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Effects of mechanical box pruning intensity on bud development, vegetative growth, and yield components on cv. Cabernet-Sauvignon in Mendoza, Argentina

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ABSTRACT

Mechanised winter pruning is increasingly being used to reduce the high costs of manual pruning. Mechanised pruning is non-selective, and for its optimal application, the definition of pruning intensity is necessary to achieve the target yield and grape quality. Our objectives were to evaluate the effects of three intensities of mechanical box pruning compared to a manual pruning treatment on spur length, the number of retained buds, budburst, shoot growth, and development; yield components and berry composition, and vine balance. Treatments corresponded to three mechanical pruning intensities leading to different bud loads at the beginning of the experiment by pruning at different distances from the cordon (i.e., box size): MP1 (1 cm height × 7 cm width), MP2 (14 cm × 14 cm), and MP3 (21 cm × 21 cm). MP2 treatment was also compared to a traditional manual pruning treatment (SP) that was pruned to the same bud/plant in the first season. In the first season, MP2 retained the same bud/plant as SP, but in the second and third seasons, MP2 retained 88 % and 100 % more buds/plant than SP, respectively. Accordingly, MP2 and SP showed no difference in yields in the first season, but in the second season, MP2 showed a 39 % higher yield than SP. Regarding the intensity of mechanical pruning, after treatments were applied, the number of retained buds increased as box size increased. Treatments modified the length of spurs, budburst percentage and their pattern. In the first season, the yield on the highest box (MP3) was four times higher than the smallest box (MP1), but they showed similar yields in the second season. The intensity of mechanical box pruning, applied after two seasons, generated regulatory and compensation mechanisms (i.e., lower budburst and cluster weight), leading to similar yields independently of the pruning intensity. Our results show that (i) after two seasons, mechanical pruning was more productive than manual pruning, and (ii) mechanical pruning intensity had a marked influence on yield only during the first year of application, while some compensation mechanisms among yield components equilibrate yield afterwards.

KEYWORDS: manual pruning, mechanisation, training system, box size, retained buds, berry composition, balance index

INTRODUCTION

Dormant pruning and harvest, when performed manually, represent the highest operating costs within vineyard management. In addition, dormant pruning requires skilled manual labour, which is now difficult to find in many viticultural regions (Battistella *et al*., 2013). Full vineyard mechanisation reduces costs, labour requirements and allows for more rapid management interventions (Pezzi and Bordini, 2008; Kurtural *et al*., 2012). The mechanisation of pruning and harvesting, which is expanding in several wine regions around the world, requires special attention regarding vineyard design, training systems, canopy management, and variety adaptation to generate a canopy adapted and compatible with the machinery available, to maintain high production and quality levels (Kurtural and Fidelibus, 2021). Currently, in Argentina, vineyards are managed with high labour intensity; mechanisation has been implemented for harvesting in large vineyards, while pruning remains manual or is only partially done by pre-pruning.

Manual pruning is selective; it allows precise control of the number of nodes in relation to plant vigour, cultivar, and management criteria (Greven *et al*., 2014), although it has been associated with excessive pruning intensity (Poni *et al*., 2016). In contrast, mechanical pruning is non-selective; it is less likely to control bud load precisely and can lead to over-cropping in relation to plant growth capacity. In grapevine, several studies have compared mechanical vs manual pruning in productive terms. Some authors have found that mechanical pruning increases yield after several years of application, mainly related to a higher number of clusters (De Toda and Sancha, 1999; Peppi *et al*., 2017), while others did not find differences associated with a partial compensation through budburst percentage and other yield components (Gatti *et al*., 2011; Caprara and Pezzi, 2013). Mechanised pruning can lead to excessive production with a marked decrease in plant vigour and yield components, such as budburst, shoot fertility, and number of berries per cluster in the following seasons (Poni *et al.*, 2016). Moreover, mechanical box pruning applied with different intensities may modify canopy architecture through changes in spur number and length and altering shoot number and fertility, with consequences on yield components (McLoughlin *et al.,* 2011; Kurtural *et al*., 2012). In consequence, mechanical pruning intensity requires special consideration because it will determine spur length, producing variations in microclimate, and, if not well performed, it could lead to different inter-annual node numbers and, consequently, to variations in yield. (Poni *et al*., 2004; Peppi *et al*., 2017).

The expansion of mechanical box pruning is limited by, among other factors, the use of training systems that are not appropriate for mechanical pruners, inadequate support structures and insufficient knowledge about the management required to maintain productive canopies and grape quality over the lifespan of the vineyard (Poni *et al*., 2004; Palliotti, 2012; Caprara and Pezzi, 2013). In this study, we evaluated the effects of three pruning intensities on cv. Cabernet-Sauvignon trained in a single wire system and mechanically box pruned. A manual spur pruning treatment was included with a similar bud load to a mechanical treatment to compare both methods. The canopy of mechanically pruned vines was trained with no catch wires (i.e., a sprawling canopy), while manually pruned vines were trained to a conventional vertically shoot-positioned (VSP) system. Our objectives were to evaluate the effects of mechanical box pruning intensity on (i) spur length, bud number retained, budburst, shoot growth and development, (ii) yield components and berry composition, and (iii) vine balance.

