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ORIGINAL RESEARCH ARTICLE

Limiting carbohydrates to trunk and roots improves bud fruitfulness, fruit set and yield in cv. Malbec

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ABSTRACT

Many commercial vineyards of Malbec, the most cultivated grapevine in Argentina, show unstable yield because of variations in bud fruitfulness and the occurrence of "shatter", characterised by poor fruit set and fruitlet abscission. Shatter can be due to plant material, growing conditions and meteorological events. Among the parameters that determine fruit set efficiency and vine yield, the availability of carbohydrates (CH) plays an essential role. We previously showed that controlling CH partitioning by removing part of the phloem tissue through an annular incision at the base of the fruit shoots (shoot girdling) reduced shatter in Malbec. The objective of this research was to evaluate the partitioning of CH for the different sink organs of the aerial part of the plant when an interruption of phloem flux from leaves to storage organs is imposed by a girdle. Shoot and trunk girdling trials were conducted during the 2018 and 2019 growing seasons, respectively. At flowering, girdling was performed on different plant lots either at the base of the shoot (Base G), above the distal cluster of the shoot (Top G), to the trunk (Trunk G) or no girdling (Control). Most of the yield components were increased by Base G and Trunk G with no significant impact on vegetative growth or fruit quality. Total shoot biomass was not affected in Base G, while a 39 % reduction was observed in Top G, as compared to Control. The partitioning pattern within the shoot was modified by shoot girdling, and Base G favoured the accumulation of CH towards clusters at the expense of lateral shoots. Shoot girdling increased node diameter, bud fruitfulness and inflorescence dry weight of the shoot. Trunk-girdled vines showed an increase in fruit set and total yield at harvest, with no significant impact on vegetative growth. The restriction of CH flow to the trunk and roots by Base G and Trunk G at flowering increased yield components at harvest without affecting vegetative growth or grape quality. These results reinforce our hypothesis that the root system of the vines under study constitutes a strong sink during the flowering period that competes for CH.

KEYWORDS: girdling, yield, Malbec, carbohydrates

INTRODUCTION

Malbec is the most cultivated grapevine in Argentina, and its varietal wines represent 61.4 % of the total bottled wines exported in 2020. In the last 10 years, the exported volume of Malbec wines has increased by 57.4 % (Instituto Nacional de Vitivinicultura, 2021). It is considered a high-quality cultivar with a polyphenolic richness and a high potential to produce quality wines (Fanzone *et al*., 2010); also, it presents high phenotypic plasticity, i.e., the ability to express different phenotypes as a function of the environment (Marfil *et al.*, 2019). It has been described locally as a vigorous cultivar of medium to low productivity susceptible to shatter and millerandage (Rodríguez *et al*., 1999) that is better adapted to high plant density and weak vigour rootstocks (Zuluaga *et al*., 1959). According to Weber (2003), the Malbec cultivar presents limited fruit set efficiency and very low yields. It was introduced in Argentina in 1868 from France, and despite intensive efforts to improve these traits (Ojeda *et al.*, 2001), many commercial Malbec vineyards still present a marked instability of their productive potential. Yield loss is usually associated with three main groups of factors, namely technological practices (vineyard management), biological factors (plant material, pathogens, weeds) and environmental conditions (e.g., weather, soil fertility and water availability). Those factors, individually or combined, may cause low bud fruitfulness, inflorescence necrosis, and shatter. The shatter phenomenon is characterised by loose clusters due to poor fruit set and fruitlet abscission, a common problem of Malbec. Annual production or fruit yield results from the combined performance of the different yield components of a vineyard, such as the number of vines per surface area, the number of buds per vine, the number of shoots per vine (% of sprout), the number of clusters per shoot (fruitfulness), the number of berries per cluster (% of fruit set) and the berry weight (Tassie *et al*., 2001). Among these parameters, the number of clusters per vine explains 60 to 70 % of vine yield annual variation (Clingeleffer *et al.*, 2001), as shown using various cultivars grown in diverse agroecological conditions for a long period of time. The number of clusters per shoot or per plant, known as fruitfulness, is defined during floral induction and differentiation processes. Floral induction begins inside newly formed latent buds around the flowering stage, and differentiation continues over the growing period until the establishment of endodormancy (Srinivasan and Mullins, 1981). Many factors have been identified to affect bud fruitfulness, such as the position of the bud within the shoot, temperature, light, hormone balance, mineral nutrition and CH availability (revised by Vasconcelos *et al*., 2009). The percentage of fruit set represents the ratio between developing berries per cluster and the number of flowers per inflorescence. At flowering, the inflorescences are weak sinks for CH compared to the shoot's apexes and young expanding leaves (Coombe, 1959). The fruit set is dependent on the supply of CH to the inflorescences, determined by the carbon balance between vine reserve status, current photosynthesis, and demand by competing sinks (Zapata *et al*., 2004b). Many researchers have proven that CH availability to shoots and buds during the growing period is critical for bud fruitfulness and fruit yield (Candolfi and Koblet, 1990; Bennett *et al.*, 2005; Caspari *et al*., 1998; Eltom *et al.,* 2014; Eltom *et al.,* 2015; Carrillo *et al.*, 2020). Moreover, CH partitioning, i.e., the distribution of exported CH to the different vine´s sink organs, determines crop yield (Génard *et al*., 2008). Sinks compete for CH supply, and the relative strength of different sinks changes during plant phenology. Generally, after budburst, shoot tips and young leaves are high-priority sinks, whereas inflorescences are low-priority sinks (Coombe, 1959; Koblet, 1969). At bloom and following fruit set, newly formed clusters dominate the sink hierarchy for photoassimilates, especially for nearby leaves (Hale and Weaver, 1962; Williams, 1996). Then, starch accumulation in perennial tissues begins again from fertilisation until the beginning of berry ripening (Mullins *et al*., 1992; Zapata *et al*., 2004a) and reserves are progressively restored (Candolfi-Vasconcelos *et al*., 1994).

