apical bunches compared with basal bunches, and was statistically similar between all treatments (approximately 90 %).

Progression of flowering between treatments, inner and outer arm bunch components: Regardless of the girdling treatment, flowering generally progressed in the order starting with the basal inflorescence inner arm, apical inflorescence inner arm, basal outer arm and finally the apical outer arm (Tab. 2).

On an individual shoot, flowering took approximately 16 d (from the date at which the basal inflorescence inner

Table 1

Variation in the structure observed at the primary branch point of the main grapevine rachis during flowering

Treatment	Bunch no.	Basal bunches (%)			Apical bunches (%)		
		Outer arm	Tendril	Nothing	Outer arm	Tendril	Nothing
Control	1.8^{b}	83.3^{b}	12.5^{a}	4.2^{ns}	5.3^{ns}	94.7^{ns}	0.0^{ns}
20-cm	1.9^{b}	81.8^{b}	18.2^{a}	0.0^{ns}	4.8^{ns}	90.5^{ns}	4.8^{ns}
10-cm	1.2^{a}	25.0^{a}	58.3^{b}	16.7^{ns}	14.3^{ns}	71.4^{ns}	14.3^{ns}
5-cm	1.2^{a}	40.0^{a}	50.0^{b}	10.0^{ns}	0.0^{ns}	100^{ns}	0.0^{ns}

Values in the table are means. Percentage values were calculated from the total number of bunches in the basal or apical bunch position within a treatment. Values were separated using Fisher's unprotected LSD test, where values with different letters in superscript are statistically different from one another ($P < 0.05$) between treatments (basal and apical positions calculated separately). ns = not significant ($P > 0.05$).

Table 2

The influence of grapevine cane girdling pre-budbreak on flowering

	Basal IA	Apical IA	Basal OA	Apical OA
Start of flowering (5 %, 2012 date)				
Control	5-Dec$^{a/1}$	8-Dec$^{a/2}$	12-Dec$^{a/3}$	15-Dec$^{a/4}$
20-cm	5-Dec$^{a/1}$	8-Dec$^{a/2}$	12-Dec$^{a/3}$	15-Dec$^{a/4}$
10-cm	8-Dec$^{b/1}$	11-Dec$^{b/2}$	14-Dec$^{b/3}$	18-Dec$^{b/4}$
5-cm	8-Dec$^{b/1}$	12-Dec$^{b/2}$	15-Dec$^{b/3}$	np
Duration of flowering (days)				
Control	6.0^{ns}	5.8^{ns}	5.9^{ns}	6.3^{ns}
20-cm	5.2^{ns}	5.4^{ns}	5.7^{ns}	5.6^{ns}
10-cm	5.8^{ns}	6.2^{ns}	5.7^{ns}	6.3^{ns}
5-cm	6.1^{ns}	6.2^{ns}	5.6^{ns}	np

Values in the table are means. Values were separated using Fisher's unprotected LSD test, where values with different letters (between treatments for a given bunch component) and numbers (within a treatment across all bunch components) in superscript are statistically different from one another ($P < 0.05$). ns = not significant ($P > 0.05$). np = no structure present. IA = inner arm. OA = outer arm.