MATERIALS AND METHODS

1. Site and plant material

The experiment was carried out during two consecutive growing seasons, from winter 2017 to winter pruning 2019, in a commercial vineyard cv. Cabernet-Sauvignon is located in Rivadavia (33° 17´S, 68° 25´ W, 671 m a.s.l.), Mendoza, Argentina. Vines were planted in 2000, trained in a vertically shoot-positioned (VSP) system, and spur pruned to a bilateral cordon. The permanent cordon was 0.9 m above ground, and shoots were trellised with three catch wires to form a canopy of 1.2 m height from the permanent cordon. Rows were N-S oriented, and plants were spaced 2.0 m between each other and 2.5 m between rows. The climate is arid, with 196 mm of annual rainfall concentrated mostly during the summer. The Heliothermal Index of Huglin (HI) and Cool Night Index (CI) estimated for the 1996–2019 period classified the region as "warm" with a value in HI of 2835 and "cool nights" with a value in CI of 13.1 ºC (Tonietto and Carbonneau, 2004). Vines were irrigated with a single drip line per row, with emitters (2 L/h) spaced at 0.6 m. Irrigation was managed to restore 100 % of estimated crop evapotranspiration during whole growing seasons.

2. Treatments and experimental design

From winter 2017 to 2019, four pruning treatments were applied: one manual spur pruning and three mechanical box pruning systems. Spur pruned (SP) vines were manually pruned to retain 36 buds and 18 spurs per meter of row (i.e., 14 buds/m^2 and 7 spurs/m^2). The canopy was trained to VSP, and shoot trimming was performed twice per season at 20 cm above the last catch wire. The mechanical pruning treatments consisted of three different box pruning sizes: MP1 (1 cm height × 7 cm width), MP2 (14 cm height × 14 cm width), and MP3 (21 cm height × 21 cm width). This scheme allowed us to compare the three mechanically pruned intensities among them and, at the same time, since SP and MP2 retained the same bud number per m^2, we could compare mechanical vs manual pruning at the same bud load. In mechanically pruned vines, summer pruning (once per season) was applied to trim shoots extending 40 cm above the ground to allow weed control and machinery transit. The same box pruning treatments were applied to the same vines over three consecutive winters.

The experiment was outlined in a completely randomised block design with five replicates. The blocks were selected according to previous measurements of trunk diameter.

Each replicate consisted of 12 plants distributed over four consecutive rows (3 plants per row), and the central vines from the two central rows were used for data collection.

3. Buds retained after pruning and their posterior development

After pruning, spur number, length, and node number per spur were recorded in two vines per replicate. The total node number per m^2 was calculated. Three weeks prior to flowering, we selected 10 and 15 contiguous spurs on a cordon of one vine per replicate in 2017–2018 and 2018–2019, respectively. For each spur, we noted the node position from the spur base to the last node and classified them as blind when budburst did not occur, fruitful when the bud developed a shoot with at least one inflorescence, and unfruitful when the bud developed a vegetative shoot without inflorescences (Di Lorenzo and Pisciotta, 2019). The frequency of each node type was then estimated for each node position. Spurs with five nodes were scarce and thus not considered. In addition, the number of fruitful and unfruitful shoots was counted along the entire cordon, considering their position in the plant (old wood, base of spur, and node of spur) in one vine per replicate. Before pruning, the total number of shoots per plant was counted in two vines per replicate, and budburst was expressed as the percentage of the total number of shoots divided by the total node number retained by the previous pruning.

4. Leaf area and vegetative growth

The leaf area index (LAI, m^2/m^2) per plant was determined after harvest on one vine per replicate. Leaves were extracted from the southern half of one plant per replicate, weighted immediately, and a subsample of 20 leaves was separated. The length of the section of cordon defoliated was determined. The petioles of all leaves were removed, and the blades were extended over a white surface, avoiding overlapping. The leaf area of those leaves was estimated through digital pictures and subsequently processed using CobCal ver. 2.0 (INTA, BsAs, Argentina), and a regression was established between fresh weight and leaf area. The resulting equation was applied to calculate LAI using data on total leaf fresh weight and length of cordon defoliated. After leaf fall, shoot length and node number on primary and lateral shoots were registered in two vines per replicate and pruning weight was measured in two vines per replicate during winter.

5. Yield components, berry composition and vine balance index

Total soluble solids concentration (TSS, ºBrix) was monitored in three vines per treatment from the beginning of March, and harvest was performed when TSS ranged between 23–24 °Brix, which occurred on March 20th and March 13th in 2018 and 2019, respectively. At harvest, two vines from the two central rows of each replicate (10 vines per treatment) were harvested, and yield was recorded and expressed as kg per plant. Then a sample of approximately 20 kg was obtained by combining clusters of two plants per replicate and transported to the laboratory to register cluster number and weight. Forty clusters from each replicate were placed on a table in the laboratory. Next, a sample of 45 berries was randomly picked, considering berry position within each cluster (i.e., 15 berries from apical, 15 from median, and 15 from basal positions). The sample of berries was weighed, and the mean berry weight was calculated. Then, the number of berries per cluster was estimated considering mean berry weight and cluster weight (without the rachis).

Clusters per shoot were calculated in one vine per replicate using the total number of clusters harvested and the number of shoots counted before pruning on the same vine. The Ravaz index (RI, yield/pruning weight) and the leaf-to-fruit ratio (LA/Y) were estimated as indicators of vegetative-productive balance.