Traditional bibliography from the 1990s stated that roots had two peaks of growth, one around anthesis and early fruit development and another one at the time of ripening (Mullins *et al*., 1992; Reimers *et al*., 1994). More recent studies found that most root growth occurred mainly between bloom and veraison (Eissenstat *et al*., 2005). Around bloom, assimilates export from the shoots normally goes to storage reserves (trunk and root) and also to sustain secondary growth (lateral shoots). Normally, secondary growth is limited from budburst to flowering due to the basipetal auxin flow from the shoot tip and young leaves that stimulates internode elongation and inhibits the growth of laterals (Woodward and Bartel, 2005; Mason *et al*., 2014).

Girdling (phloem removal) is an ancient technique proven to be effective in the accumulation of CH in the portion above the incision (Hunter and Ruffner, 2001) and in the increase of the source:sink ratio (Caspari *et al*., 1998). Roper and Williams (1989) found that girdling the trunk of vines shortly after anthesis increased CH's accumulation in leaves and clusters, i.e., above the girdle, at the expense of its partitioning to the roots. Moreover, girdling the trunk of vines at flowering was effective in improving the total fruit set (Coombe, 1959) and yield (Brown *et al*., 1988). Considering vegetative growth, girdling during the flowering–fruit set period has not been shown to alter either the final length of shoots or the number of leaves per shoot (Caspari *et al*., 1998; Carrillo *et al.*, 2020); however, its effect on leaf area is not clear. Regarding yield components, it is not clear whether girdling improved them in entire plants or a compensation effect may appear. Most of the research has been done to enlarge berries and improve the quality of table grapes. In that field, it is demonstrated that girdling the arms or the trunk of vines after fruit set increases the accumulation of Total Soluble Solids (TSS) in berries and improves quality (Basile *et al*., 2018; Crupi *et al*., 2016; Fawzi *et al*., 2019).

Based on our previous research, we propose to evaluate the effect of girdling, both at the shoot and vine level, on CH partitioning, bud fruitfulness, yield components,

vegetative growth and grape quality on field-grown vines. Our hypothesis is that a limitation of CH export to the reserve organs from flowering onwards, imposed by a girdle, increased the availability of CH to the canopy sinks, favouring reproductive to vegetative growth.

MATERIALS AND METHODS

1. Plant material and experimental design

The trials were carried out in a commercial vineyard of *Vitis vinifera* L. cv. Malbec, located in Gualtallary, Mendoza, Argentina (33°26´S, 69°13´W and 1205 m asl) during the 2018/19 and 2019/2020 growing seasons. Vines were 15 years old, grafted onto 1103 Paulsen rootstock, trained in a bilateral cordon (1.3 m between vines N–S oriented rows and 2.5 m between rows), and protected with anti-hail nets (black polyethylene). The parcel was drip irrigated to maintain vines without soil water limitations during the whole experiment. For the different treatments, an area with vines showing homogeneous vigour was selected within the parcel. NDVI images and trunk diameter measurements were used to assess homogeneity (data not shown).

Plants were spur-pruned during winter dormancy to retain eight nodes per arm (16 nodes per plant) and shoot-thinned to 16 fruitful shoots. To evaluate the effect of shoot girdling and trunk girdling, two different types of treatments were performed using two different lots of plants within the same area of the parcel. (1) Girdling of the shoot internode was performed either below the cluster (Base G) or above the apical cluster (Top G) on 14 plants selected in 2018. For each selected plant, additional non-treated shoots (Control) were also selected. At the start of flowering (first flower caps loosening; E-L 19, Coombe, 1995), which occurred in mid-November, the treatments were randomly applied to three shoots per plant arm (i.e., six shoots per plant). In total, each treatment was performed on two shoots, one of each arm of the 14 selected plants (n = 28), as shown in Figure 1. (2) Girdling of the trunk (Trunk G) was performed in 2019 at the start of flowering (E-L 19, Coombe, 1995) on 10 randomly selected plants of similar vigour, whereas an identical number of similar plants were not subjected to the treatment and used as Control (n = 10). All shoot girdles were performed at the midpoint of the internode, whereas trunk girdles were made at 20 cm below the permanent arms. In all cases, girdling was done on the whole circumference of the shoot/trunk using a specific tool made of two blades separated by 3 millimetres. This tool allows one to extract the phloem down to the xylem completely.

1.1. Shoot girdling trial

1.1.1. Shoot´s CH partitioning, node´s diameter, sugars and bud fruitfulness

At harvest, when Control berries reached 24 °Brix (measured with a Pocket PAL-1 digital hand-held refractometer; Atago, Tokyo, Japan), 5 shoots per treatment were cut from the base and placed in a sealed plastic bag. Then, the different shoot organs were separated (leaves, laterals, clusters and cane) and placed in the stove at 65 °C until constant dry weight (DW). Shoot´s CH partitioning to the different organs was expressed as the proportion of each organ DW in relation to Total shoot DW (sum of leaves, laterals, clusters and cane DW).