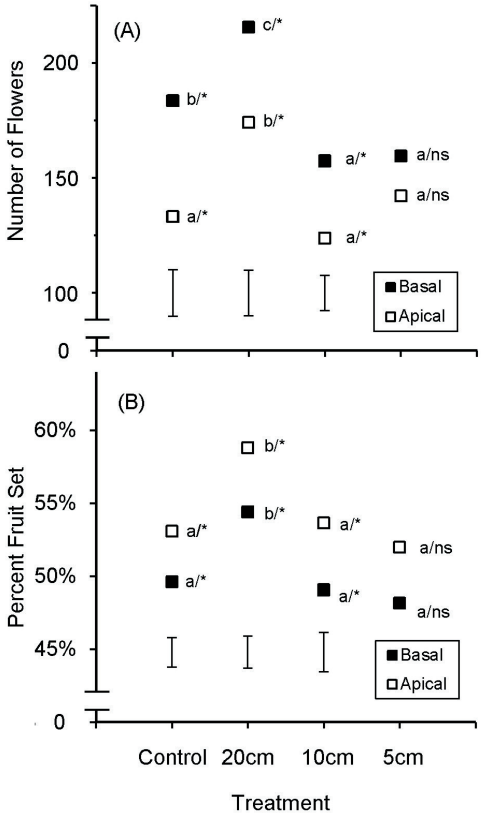

Fig. 2: The effect of pre-budbreak cane girdling on the number of grapevine flowers per inflorescence structure and on percentage fruit set. All data points are mean values. See text for explanation of treatments. Values were separated using Fisher's unprotected LSD test, where values with different letters (between treatments) and an asterisk (within a treatment) in superscript are statistically different from one another ($P < 0.05$) between treatments. Vertical bar represent the LSD. ns = not significant ($P > 0.05$).

arm reached 5 % flowering to the date at which the apical inflorescence outer arm achieved 95 %). Girdling had no effect on the duration of flowering within or between treatments, although the start of flowering for the 5-cm and 10-cm treatments was approximately 3 d behind the 20-cm and Control treatments. Flowering started on the outer arm approximately 7 d after its inner arm component, and was unaffected by the presence of a girdle and/or bunch position (Tab. 2).

There was a decrease in flower number in apical inflorescences compared with basal inflorescences in all treatments, except for the 5-cm treatment (Fig. 2a). Inflorescences in the 20-cm treatment had the greatest number of flowers in the basal and apical bunch positions, followed by those in the Control, and then those in the 10-cm and 5-cm treatments (which were statistically similar). Conversely, there was an increase in percentage fruit set (% FS) in the apical position compared with the basal position (Fig. 2b). Percentage fruit set in the basal and apical positions was the greatest for the 20-cm treatment, followed by those in the Control, 10-cm and 5-cm treatments, which had statistically similar % FS values in the basal and apical bunch positions.

Total shoot bunch weight was statistically similar in the Control and 20-cm treatments, whereas the 10-cm and 5-cm treatments resulted in decreased values compared with that in the Control. While the average berry number in the 20-cm treatment increased compared with that in the Control, average berry weight decreased, resulting in statistically similar total shoot bunch weights between the 20-cm and the Control treatments. Average inner arm berry number was greater than that for the outer arm in all treatments and bunch positions, where the observed decrease in berry number from basal to apical bunch positions was not affected by girdling. Average inner arm berry weight and SS were greater than those for the outer arm in all treatments and bunch positions. However, the Control treatment had the greatest average berry weight and SS values for all bunch positions and components, followed in descending order by the 20-cm, 10-cm and then the 5-cm treatments. As well, there was a decrease in berry weight and SS from the basal to apical bunch position (Tab. 3).

The differences in SS (°Brix) between the inner and outer arm within a bunch was a reflection of the relative number of berries on each structure. The more alike the berry numbers between the inner and outer arm structures were, the more similar the SS were at harvest (Fig. 3a). This is a reflection of the relative timing of flowering between the two components (Fig. 3b), where the more similar in berry number the inner and outer arm were, the more closely the flowering start times were relative to one another (Fig. 3c).

Calculating the total bunch SS indicated that as the proportion of fruit on the outer arm decreased, the overall bunch soluble solids initially decreased, reflecting the decreasing SS value of the outer arm. However, as the outer arm berry number continued to decrease relative to the inner arm berry number total bunch SS increased (Fig. 4).