The 45-berry sample was frozen at –20 °C for subsequent analysis. Must was obtained by manually pressing. TSS was measured using a temperature-compensating refractometer (Atago-Co Ltd., Japan), and pH was assessed with a digital pH meter (Denver Instrument UV-10) previously calibrated. Titratable acidity (TA) was measured by titrating with 0.1 N NaOH and expressed as g/L of tartaric acid equivalents.

6. Statistical analysis

Two-way ANOVA was used to test the effect of treatments, seasons, and treatment-by-season interactions on response variables. The means were separated using the LSD test at a significance level of = 0.05. All graphs were prepared with GraphPad Prism version 5.01 software (California, USA). The statistical analyses were performed with INFOSTAT software (UNC, Argentina).

RESULTS

1. Buds retained after pruning

As expected, in the first season, the treatments generated three contrasting levels of bud number per m^2: 7 buds/m^2 in MP1, 13 buds/m^2 in MP2 and SP, and 29 buds/m^2 in the MP3 treatment (Table 1). In the successive seasons, the box size applied for each treatment was maintained, but the bud number pattern changed.

Pruning treatments, seasons, and their interactions significantly affected bud number per plant and spur characteristics after pruning (Table 1). This interaction reflected the changes in the number of buds and spurs per m^2 caused by repeated mechanical box pruning versus the uniform pattern of manual pruning over time. In mechanical pruning treatments, the bud number retained per m^2 was the lowest in MP1 (14 buds/m^2), intermediate in MP2 (22 buds/m^2), and highest in MP3 (33 buds/m^2), averaged over the three years. However, both MP1 and MP2 exhibited a significant increase in bud number from the first to the third pruning year, in contrast to MP3, where bud number remained unchanged in the last two seasons. The SP treatment showed an average of 13 buds/m^2 during the three years. In 2017, the SP treatment presented a similar bud number to MP2; while in 2018, the SP treatment presented a similar bud number to MP1 and was lower than all mechanical box pruning treatments in 2019.

TABLE 1. Bud and spur number, buds per spurs and spur length in Cabernet-Sauvignon plants box pruned mechanically at 1 cm height × 7 cm width (MP1), at 14 cm × 14 cm (MP2), at 21 cm × 21 cm (MP3), and manually spur pruned (SP) in three years (2017, 2018, and 2019) in Rivadavia, Mendoza.

Source of Variation		Buds/m² (#)	Spurs/m² (#)	Buds/spur (#)	Spur length (cm)
2017	SP	11 ab	6 a	1.76 c	5.72 bc
	MP1	7 a	7 a	0.95 a	3.01 a
	MP2	13 b	8 a	1.71 c	6.33 c
	MP3	29 de	12 b	2.53 f	11.52 e
2018	SP	13 b	8 a	1.81 cd	5.76 bc
	MP1	15 b	13 b	1.20 b	3.26 a
	MP2	25 d	14 bc	1.83 cd	6.52 c
	MP3	35 f	15 cd	2.27 e	8.93 d
2019	SP	14 b	7 a	2.02 d	4.89 b
	MP1	20 c	16 de	1.20 b	2.91 a
	MP2	29 e	16 de	1.76 c	5.11 b
	MP3	34 f	18 e	1.92 cd	6.46 c
Pruning	SP	13	7	1.86	5.46
(P)	MP1	14	12	1.12	3.06
	MP2	22	12	1.77	5.99
	MP3	33	15	2.24	8.97
Year (Y)	2017	15	8	1.74	6.64
	2018	22	12	1.78	6.12
	2019	24	14	1.73	4.84
P	P-value	<0.0001	<0.0001	<0.0001	<0.0001
Y	P-value	<0.0001	<0.0001	0,6581	<0.0001
P × Y	P-value	0.0012	<0.0001	0.0001	<0.0001

Values with the same letter are not significantly different within each year, according to the LSD test at P ≤ 0.05. Letters are only presented when ANOVA indicates a significant effect.

In mechanical treatments, the number of spurs per m^2 increased significantly from the first to the third pruning season. In the first season, MP1 and MP2 presented lower spur numbers (7) than the MP3 treatment (12). After the third pruning, spur numbers were not different among mechanical box pruning intensities. Over the three pruning seasons, the SP maintained an average of 7 spurs/m^2, which was significantly lower than the mechanical pruning treatments in the second and third seasons.

Pruning, season, and their interactions had similar effects on spur length and buds per spur. On average, over the three pruning seasons, MP1 presented the shortest spur (3.1 cm) with the smallest number of buds per spur (1.12 buds/spur), which was significantly lower than MP3, which presented the longest spurs (9.0 cm) and highest bud numbers per spur (2.2). The number of buds per spur varied across seasons for each of the three mechanical pruning treatments. Buds per spur increased from the first to the third season in MP1, remained stable in MP2, and decreased toward the third season in MP3. In SP, spur length was similar among seasons, but the bud number per spur ranged from 1.8 to 2.0. SP and MP2 treatments showed similar spur length and bud numbers except for the third season when MP2 presented a lower bud number per spur than SP.