During winter dormancy, 10 treated canes per treatment were collected and used for the evaluation of nodes diameter, sugars and bud fruitfulness along the shoot. The diameters of the nodes in positions 1 to 10 were measured with a digital calliper. Following, the canes were divided into single node cuttings (SNC) from node positions 1 to 10. The SNCs derived from half of the canes were ground with an analytical mill (IKA A11, Staufen, Germany), and the powder was kept in the freezer until analysis. Total soluble carbohydrates (TSC) were extracted by percolation with 80 % ethanol, and starch was extracted by percolation with 35 % perchloric acid. Antrone reagent was added to both extracts and analysed by spectrophotometry using the protocols of Sydney University (Hansen and Møller, 1975). The SNCs from the other half of the canes were randomly placed in trays, with perlite as substrate and kept in a growth chamber. Daytime and night-time temperatures were maintained at 23±1 °C and 19±1 °C, respectively, with day length fixed at 12 hours. Trays were irrigated every two days with regular water.

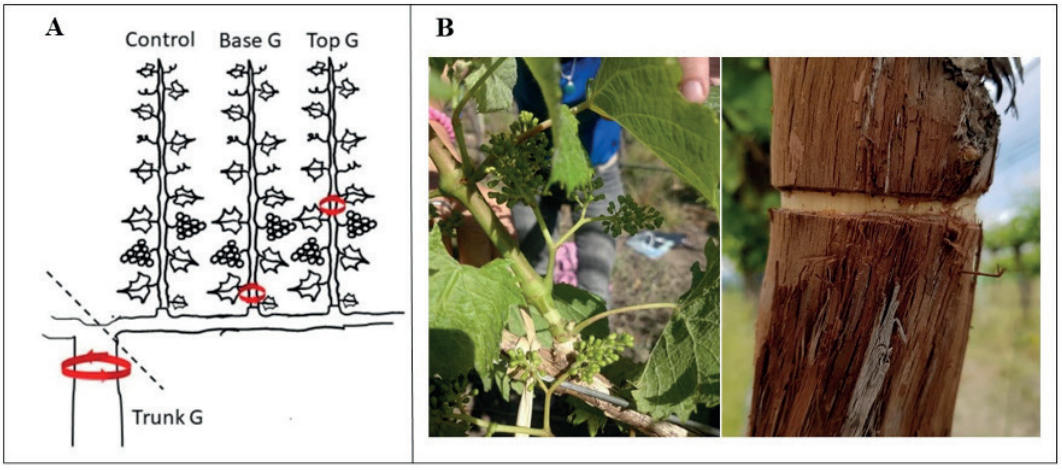


FIGURE 1. Schematic representation (A) and images (B) of shoot girdling and trunk girdling treatments.

Leaves were removed from the SNCs when appearing, and the number of inflorescences per node was counted (modified from Candolfi and Koblet, 1990). Bud fruitfulness along the basal 10 nodes per shoot and the mean shoot fruitfulness were calculated. When the inflorescences reached stage 17 (single flowers separated; Coombe, 1995), they were cut and dried in the stove at 60 °C until constant weight. The mean shoot inflorescence DW was assessed by the mean DW of each individual inflorescence present in nodes 1 to 10 of treated shoots.

1.1.2. Vegetative growth, fruit yield and quality components

The shoot length and the number of leaves per shoot were assessed at veraison, 67 days after flowering (E-L 36, Coombe, 1995). Leaf area (LA) of the 2nd, 4th and 8th leaf from the base of the shoot was non-destructively estimated by measuring the leaf central vein length, as described by Berli *et al*. (2013), also at veraison. Chlorophyll relative content (CRC) was assessed in the same leaves and dates as LA with a SPAD 502 (Konica Minolta, Osaka, Japan).

The clusters were harvested when the total soluble solids (TSS) of Control berries reached 24 °Brix (refractometer described above). Cluster fresh weight (FW) and the number of berries per cluster were determined. Berries within a cluster were sieved using a strainer and classified as normal (≥12 mm diameter) and small (<12 mm diameter). For each group, the total berries FW and the number of berries were recorded. In parallel, according to the proportion of normal and small berries within a cluster, subsamples of 15 berries were used to evaluate total soluble solids (TSS) and phenolic compounds (anthocyanins and total polyphenols) concentration. Phenolic extractions were done as described in Berli *et al*. (2008), but instead of using a fixed volume of the extraction solution, we adjusted it with the volume of the berries subsample.

1.2. Trunk girdling trial

1.2.1. Fruit set, fruit yield, quality components and vegetative growth

To measure the fruit set, gauze bags were placed over two basal inflorescences per plant (n = 20) from the beginning of flowering up to one month after the fruit set to collect the fallen cups. The number of flower cups was assessed as described by Keller *et al*. (2010) and the fruit set (proportion of flowers that set a berry) was calculated by counting the final number of berries at harvest.

All clusters per plant were harvested when the Control berries reached 24 °Brix (refractometer described above) in mid-March. The number of clusters per plant was counted, and fruit yield was measured in the field with a portable digital scale. Four clusters per plant were randomly selected, placed in plastic bags and transported to the laboratory in a portable cooler. Clusters were freshly weighed, and the number of berries per cluster was counted. The number and weight of normal and small berries and quality components were determined as described for the shoot girdling treatment.