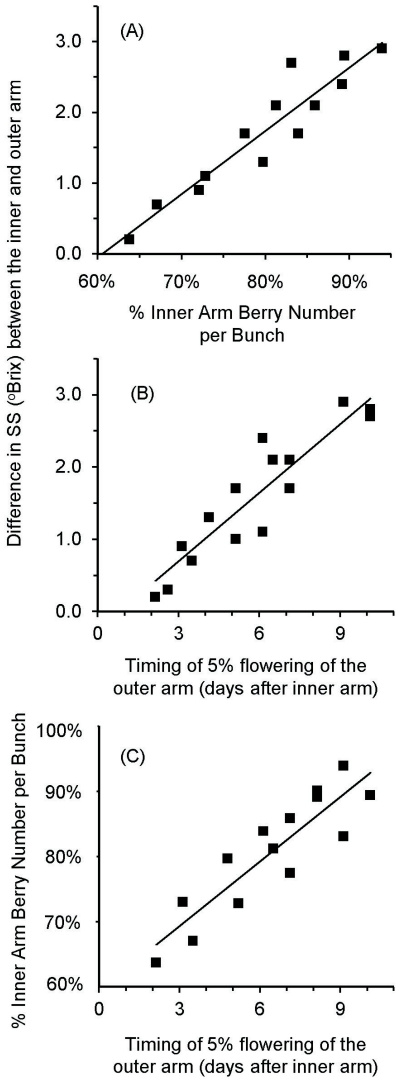

Fig. 3: A comparison between inner and outer arm grapevine bunch components by differences in berry number, date of flowering and soluble solids (SS) at harvest for the control treatment. (**A**) Linear regression y = 8.9x - 5.4, R^2 = 0.88; (**B**) linear regression y = 0.32x - 0.24, R^2 = 0.85; (**C**) linear regression y = 0.03x + 0.6, R^2 = 0.79.

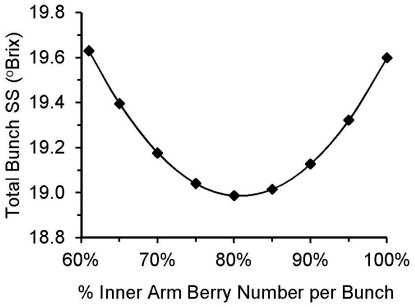

Fig. 4: The effect of the relative difference in grape berry number between the inner and outer arm compared with the overall bunch soluble solids (SS) for the control treatment. Total bunch SS was calculated using the regression of percentage inner arm berry number per bunch versus the difference in SS between the inner and outer arm (Fig. 3a). The relative proportions of berries for the inner and outer arm components were multiplied by their respective °Brix values, and then added together. The resulting calculated quadratic equation is y = 16.6x² -26.7x + 9.8.

Table 3

Grape berry number, berry weight and % soluble solids at harvest

		Basal IA	Apical IA	Basal OA	Apical OA	Total shoot bunch weight (g)
Total bunch weight (g)	Control	$140.9^{d/4}$	$99.0^{c/3}$	$25.3^{b/2}$	$12.3^{a/1}$	294.8^{c}
	20-cm	$129.8^{c/4}$	$112.2^{c/3}$	$26.3^{b/2}$	$7.8^{a/1}$	298.2^{c}
	10-cm	$101.0^{b/4}$	$77.0^{b/3}$	$17.3^{a/2}$	$1.8^{a/1}$	215.1^{b}
	5-cm	$41.6^{a/3}$	$36.1^{a/2}$	$11.9^{a/1}$	np	94.5^{a}
Berry no.	Control	$78^{b/3}$	$64^{a/2}$	$17^{a/1}$	$8^{ns/1}$	
	20-cm	$95^{c/4}$	$82^{b/3}$	$34^{b/2}$	$9^{ns/1}$	
	10-cm	$74^{b/3}$	$61^{a/2}$	$20^{a/1}$	$2^{ns/1}$	
	5-cm	$64^{a/3}$	$53^{a/2}$	$14^{a/1}$	np	
Berry weight (g)	Control	$1.8^{c/2}$	$1.8^{c/2}$	$1.6^{c/1}$	$1.5^{b/1}$	
	20-cm	$1.5^{b/2}$	$1.4^{b/2}$	$0.9^{b/1}$	$0.8^{a/1}$	
	10-cm	$1.5^{b/2}$	1.4b/2	$1.0^{b/1}$	$0.9^{a/1}$	
	5-cm	$0.7^{a/ns}$	$0.7^{a/ns}$	$0.7^{a/ns}$	np	
SS (°Brix)	Control	$19.6^{d/3}$	$19.4^{d/3}$	$18.2^{c/2}$	$16.4^{b/1}$	
	20-cm	$16.0^{c/3}$	$14.7^{c/2}$	$12.8^{b/1}$	$10.8^{a/1}$	
	10-cm	$14.3^{b/3}$	$12.6^{b/2}$	$12.7^{b/2}$	$10.3^{a/1}$	
	5-cm	$9.0^{a/ns}$	$9.6^{a/ns}$	$9.2^{a/ns}$	np	