2. Budburst and shoot development

Bud number per spur and bud positions within spurs are shown in Figure 1. In the first season, MP1 presented a similar proportion of spurs with only basal buds and spurs with one node (Figure 1A). In the second season, the percentage of spurs with one node increased from 54 % to 66 %, while the rest were spurs with a basal bud and two nodes (Figure 1B). In the first season, MP2 showed similar proportions of spurs with only basal buds and one or two nodes (Figure 1C). For the second season, MP2 increased the proportion of spurs with one node from 32 % to 42 %. Spurs with basal buds decreased up to 9 %, in contrast to spurs with two and three nodes that increased up to 37 % and 10 %, respectively (Figure 1D). MP3 in the first season presented a higher proportion of spurs with one and two nodes, 31 % for both, and a lower proportion of spurs with basal buds (5 %), three (21 %) and four nodes (11 %) (Figure 1E). In MP3 the second season, spurs with one node increased from 31 % to 50 %, spurs with only basal bud did not change, and spurs with two, three, and four nodes slightly decreased their percentages compared to the previous season (Figure 1F). SP showed a similar distribution in both seasons, with a low percentage of spurs with a basal bud (12 % in both

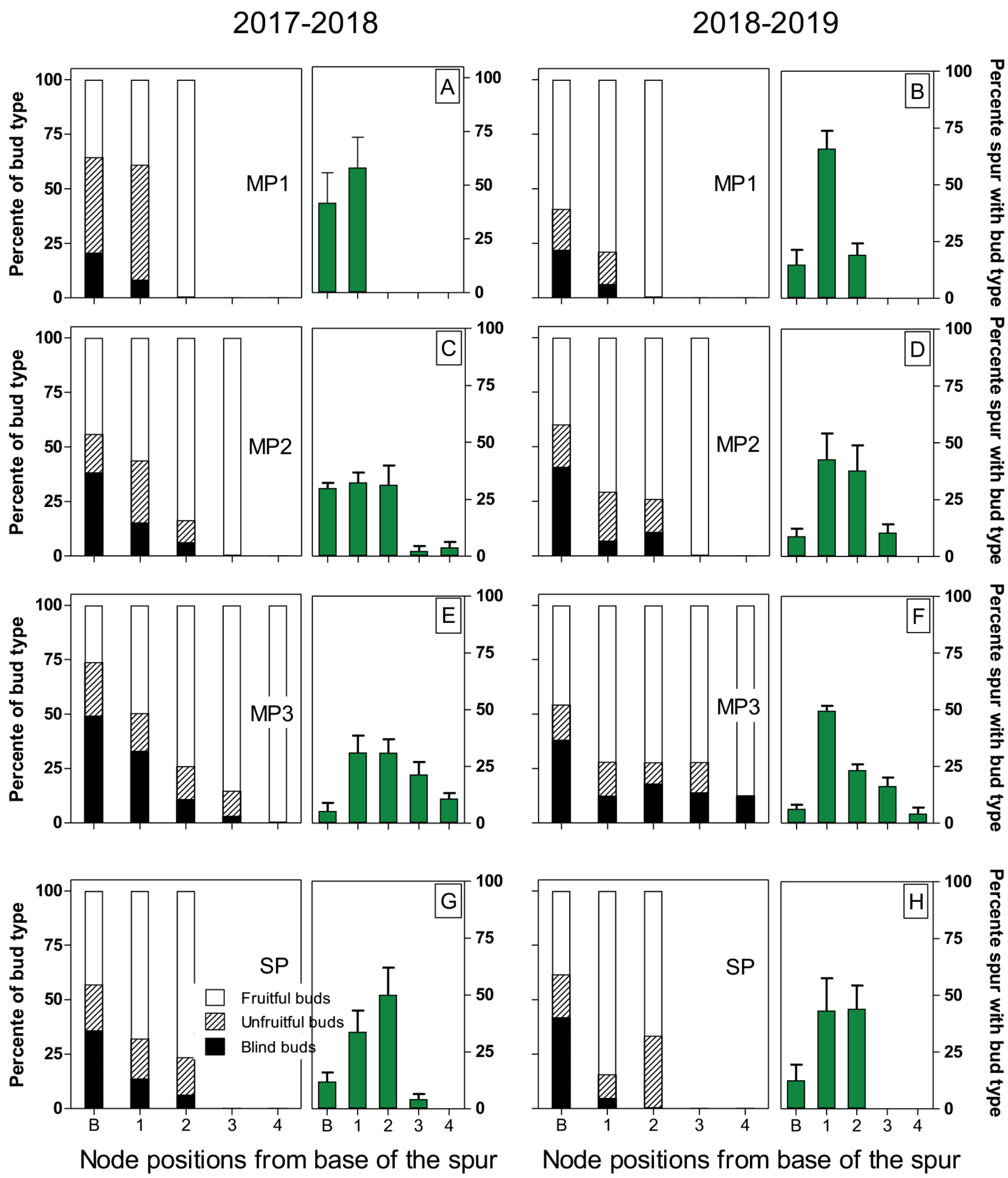

FIGURE 1. Left panels within each 2017–2018 and 2018–2019 seasons: Percentage of blind buds, unfruitful, and fruitful shoots emerging at each node position from the base of spurs in Cabernet-Sauvignon plants box pruned mechanically at 1 cm height × 7 cm width (MP1, A and B), at 14 cm × 14 cm (MP2, C and D), at 21 cm × 21 cm (MP3, E and F), and manually spur pruned (SP, G and H). Right panels within each season: Percentage of spurs with only basal buds and with 1, 2, 3, and 4 nodes. Data pooled from five single-vine replicates.

seasons) and the majority of spurs with one or two nodes (approximately 43 % each) (Figure 1G, H).