At veraison (E-L 36, Coombe, 1995), one shoot per plant was randomly selected to measure the total shoot length and the number of leaves per shoot (n = 10). Moreover, total shoot LA was estimated through the measurement of the central vein length of all the leaves, as described by Berli *et al*. (2013). During winter dormancy, plants were spur-pruned, and the pruning weight registered as a measurement of the growing season's vegetative growth.

1.3. Statistical analysis

The effect of shoot and trunk girdling treatments was evaluated against the Control by one-way ANOVA and Fisher's multiple comparisons test (InfoStat version 2017; Grupo InfoStat, Universidad Nacional de Córdoba, Argentina). Non-normally distributed data were analysed with Fisher´s generalised linear model (GLM).

RESULTS

1. Shoot girdling

As shown in Figure 2, a 42.8 % reduction in total shoot DW was observed in Top G as compared to Control shoots, whereas no differences were perceptible between Base G and Control. However, the shoot girdling treatments changed the distribution of biomass between the different shoot organs. In Control shoots, clusters represent 40.3 % of the total shoot DW, canes 26.8 %, whereas leaves and laterals account for 15.4 % and 17.4 %, respectively. Base G treatment increased the proportion of clusters DW in the total shoot DW, representing 60.4 %, while laterals contribution to the total shoot biomass was reduced to 6.5 %; leaves and canes were not affected.

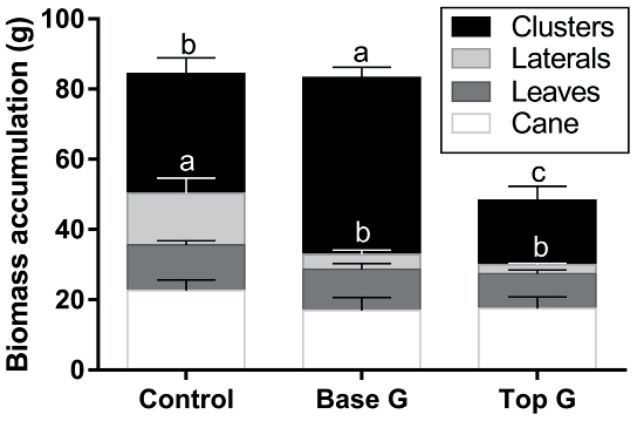


FIGURE 2. Total shoot biomass accumulation and distribution between different organs.

Values are means +/- SEM (n = 5), and different letters within each organs indicate statistically significant differences between treatments (Fisher's LSD, $p \leq 0.05$).

Node diameter (ND) of Control shoots did not significantly vary all along the shoot, although a limited but constant decrease was observed from the bottom to the top. (Figure 3). The evolution of ND was strongly affected after girdling, with a strong impact on the node located immediately above the girdle.

In the Top G shoots, ND increased at node 5, located just above the girdle, with no further variation from node 6 up to node 10. Similarly, in Base G shoots, a strong increase in ND was observed at node 3, also located just above the girdle, and no significant variations were measured up to node 10.

Figure 4 shows that the position of the bud had a marked influence on its fruitfulness (Figure 4A) and that both girdling treatments raised the mean bud fruitfulness along the shoot (1–10 nodes) as compared to Control (Figure 4B). For Control shoots, bud fruitfulness increased from node 3 upward. Base G shoots resulted in an increase in node 3 fruitfulness, with a similar trend at node 4, not significant, though. For Top G, bud fruitfulness tended to increase progressively along the cane up to node 8, where it was statistically significantly higher than in the Control shoots.

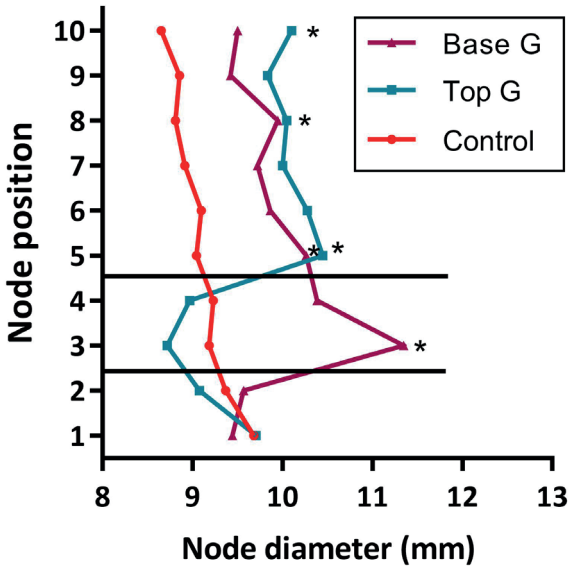


FIGURE 3. Node diameter along the shoot as measured in the treated (Base G and Top G) and Control during winter dormancy.

Values are means (n = 10) and asterisks represent significant differences between treatments for each node position as compared with the Control (*p value < 0.1; Fisher's test). Lines between nodes 2–3 and 4–5 indicate the position of the girdles in Base G and Top G, respectively.