Values in the table are means. Values were separated using Fisher's unprotected LSD test, where values with different letters (between treatments for a given bunch component) and numbers (within a treatment across all bunch components) in superscript are statistically different from one another ($P < 0.05$). np = no structure; IA = inner arm; OA = outer arm. SS = soluble solids.

Restricting the developing shoot leaf area by girdling also limited the total soluble solids content of bunches on a shoot. Increases in leaf area beyond approximately 4,900 $cm^2 \cdot shoot^{-1}$ had little effect on the total bunch soluble solids content (48.9 $g \cdot shoot^{-1}$), while the accumulation of soluble solids content on shoots with lower total leaf areas was significantly lower (Fig. 5).

Discussion

Inter-conversion of the structure at the primary branch point between a tendril and inflorescence structure is not uncommon, as the two structures are considered homologous, based on the following evidence: both structures are derived from uncommitted primordia (anlagen); inter-conversion between the two structures is based on cytokinin/gibberellin balance and temperatures; and intermediate structures between the two exist (Srinivasan and Mullins 1981, Boss and Thomas 2000, Boss *et al.* 2003, Calonje *et al.* 2004). In addition to this evidence, our study demonstrated that limiting early season CHO availability reduced the occurrence of an outer arm and promoted the presence of a tendril. This gives new evidence that the final identity of the structure observed at the outer arm position can be influenced by local CHO status in season two. However, we acknowledge that the presence of a girdle blocks the

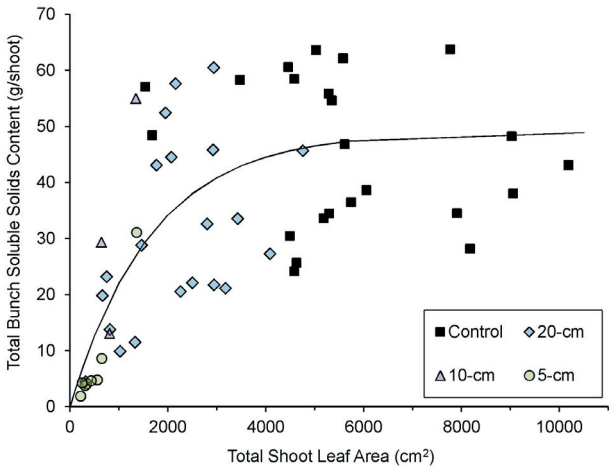

Fig. 5: The relationship between total grape bunch soluble solids content (g·shoot^{-1}) and the total shoot leaf area (main and lateral). The data series was fitted with a single, three-parameter exponential rise to maximum curve, y = 48.9*(1-exp(-0.0006*x)), $R^2 = 0.59$.

effects of all phloem transported metabolites, including CHOs, which may influence inflorescence development (Kehr 2006, Robert and Friml 2009). Based on previous work indicating that the presence of the girdled caused a CHO deficit at the start of season two (Eltom *et al.* 2013), we propose possible explanation for the observed decrease of an outer arm at the basal bunch position in the 5-cm and 10-cm treatments. The first is that a CHO deficit caused the conversion of an outer arm with floral identities into a tendril. The second explanation is that the CHO deficit caused incomplete development of the structure at the outer arm position formed during the previous season. The second explanation is likely correct, as evidence in the literature indicates that floral identities at both the inner and outer arm positions develop in season two of IP development (Srinivasan and Mullins 1981, Morrison 1991, May 2000).