Bud development, classified as blind, fruitful, and unfruitful shoots, according to their position within spurs, are summarised in Figure 1. There was a marked decrease in the percentage of blind buds from base to distal node positions over all pruning treatments in both seasons (Figure 1, left panels). Furthermore, the proportion of blind buds increased with spur length and bud number per spur. In MP1, the proportion of buds in the basal position that did not burst was around 20 %, decreasing to 7 % in the one-node position. Fruitful shoots at the base increased from 36 % to 60 % from the first to the second season. Similarly, in position 1, there were 40 % and 80 % of fruitful shoots in 2017–2018 and 2018–2019, respectively. In MP2, which showed similar shoot development patterns in both seasons, the proportion of basal buds that remained blind was 40 % (averages for 2017–2018 and 2018–2019), decreasing to 10 % in the first and second-node positions. For both seasons, the proportion of fruitful shoots increased from 40 % at the basal position to 63 %, 79 %, and 100 % at the first, second, and third-node positions, respectively. In MP3, the proportion of buds in the basal position that did not burst was 43 % (averages for 2017–2018 and 2018–2019).

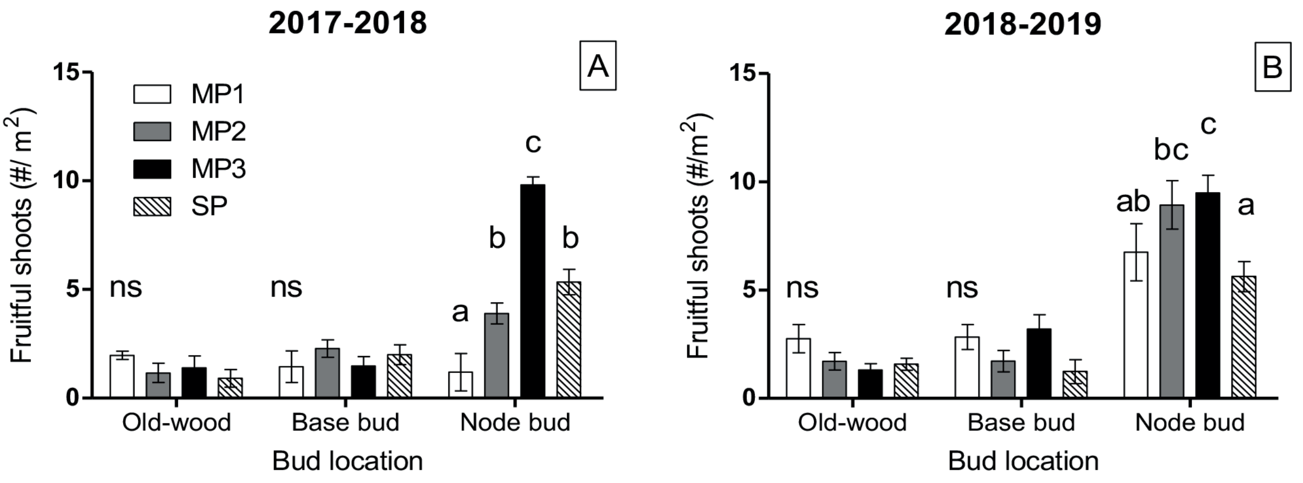

FIGURE 2. In the 2017–2018 (A) and 2018–2019 (B) seasons, fruitful shoot number relative to bud location within spurs in Cabernet-Sauvignon plants box pruned mechanically at 1 cm height × 7 cm width (MP1), at 14 cm × 14 cm (MP2), at 21 cm × 21 cm (MP3), and manually spur pruned (SP). Values with the same letter are not significantly different within each year, according to the LSD test at P $\leq$ 0.05. Ns is non-significant difference.

The proportion of buds that remained blind in the first, second and third-node positions were 33 %, 11 % and 3 % for 2017–2018 and 12 % from the first to fourth node positions. The proportion of unfruitful and fruitful buds in MP3 changed with the seasons. In 2017–2018, basal buds developed a similar proportion (25 %) of unfruitful and fruitful shoots, but in 2018–2019, fruitful shoots increased up to 46 %, while 16 % were unfruitful. The majority (70 %) of shoots developed from the first to fourth node positions of all buds in this treatment were fruitful. Finally, in SP, 39 % of basal shoots were blind, while 70 % of shoots coming from the first and second node positions were fruitful.

The number of fruitful shoots was analysed in relation to whether the bud was in the old wood, at the base of the spur, or in a spur node (Figure 2). In the first season, the number of fruitful shoots from node buds increased significantly with box size, from 1 fruitful shoot per m^2 in MP1 to 4 shoots in MP2, and 10 fruitful shoots in MP3. Similarly to MP2, the SP treatment presented 5 fruitful shoots from the node buds. In all treatments, the most fruitful shoots developed from spur nodes, while those developed from old wood and basal buds were less fertile. From the total fruitful shoots counted within each treatment, the fruitful shoots from the base and old-wood buds accounted for 74 %, 47 %, 23 %, and 35 % of the population in MP1, MP2, MP3, and SP, respectively. In the second season, the number of fruitful shoots developed from spurs was significantly affected by pruning treatments. Shoot fruitfulness from node buds considerably increased in MP1 (7 shoots/m^2) and MP2 (9 shoots/m^2), reducing the difference with MP3 (10 shoots/m^2) that was observed in the first season. The SP treatment showed 6 shoots fruitful/m^2, which was significantly lower than MP2 and MP3. During the second season, fruitful shoots developed from old-wood and basal buds were similar among treatments and accounted for 45 % in MP1 and around 30 % in MP2 and MP3.