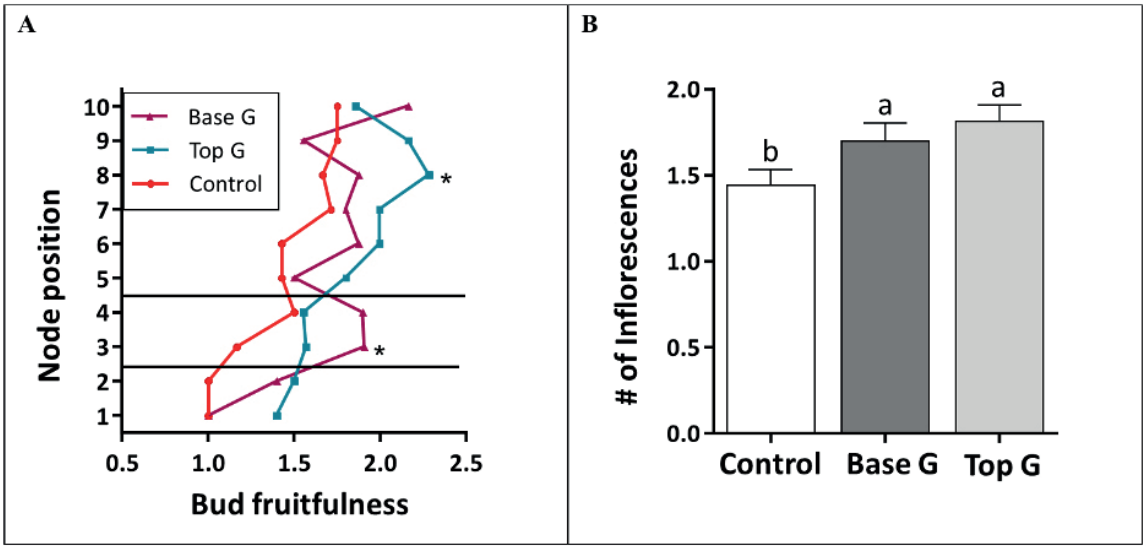


FIGURE 4. Bud fruitfulness along the shoot measured by single-node cuttings cultivation (A) and mean bud fruitfulness along the shoot (B).

Statistically significant differences were analysed with Fisher's GLM comparing each treatment with the Control, n = 5 (*α < 0.05). Lines between nodes 2–3 and 4–5 indicate the position of the girdles in Base G and Top G, respectively.

The mean shoot inflorescence DW for Control shoots was close to 20 mg. Girdling treatments resulted in a 58 % and 62 % increase for Top G and Base G, respectively (Figure 5).

In all conditions tested (Control, Base G and Top G), LA measured at veraison increased from the base to the middle of shoots (p (leaf) < 0.0001), with no significant difference between treatments. Chlorophyll Relative Content was similar for all treatments at leaves 2 and 4; however, it was reduced by girdling treatments at leaf 8 (Table 1).

TSC at winter dormancy, measured in the same nodes as leaves, varied depending on the node position relative to the girdle (p ($_{node*Treatment}$) = 0.0254). That is, TSC in node 2 were higher for Top G than for Base G, whereas in node 4, they were lower. Node 8 did not present differences in TSC due to girdling treatments (Table 1). Starch accumulation in the nodes was unaffected by treatments (data not shown).

Table 2 shows that the number of berries per cluster and the cluster FW were increased in Base G shoots as compared to Control.

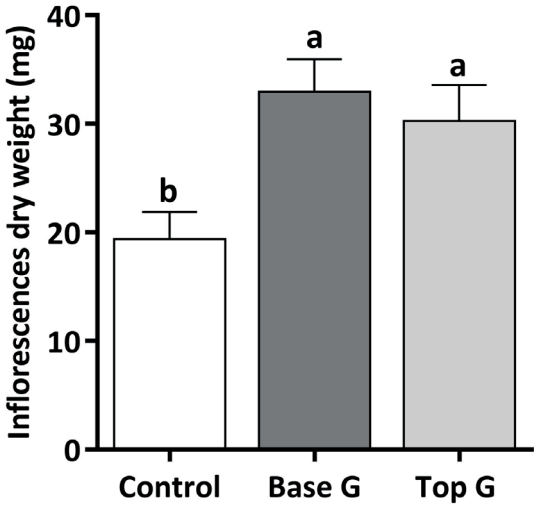


FIGURE 5. Shoot average inflorescences dry weight, normalised per node, evaluated in 2018.

Statistically significant differences were analysed with Fisher multiple comparisons test, comparing each treatment with the Control, n = 5 (p = 0.0371).

TABLE 1. Leaf area (LA), chlorophyll relative content (CRC) and total soluble carbohydrates (TSC) at different node positions (2nd, 4th and 8th) in Malbec treated shoots (Control, Base G, Top G). Values are means and different letters within each column indicate statistically significant differences (Fisher's LSD, p ≤ 0.05).

		L A (cm2)			CRC (%)			TSC (mg/g DW)		
		leaf 2	leaf 4	leaf 8	leaf 2	leaf 4	leaf 8	node 2	node 4	node 8
Treatments	Control	47.59 a	123.20 b	152.49 c	38.12 a	39.06 a	41.32 a	226.61 abc	243.44 ab	221.37 abc
	Base G	46.75 a	121.66 b	145.33 c	30.08 a	31.32 a	30.50 b	169.99 c	248.75 a	216.82 abc
	Top G	53.22 a	127.04 b	150.63 c	35.04 a	32.88 a	35.75 b	249.92 a	186.13 bc	240.20 ab
ANOVA	$P_{(Treatment)}$	0.8798	0.8560	0.1696	0.2737	0.3368	0.004	0.0343	0.0890	0.7624
	$P_{(Leaf/Node*Treatment)}$		0.9558			0.9523			0.0254	

TABLE 2. Yield components in Control, Base G and Top G shoots. Values are means of 14 replicates; different letters within each treatment and column indicate a statistically significant difference (Fisher's LSD, α ≤ 0.05).