Additionally, a CHO deficit at the start of season two was likely the cause of the observed decrease in the 10-cm and 5-cm treatment bunch numbers from the Control. This suggests that a decrease in CHO availability at the start of season two can cause the abortion of entire pre-formed inflorescence primodia structures at, or shortly after budbreak. This result is consistent with the literature which indicates that the number of inflorescence structures per bud is determined during season one of bud development (Buttrose 1974, Morrison 1991, Mullins *et al.* 1992), which can be further modified during BB of the following season.

Interestingly, there was a greater percentage decrease in the presence of an outer arm in the 10-cm and 5-cm treatments over that in the Control *versus* the percentage decrease in bunch number (60 % *versus* 35 % respectively, Tab. 1). This indicates that the development of a structure with floral identities at the outer arm position is more sensitive to changes in the local CHO status of the vines than the inflorescence primordia as a whole. However, a bunch must be present for an outer arm to be present, meaning that a decrease in bunch number is more detrimental to the overall yield of the vine than a decrease in the presence of an outer arm. Bunch architecture is further affected by the local CHO status of the vine during the initial stages of growth in season two, where an increase or decrease in branching of inflorescence primordia structures occurs, depending on the treatment applied. Previous studies have indicated that the degree of branching of inflorescence primordia is directly correlated to the number of flowers formed (Petrie and Clingeleffer 2005, Dunn and Martin 2007), and that a decrease in CHO availability during IP initiation during season one (and the resulting CHO deficit in season two) can cause a decrease in flower number in season two (Bennett *et al.* 2005). However, we know of no studies which investigate alterations of available CHOs to IP at the start of season two, such as in our study. In our experimental setup, the Control treatment buds had access to a larger share of the CHO resources from the vine's trunk and root system, but to less cane-stored CHOs compared with buds in the 20-cm treatment (Eltom *et al.* 2013). Since the 20-cm treatment buds had access to a greater amount of cane-stored CHOs than those in the Control, and the presence of a girdle would have prevented any export of CHOs to the rest of the vine, a state of cane-stored CHO "excess" compared with that in the Control may have occurred during budbreak, resulting in the observed increased branching for the 20-cm treatment inflorescence structures. Following this logic, the 10-cm and 5-cm treatment buds would have had access to less cane-stored CHOs than the Control and 20-cm treatment buds, creating a potential CHO deficit for the developing 10-cm and 5-cm treatment buds.

In addition to the variation in bunch architecture, the timing of flowering between treatments was also influenced by the girdling treatment applied. Previous work has indicated that the 10-cm and 5-cm treatment shoots had a decreased CHO status at the time of flowering (Eltom *et al.* 2013), which may have been the cause in the delay in flowering. Additionally, vines in the 5-cm treatment were not further delayed in flowering compared to those in the 10-cm treatment, indicating that a minimum CHO threshold in the shoot/vine may be required for the "normal" timing of flowering, and shoots that are below this threshold will encounter a delay in the start of flowering. This finding is consistent with recent literature which indicates that carbohydrates, and their associated biochemical pathways are critical in the timing of flowering (Bernier *et al.* 1993, Srikanth and Schmid 2011, Proveniers 2013, Wahl *et al.* 2013).

Wherein the timing of flowering between treatments may to be due to differences in the CHO status of the treatment shoots, the observed sequence in the start of flowering between all bunch components and bunch positions is intrinsic to all developing grapevine buds. The delay in the start of flowering between bunch positions along a shoot is explained by the acropetal delay in IP development (Snyder 1933, Pratt 1971, Srinivasan and Mullins 1981, Morrison 1991). Possible explanations for the delay between the inner and outer arm bunch components are that the final identity of the outer arm is not determined until season two, causing a further delay in its development, or that the timing of flowering of the outer arm is a function

of the relative difference in berry number between the two structures. Although the duration of flowering between all treatment bunch components was similar (Tab. 2), the duration of flowering on a whole can be very susceptible to changes in temperature and humidity before and during flowering. Lower temperatures before and during flowering can cause flowering to occur over a longer period of time (Staudt 1982, 1999, Friend 2005, Vasconcelos *et al.* 2009, Keller *et al.* 2010). As well, high amounts of humidity can cause flowering to occur over a longer period of time (Cunha *et al.* 2003). In our study, the daily maximum, minimum and average temperatures during flowering were consistent between all treatments, giving further evidence that the CHO status of the shoot, the presence of a girdle, and the shoot's total photosynthetic ability all have a role in the timing of flowering between treatments.