3. Vegetative growth

Variables associated with vegetative growth were affected by pruning and year, and budburst percentage, internode length, and number of nodes per shoot showed a significant additional interaction between pruning and year. The exception was shoot number, which was not affected by pruning or year (Table 2).

Budburst percentage showed a general decreasing pattern with increasing box size. Within treatments, the budburst percentage was differently affected by year. In MP1 and MP2, the budburst percentage decreased from the first to the second season, but in MP3, the budburst was not different between seasons. Similarly, SP showed similar budburst in both seasons (average 133 %), which partly explained a portion of the observed pruning by year interaction. This marked difference in budburst compensated for bud numbers retained at pruning, resulting in similar shoot numbers among treatments.

Regarding shoot growth, MP1 and SP showed longer shoots with longer internodes than both MP2 and MP3 treatments, with no difference between them. In addition, shoots from MP1, MP2, and SP treatments showed a higher node number than shoots from MP3 in 2017–2018, with no differences among treatments in 2018–2019. Pruning weight was higher in SP and MP1 treatments (0.47 kg/m^2) than in MP2 and MP3 (0.36 kg/m^2), while LAI was not significantly affected by pruning treatment. In the 2018–2019 season, shoots presented lower growth, lower shoot length and lower nodes than in 2017–2018. As a result, LAI and pruning weight decreased in the last season.

4. Yield components and vine balance

Pruning treatments, seasons, and their interactions significantly affected yield, cluster per m^2, and cluster weight (Table 3). This interaction reflected the marked influence of pruning during the first but not the second season.

TABLE 2. Vegetative development in Cabernet-Sauvignon plants box pruned mechanically at 1 cm height × 7 cm width (MP1), at 14 cm × 14 cm (MP2), at 21 cm × 21 cm (MP3), and manually spur pruned (SP) during 2017–2018 and 2018–2019 seasons in Rivadavia, Mendoza.

Source of Variation		Bud burst (%)	Shoots (#/m^2)	Shoot length (cm)	Internode length (cm)	node/shoot (#)	Leaf area (m^2/m^2)	Pruning wt (kg/m^2)
2017–18	SP	145 c	16	97.8	5.3 a	18.1 c	2.8	0.54
	MP1	218 d	15	92.8	5.4 a	17.0 c	2.1	0.51
	MP2	127 bc	16	83.3	5.2 a	16.1 c	2.1	0.46
	MP3	60 a	17	66.8	5.4 a	12.1 b	2.1	0.36
2018–19	SP	122 bc	16	65.4	6.8 b	9.7 a	1.9	0.39
	MP1	111 b	16	63.6	6.6 b	9.8 a	1.7	0.44
	MP2	72 a	18	53.2	5.2 a	10.3 ab	1.4	0.33
	MP3	50 a	18	52.1	4.9 a	10.4 ab	1.4	0.31
Pruning	SP	133	16	81.6 b	6.1	13.9	2.3	0.47 b
(P)	MP1	164	16	78.2 b	6.0	13.4	1.9	0.47 b
	MP2	99	17	68.3 a	5.2	13.2	1.8	0.39 a
	MP3	55	17	59.4 a	5.2	11.3	1.8	0.33 a
Season	2017–18	137	16	85.2 b	5.3	15.8	2.3 b	0.47 b
(S)	2018–19	89	17	58.6 a	5.9	10.0	1.6 a	0.37 a
P	P-value	<0.001	0.3271	<0.001	0.0128	0.0141	0.1022	0.0013
S	P-value	<0.001	0.0663	0.0001	0.0289	<0.001	0.0010	0.0005
P × S	P-value	0.0001	0.6142	0.2170	0.0190	0.0016	0.8284	0.4240

Values with the same letter are not significantly different within each year by LSD test at P ≤ 0.05. Letters are only presented when ANOVA indicated a significant effect.

TABLE 3. Yield components in Cabernet-Sauvignon plants box pruned mechanically at 1 cm height × 7 cm width (MP1), at 14 cm × 14 cm (MP2), at 21 cm × 21 cm (MP3), and manually spur pruned (SP) during 2017–2018 and 2018–2019 seasons in Rivadavia, Mendoza.