		# berries per cluster	Cluster FW (g)	# normal berries	Normal berries FW (g)	# small berries	Small berries FW (g)
Treatments	Control	32.69 b	53.33 b	25.69 a	45.96 a	7.00 b	4.19 b
	Base G	73.09 a	89.81 a	34.13 a	59.77 a	38.96 a	19.04 a
	Top G	34.06 b	53.34 b	26.12 a	46.18 a	7.94 b	4.37 b
ANOVA	$P_{(Treatment)}$	0.0001	0.0001	0.123	0.1815	0.0001	0.0001

TABLE 3. Berry total soluble solids (TSS) and phenolic compounds (anthocyanins and total polyphenols) concentration at harvest. Treatments: Control, Base G and Top G. Values are means and different letters within each factor and column indicate statistically significant differences (Fisher's LSD, $\alpha \leq 0.05$).

		TSS		Anthocyanins		Total Polyphenols	
		(mg 100 mg-1 berries)		DO520 g-1 skin DW		DO280 g-1 skin DW	
Treatments	Control	25.18	a	21.28	b	15.83	b
	Base G	22.03	b	37.53	a	25.65	a
	Top G	21.58	b	24.85	b	18.67	b
ANOVA	$P_{(Treatment)}$	0.0001		0.0290		0.0876	

Clusters from Base G shoots raised 124 % and 68 % of the number and FW of berries, respectively. Considering the proportion of small berries (< 12 mm), their number and FW increased in Base G shoots only, whereas normal berries (≥12 mm) were in similar quantities in clusters of all treatments. Yield components of clusters from the Top G shoot were not modified by girdling.

TSS of Control clusters at harvest were around 25 °Brix, and girdling treatments reduced its accumulation (Table 3). Anthocyanins and Total polyphenols, both expressed on a concentration basis, were increased in Base G berries (Table 3).

2. Trunk girdling

Yield components were markedly improved by Trunk G (Table 4). More specifically, the percentage of fruit set, the number of berries per cluster and cluster FW increased 62 %, 57 % and 74 %, respectively, as compared to Control. The percentage of fruitlet abscission was reduced by 21.64 % by Trunk G. The number of clusters per shoot didn´t show differences for any treatment. In addition, Trunk G clusters showed a higher number of small berries (+114 %) and a higher normal and small berries FW (+68 % and +98 %, respectively); therefore, girdled vines produced an average of one more kilogram of fruit per plant than Control vines.

TABLE 4. Yield components in Control and Trunk G plants. Values are means of 20 replicates; different letters within each treatment and column indicate a statistically significant difference (Fisher's LSD, $\alpha \leq 0.05$).

	Fruit set (%)		# berries per cluster		Cluster FW (g)		# clusters per shoot		# normal berries		Normal berries FW		# small berries		Small berries FW		Yield per plant (kg)	
								Treatments										
Control	33.98	b	35.72	b	50.37	b	1.67	a	27.89	a	43.96	b	8.29	b	5.36	b	1.86	b
Trunk G	55.13	a	56.24	a	87.75	a	1.93	a	38.47	a	73.66	a	17.76	a	10.59	a	2.83	a
								ANOVA										
$P_{(Treatment)}$	0.0030		0.0137		0.0088		0.1704		0.1562		0.0309		0.0175		0.0278		0.0060	

TABLE 5. Vegetative growth measured at veraison and during winter dormancy; vine balance indexes in Control and Trunk G plants. Values are means of 10 replicates; different letters within each treatment and column indicate a statistically significant difference (Fisher's LSD, $\alpha \leq 0.05$).

		Shoot Length (cm)		Number of leaves		Vine LA (cm^2)		Pruning weight (g)		Ravaz Index		LA/Fruit (cm^2/g)	
Treatments	Control	113.32	a	22.11	a	40180.79	a	507.2	a	3.85	a	26.04	a
	Trunk G	122.56	a	22.67	a	50744.92	a	573.06	a	5.23	a	16.16	b
ANOVA	$P_{(Treatment)}$	0.3224		0.7945		0.1597		0.4052		0.1846		0.0207	

TABLE 6. Berry total soluble solids (TSS) and phenolic compounds (anthocyanins and total polyphenols) concentration at harvest. Treatments: Control, Trunk G. Values are means and different letters within each factor and column indicate statistically significant differences (Fisher's LSD, $p \leq 0.05$).

		TSS		Anthocyanins		Total Polyphenols	
		(mg 100 mg-1 berries)		DO520 g-1 skin DW		DO280 g-1 skin DW	
Treatments	Control	25.33	a	20.86	a	34.70	a
	Trunk G	24.70	a	24.02	a	32.53	a
ANOVA	$P_{(Treatment)}$	0.3591		0.5121		0.7493	

Vegetative growth variables measured at veraison were not affected by girdling, although some parameters tend to increase (Table 5). For example, shoot at the end of the vegetative cycle were 113 cm and 122 cm in length in Control and Trunk G vines, respectively, and carried a similar number of 22 ± 1.5 leaves. Total vine LA and pruning weight tended to be higher in Trunk G than in Control vines, but differences were not statistically significant. However, the ratio between LA/fruit (cm2/g) was 1.6-fold lower in Trunk G vines.