In addition to differences in the timing of flowering between treatments, the local CHO status of the shoot may also affect percentage fruit set (% FS), where previous studies indicate that a decrease in the CHO status of the shoot during flowering can cause a decrease in % FS values (Keller and Koblet 1994, Caspari *et al.* 1998, Lebon *et al.* 2008). However, the 20-cm treatment inflorescences had increased % FS values, despite their decreased CHO status during flowering (Eltom *et al.* 2013). The increase in % FS may likely be a result of the increase in flower number per inflorescence structure, and not an effect of the CHO status of the shoot.

The CHO status of the vine not only affects bunch architecture and the timing of flowering and % FS, but also has an important role in determining SS of the berries at harvest (Brown *et al.* 1988, Caspari *et al.* 1998, Bennett *et al.* 2005). A alteration in the start of flowering for the basal inner arm components in the 20-cm, 10-cm and 5-cm treatments (0, 3, and 3 d after the Control respectively; Tab. 2) does not sufficiently account for the decrease in SS values at harvest (3.6, 5.3 and 10.6 °Brix respectively, Tab. 3). Therefore, it is likely that the decreased photosynthetic capability of the shoots resulted in fewer sugars that could be sequestered by the berries, resulting in a decrease in SS values. Evidence for this is provided in Fig. 5, where the total bunch soluble solids content ($g \cdot shoot^{-1}$) is directly related to total leaf area of a shoot. Additionally, at a leaf area of approximately 4,900 cm^2, the total bunch soluble solids content is at 95 % of the maximal value (48.9 $g \cdot shoot^{-1}$). Increasing the leaf area past this point would have little effect on the bunch soluble solids content on a shoot.

The factors causing differences in berry number between the inner and outer arm components still have to be elucidated. One hypothesis we propose is that temperature during IP branching is a major influencing factor on inflorescence development and branching, where CHO availability is only a modifying factor.

However, for the first time we show that the difference in SS between the inner and outer arm bunch components is related to the relative difference in berry number between the two components. Fig. 3 indicates that the more similar the inner and outer arm are in berry number, the earlier the outer arm starts flowering after the inner arm, resulting in more similar SS values at harvest. However, the total bunch SS is a result of the relative berry numbers of the inner and outer arms and their SS values at harvest. For bunches with a relatively small outer arm component, the SS values between the two components are at their maximum difference (Fig. 3a). However, the total bunch SS is not affected as much, since the outer arm contributes relatively little to the overall bunch (Fig. 4).

Conclusions

In our study, pre-budbreak cane girdling had a direct influence on bunch architecture. When available CHOs to developing shoots were severely restricted, entire preformed inflorescence structures aborted. The final identity of the structure at the primary branch of the rachis can also be influenced by CHO availability early in season two, where shoots in a CHO-restricted state favoured the formation of a tendril rather than an outer arm in the basal bunch position. As well, an "excess" of cane-stored CHOs may be the cause of increased branching of inflorescence primordia structures, which is reflected in the flower number. It was also found that there was a significant delay in flowering between basal and apical bunches as well as between inner and outer arm components. This delay in flowering is hypothesised to cause a decrease in SS between bunch positions and components within a single treatment, and the shoot's overall photosynthetic capability is thought to cause the differences in SS between treatments. As well, the more similar in berry number the outer arm is to the inner arm, the more closely flowering starts between the two components, resulting in more similar SS values at harvest. However, total bunch SS depends on the relative size of the outer arm and its SS value.

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