Source of variation		Yield (kg/m^2)	Clusters (#/m^2)	Cluster wt (g)	Berry/cluster	Berry wt (g)	Berry/cluster	Clusters/shoot	Ravaz Index (kg/kg)	Leaf Area/Yield (m^2/kg)
2017–18	SP	1.17 ab	13.28 ab	85.28 bc	68.03	1.21	0.95 ab	0.87	2.49	2.37 c
	MP1	0.63 a	8.36 a	70.87 a	57.81	1.24	0.94 a	0.63	1.74	3.62 d
	MP2	1.37 b	16.58 b	77.65 ab	61.42	1.27	0.95 a	0.92	2.68	1.79 bc
	MP3	2.39 c	30.08 c	77.40 ab	64.92	1.21	0.94 a	1.59	7.35	1.00 ab
2018–19	SP	2.46 c	26.59 c	88.47 bc	67.81	1.31	0.95 ab	1.65	8.09	0.77 ab
	MP1	3.15 d	30.14 c	97.41 c	67.88	1.44	0.96 bc	1.81	6.86	0.54 a
	MP2	3.42 d	38.99 d	85.72 bc	67.26	1.28	0.94 a	2.17	11.35	0.41 a
	MP3	3.41 d	42.75 d	77.94 ab	64.72	1.21	0.96 c	2.66	14.51	0.42 a
Pruning	SP	1.82	19.93	86.88	67.91	1.27 ab	0.95	1.26 a	5.29 a	1.57
(P)	MP1	1.89	19.25	84.14	62.84	1.34 b	0.95	1.22 a	4.30 a	2.08
	MP2	2.40	27.79	81.68	64.34	1.28 ab	0.95	1.55 a	7.02 a	1.10
	MP3	2.90	36.42	77.67	64.82	1.21 a	0.95	2.13 b	10.93 b	0.71
Season	2017–18	1.39	17.08	77.80	62.78	1.23 a	0.94	1.00 a	3.57 a	2.20
(S)	2018–19	3.11	34.62	87.38	66.92	1.31 b	0.95	2.07 b	10.20 b	0.53
P	P-value	0.0001	<0.0001	0.2405	0.5428	0.0496	0.2841	<0.0001	0.0002	0.0050
S	P-value	<0.0001	<0.0001	0.0058	0.1136	0.0260	0.0013	<0.0001	<0.0001	<0.0001
P × S	P-value	0.0077	0.0498	0.0334	0.3699	0.1216	0.0054	0.5413	0.5578	0.0155

Values with the same letter are not significantly different within each year, according to the LSD test at P ≤ 0.05. Letters are only presented when ANOVA indicates a significant effect.

TABLE 4. Total solid soluble concentration (TSS), total acidity (TA) and pH in berries of in Cabernet-Sauvignon plants box pruned mechanically at 1 cm height × 7 cm width (MP1), at 14 cm × 14 cm (MP2), at 21 cm × 21 cm (MP3), and manually spur pruned (SP) during 2017–2018 and 2018–2019 seasons in Rivadavia, Mendoza.

Source of variation		TSS (°Brix)	TA (g/l)	pH
2017–18	SP	23.8	4.73	4.13 c
	MP1	23.6	5.13	4.07 c
	MP2	23.6	4.61	4.11 c
	MP3	23.9	4.80	4.09 c
2018–19	SP	24.4	4.75	3.94 b
	MP1	24.6	4.83	3.93 b
	MP2	23.6	4.63	3.85 a
	MP3	23.4	5.00	3.85 a
Pruning	SP	24.1	4.74	4.03
(P)	MP1	24.1	4.98	4.00
	MP2	23.6	4.62	3.98
	MP3	23.7	4.90	3.97
Season	2017–18	23.7	4.82	4.10
(S)	2018–19	24.0	4.80	3.89
P	P-value	0.1752	0.1487	0.0383
S	P-value	0.2000	0.9030	<0.0001
P × S	P-value	0.0709	0.4995	0.0412

Values with the same letter are not significantly different within each year, according to the LSD test at $P \leq 0.05$. Letters are only presented when ANOVA indicates a significant effect.

In contrast, berry weight and clusters per shoot were affected by pruning and season with no significant pruning × season interaction.

During the first season, the yield was significantly higher in MP3 (2.39 kg/m^2), followed by MP2 (1.37 kg/m^2), SP (1.17 kg/m^2), while MP1 (0.63 kg/m^2) presented the lowest yield (Table 3). The number of clusters increased with box size, and the yield component most influenced yield responses to pruning treatments in the first season. In contrast, cluster weight was not different among treatments. The manual SP treatment presented a similar yield (1.17 kg/m^2) to MP1 and MP2 but was lower than MP3. During the second season, yield was unaffected by mechanical box pruning intensity, and all mechanical pruning treatments were 26 % more productive than the manual SP treatment. Similar to the first season, cluster number increased with box size, but cluster weight decreased. Across pruning treatments, the average yield in the second season was 2.2-fold higher than the first season. Yield differences between seasons were higher in MP1 and lower in MP3. Thus, averaging the two seasons, MP3 was the most productive, and MP1 and SP were the lowest.

The balance between productive and vegetative growth was evaluated through the yield-to-pruning weight ratio (i.e., Ravaz index) and the leaf-to-fruit ratio (LA/Y; Table 3). Our results showed that both indexes responded to pruning and season, but the interaction effect was only significant for the LA/Y ratio. The Ravaz index increased from 4 in MP1 to 11 in MP3, while it was 5 in plants that were manually pruned (SP), with no significant differences between MP1 and MP2. In the second season, the Ravaz index increased 2.8-fold compared with the first season. In the first season, the LA/Y ratio was higher in MP1 (3.62) than in the rest of the treatments, whereas SP (2.37) was higher than MP2 (1.79) and MP3 (1.00) and no significant differences were found between these two last treatments. In the second season, the LA/Y ratio was not significantly affected, and, except for MP3, which was similar to the first season, all treatments showed lower values than the first season.

5. Berry composition

At harvest, the TSS and TA were unaffected by treatments, showing similar values in both seasons. In contrast, pH responded to pruning, season, and their interaction (Table 4). In 2017–2018, pH was similar for all treatments, but it was higher compared to the second season, where SP and MP1 presented a higher pH compared to MP2 and MP3.