TSS for Control vines at harvest were 25.3 °Brix, while for Trunk G, they were 24.7 °Brix, although differences were not statistically significant (Table 6). In addition, secondary metabolites like anthocyanins and total polyphenols were similar in berries of girdled and control vines.

DISCUSSION

The effect of shoot girdling at different positions on shatter, vegetative growth and grape quality was evaluated in 2016 and 2017 (Carrillo *et al.*, 2020). To deepen the study of girdling in relation to bud fruitfulness, carbohydrate partitioning and at the whole plant level, two trials were conducted in 2018 and 2019: Shoot and Trunk girdling, respectively.

The vine holds a balanced system of assimilates allocation based on a ranking of sink priority (Edson *et al*., 1995). Girdling the base of shoots was consistent in its effect on the increase in cluster weight, mainly due to an increase in the number of berries set. In Base G, a significant rise in the proportion of small berries was observed. Earlier research has shown that girdling shoots at flowering has a significant effect on fruit set, berry number and cluster weight (Coombe, 1959; Caspari *et al*., 1998; Carrillo *et al.*, 2020). Base G did not affect the total DW of the shoot but changed the CH distribution pattern, favouring the partitioning to clusters at the expense of lateral shoots. In correspondence, other authors showed that an increase in crop load enhances assimilates supply to reproductive growth over vegetative growth, especially root growth, but maintains total vine biomass (Petrie *et al*., 2000c; Williams, 1996).

In Top G shoots, the significant reduction of total DW was essentially due to clusters that showed a 49 % reduction in DW as compared to the control. Edson *et al*. (1995), using potted girdled vines, found that crop load induced shifts in the relative DW of the vine´s different organs but no differences in total DW. In our conditions, clusters in Top G were fed by the four basal leaves of the shoot and had to compete for CH with the trunk and roots, which may be responsible for the major CH allocation. Primary vegetative growth, represented by leaves and canes, was not modified by girdling (Top G and Base G), while secondary growth, represented by laterals, was higher in Control shoots. From budburst to bloom, the apex and young leaves synthesise auxins that prevent prompt buds from elongating (Woodward and Bartel, 2005). In control shoots, auxins and hormones are transported from the apices to the roots through the phloem unrestrictedly, while in girdled shoots, phloem interruption interferes with auxin flux basipetally. This may explain the limited lateral development after bloom, possibly by an overaccumulation of auxins in Base G and Top G and not solely by CH partitioning, in accordance with (Bangerth, 2000).

Inflorescence primordia initiation and differentiation occur in the latent buds around 2 weeks before and up to 4 weeks after flowering depending on bud position, inflorescence position and the cultivar; bud fertility is highly dependent on photo-assimilates availability (revised by Vasconcelos *et al*., 2009). Girdling at flowering increased the diameter of the node and bud fertility above the girdle. Consequently, total shoot fertility and mean inflorescence DW increased with girdling treatments. These results confirmed what was previously reported by Eltom *et al*. (2014), who found that girdling shoots immediately after the fruit set increased cross-sectional shoot area and raised the proportion of inflorescences with an outer arm at any cane node number, resulting in an increase in inflorescence size. The position of the leaves along the shoot had a strong influence on their size; LA increased from leaf 2 to 8, as expected. On the contrary, girdling treatments did not affect the LA of the leaves immediately below and above the girdles. Probably, because treatments were applied at flowering when the basal portion of the shoots was fully developed and the first eight leaves completely expanded. The CRC of the 8th leaf of girdled shoots (above the girdle) at veraison was reduced as compared to Control. Girdling imposed a phloem blockage from the upper leaves to the permanent and storage organs, possibly generating an oversupply situation in those leaves, which would cause a decrease in net photosynthesis due to inhibition (Hunter and Ruffner, 2001). By that time, leaves above the girdles presented a reddish colour, indicating that the excess of sugars was being used for anthocyanins biosynthesis (Figure S1). In addition, girdling treatments exclude the permanent storage organs (arms, trunk and roots) as sinks, increasing the source:sink ratio and possibly accelerating leaf senescence. Petrie *et al*. (2000a) found that leaves of vines with a high source:sink ratio decreased in chlorophyll content more rapidly than leaves of the low source-to-sink ratio treatments.

Variations in STC at winter dormancy were observed, depending on the position of the node relative to the girdle. In Base G, node 2, located just below the girdle, had a reduction in TSC as compared to Top G. In contrast, Top G presented a reduced TSC at node 4, which in this case, is also located just below the girdle, but no effect was observed for Base G nodes at this position. A possible explanation is that the permanent storage organs (root and trunk) pose a strong demand for sugars that can only be supplied in girdled shoots by the leaves below the girdles. This hypothesis is reinforced by a large body of literature that has shown that a major part of total seasonally assimilated CH is incorporated into structural cellulose compounds in roots, stems and shoots (Winkler and Williams, 1938) and that roots are the most important sites of accumulation of CH in terms of vine reserves (Bates *et al*., 2002; Winkler and Williams, 1945). No effect due to girdling treatments was observed at node 8.

These data are consistent with a local effect of girdling on CH accumulation in the shoot and its consequences on fruit development.

Berry TSS were decreased by girdling treatments, but Base G increased total anthocyanins on a concentration basis. The reduction in TSS may be explained by the significant increase in cluster FW in Base G shoots (68 %). Dokoozlian *et al*. (1995) also who found a lower fruit soluble solids content in vines girdled at fruit set due to a larger berry size and greater total yield. In our study, we found that girdled shoots and plants presented an important rise in the number of small berries. When berry size is decreased, or a major proportion of small berries per cluster are found, a higher skin/pulp ratio is obtained. This may increase the anthocyanins concentration since those compounds are accumulated in the skin of red cultivars. Ojeda *et al*. (2002) found that berry size reduction increased the skin/pulp weight of Syrah grapes and, consequently, the concentration of the different phenolic compounds within the berry skin.

Trunk girdling is an old/ancient technique used principally on table grapes to increase berry size and improve ripening. We found that trunk girdling at flowering significantly increased most of the yield components in a wine grapevine cultivar. Girdled vines produced an average of one more kilogram of fruit per plant, representing a gain of 3 t/ha (5.72 t/ha for the Control vs. 8.7 t/ha for the Trunk G). As observed in Base G shoots, there was a rise in the proportion of small berries within the clusters. This effect was previously shown in a two-season trial with the same cultivar (Carrillo *et al*., 2020) and coincided with what was previously observed by other authors (Coombe, 1959). Probably, the accumulation of auxins that cannot move basipetally by the time of berry setting would control fruitlet abscission since they prevent the formation of the abscission zone within the pedicel by decreasing ethylene sensitivity (Kühn *et al*., 2016). Moreover, in grapevines, roots are the more important sites of accumulation of CH in terms of vine reserves (Bates *et al*., 2002). Preventing the flux of phloem to the roots by girdling improved yield components through greater availability of CH. Loescher *et al*. (1990) showed that trunk girdling temporarily increased shoot starch concentrations during the first 31 days after treatment. The number of clusters per vine, defined previously to the application of girdling, did not present differences between Control and Trunk G vines.

Berry TSS, anthocyanins and total polyphenols were not affected by girdling despite the 57 % increase in berry number and 74 % increase in cluster FW for this treatment. Elsabagh (2010) showed that two different types of girdling did not significantly affect the TSS in Alfonso Lavalle cultivar. We assume that the greater accumulation of CH in the aerial part of the plant, at the expense of the root system and the trunk, was sufficient to achieve maturity despite the remarkable rise in yield.

Vegetative growth variables during the growing cycle were not affected by girdling. However, indexes that indicate vine balance was improved in Trunk G. Ravaz Index, which expresses the relationship between fruit yield and pruning weight, shifted from 3.85 for Control vines to 5.23 for girdled ones. Although not statistically significant, the tendency reflects a better-balanced vine. Ravaz (1911) established an optimal interval for his index, between 5 and 10, to indicate that a vine is balanced and capable of achieving both fruit quality and consistent production. Other authors have pointed out that vine balance may also be expressed as the amount of leaf area required to ripen a unit of crop weight, commonly expressed as LA (cm2)/crop FW (g) (Kliewer and Dokoozlian, 2005; Howell, 2001; Petrie *et al*., 2000b). In our study, we found that Trunk G vines significantly reduced LA/crop FW ratio, 16.16 cm2/g vs. 26.04 cm2/g for Control ones. Values for this index vary depending on the trellis system; for single canopy vertically shoot positioned vines, authors report a range of 7 to 14 cm2/g and 0.8 to 1.2 m2/kg to achieve balanced yields and quality and to attain long-term sustainable viticulture (Kliewer and Dokoozlian, 2005; Howell, 2001).

Numerous studies have evaluated the impact of girdling on different variables of table grape production. In turn, there are some studies that have used girdling to cause an interruption in the flow of CH at the shoot level and evaluate its consequences. However, there is virtually no research that has compared the effects of phloem flow interruption at the shoot and whole plant level on the same cultivar and under field conditions. During three consecutive seasons, it was demonstrated that girdling the base of shoots at flowering increases the percentage of fruit set, the number of berries per cluster and the final cluster, notably decreasing shatter in Malbec cultivar. These results allowed us to hypothesise that there was a strong demand for CH, during the flowering/fruit set and fruit growth period, from the reserve organs of our studied case (plants). When trunk girdling was applied, we were able to confirm our hypothesis as those results were replicated. However, it is interesting to note some differences between the two trials. If we consider the percentage increase in the number of berries, it is observed that Base G produced a raise of 124 % vs. 57 % for Trunk G. This difference, more than doubled, was not transferred to the cluster weight at harvest, which showed an increase of 68 % for Base G and 74 % for Trunk G. This can be explained by the proportion of large and small berries present in the cluster. Although both treatments showed a marked increase in the proportion of small berries and their corresponding fresh weight, Trunk G produced a 38 % increase (30 % vs. 68 %) in the fresh weight of normal berries.

CONCLUSION

The girdling allowed to modify of the flow of CH within the shoot and plant and the partitioning of carbon between the various sink organs. When the complete shoot is isolated from the reserve organs, as occurs in Base G, carbon partitioning towards the clusters is favoured, increasing berry number and weight without affecting quality. The greater number of small berries retained in the cluster and the reduced development of laterals indicate that phloem disruption may affect not only

the flow of photoassimilates but also the natural movement of phytohormones and other metabolites.

Trunk girdling reinforces our hypothesis that the root system of the vines under study constitutes a strong sink during the flowering period that competes for CH. The remarkable increases in yield components observed in Trunk G demonstrate that the results previously found at the shoot level are replicated at the whole plant level. Further studies will be necessary to evaluate the effects of phytohormones and to determine the possible adverse effects of successive trunk girdling on reserve accumulation and plant sustainability.

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