DISCUSSION

In this study, we evaluated three mechanical pruning intensities and a manual pruning treatment over three consecutive seasons. The non-selectivity of mechanical pruning produced an increase in the number of spurs per plant, which led to an increase in buds per plant, cluster number, and yield compared to manual pruning. However, after the first season, the yield was not closely related to the intensity of mechanical pruning due to some regulation mechanisms, such as a decrease in budburst and cluster and berry weight.

1. Mechanical pruning increase yield when applied in successive seasons compared to manual spur pruning

To compare the effect of mechanical pruning with traditional hand manual pruning, we retained the same number of buds per plant in MP2 and SP at the beginning of the experiment. In the first season, the bud number per spur and spur length were similar between MP2 and SP. Manual pruning, as expected, was adjusted to maintain the same number of buds per vine across seasons, whereas, in mechanical pruning treatments, the cutting distance was maintained and repeated over successive seasons. Consequently, buds per vine increased consistently as mechanical pruning was repeated at the same distance (Table 1). Furthermore, mechanical pruning is characterised by the non-selectivity of the cutting material, leading to an increase during the second season of 79 % in spur number and 88 % in retained buds compared to manual pruning.

This higher number of nodes and spurs per plant in MP2 than SP in the second season led to a decrease in budburst, and as a consequence, the shoot number was similar between both treatments (Table 2). This compensation mechanism has also been indicated by other authors in several grape varieties when comparing manual with free-shoot training systems (Poni *et al*., 2004; Greven *et al*., 2014). While the budburst percentage compensated for the initial differences in bud and spur number, the number of fruitful shoots, cluster per shoot, and yield were not significantly different during the first season. However, in the second season, mechanised pruning increased the number of fruitful shoots, the cluster per shoot, and yield by 47 %, 32 %, and 39 %, respectively, compared to manual pruning (Figure 2). Higher yield in MP2 was related to an increase in the proportion of spurs with one, two, and three buds with higher fertility than basal ones. Previous studies have also shown a similar increase in yield (between 31 % and 41 % higher) with mechanical compared to manual pruning, which was related to a higher cluster number per vine (De Toda and Sancha, 1999; Peppi *et al.,* 2017). Similarly, in a long-term study, mechanically pruned vines also showed a 30 % increase in yield during the first 8 years of implementation compared to manually pruned vines (Geller and Kurtural, 2013).

The higher bud number per plant and the higher yield of mechanised pruned vines resulted in lower vegetative growth than manual pruned vines. On average, MP2 vines presented shorter shoots with fewer nodes and lower pruning weight compared to the manual pruning treatment (Table 3), as also observed by De Toda and Sancha (1999) and Kurtural *et al.* (2013). The use of balance indexes (i.e. Ravaz Index (RI) and LA/Y ratio) to compare mechanical and manual pruning is limited because MP2 plants were trained in a free-shoot canopy system while SP plants were trained in a vertically shoot-positioned (VSP) system. Kliewer and Dokoozlian (2005) proposed for vineyards trained in VSP optimal values of LA/Y and RI between 0.8–1.2 m^2/kg and between 4–10, respectively. In the first season for SP plants, the LA/Y value (2.5) indicated excessive vegetative vigour, while the RI value (8) indicated equilibrated plants (Table 3). In the second season, the RI value was again within the range considered optimal, whereas the LA/Y value was near the lower limit and could be considered between balanced and over-cropped vines. MP2 plants showed similar values of LA/Y and RI than SP during the first season, but in the second season, LA/Y was quite low (0.41), indicating an excess yield compared to vegetative growth. This value is lower than that recently reported by Ahumada *et al.* (2021) for cv. Malbec managed with mechanical box pruning and different shoot trimming intensities to generate different LA/Y ratios. The authors showed that plants with LA/Y values of 0.63 and 0.93 achieved the same TSS concentration at harvest, while plants with 1.3 m^2/kg increased TSS relative to the former. In our study, both treatments (SP and MP2) drastically decreased LA/Y in the second season; however, there was no significant difference between seasons in soluble solids and total acidity. These results reveal that more information is yet needed to determine the range of optimal values for the different index balances for free-canopy training systems adapted to complete vineyard mechanisation.

2. Yield components compensate when different box pruning sizes are applied over three seasons

In the first season, the treatment with the smallest box pruning size (MP1) retained fewer buds than the treatment with the largest box pruning size (MP3). However, the difference in bud load decreased with pruning in the following seasons (Table 1). Retained bud number in MP1 increased 111 % in the second relative to the first season and 29 % in the third season relative to the second season. In contrast, the number of retained buds in MP3 increased only 21 % in the second pruning and remained similar after the third pruning. Similarly, Gatti *et al.* (2011) found that in mechanically pruned vines using a small box size, the number of retained buds increased progressively over the first two successive seasons. However, a stabilisation in the number of retained buds with successive pruning in the long term is produced, as observed after eight seasons of evaluation in a Geneva Double Curtain training system (Morris and Main, 2010).

Even if the same box pruning size was maintained in the same vines over time, the number of spurs and the number of buds per spur changed from one season to the other, which finally impacted the number of buds per m^2 in each season. After the first pruning, the number of spurs increased proportionally to the increase in box pruning size, whereas after the second and, more notably, the third pruning, the spur number was similar across treatments (Table 1). This is explained by the fact that the spur number was closely related to the shoot number developed, which was also similar across treatments during the second season. Regarding buds per spur, initially, the largest box pruning size (MP3) presented longer spurs with a higher bud number than MP1, but from the second season, they were similar since spur length and buds per spur decreased in MP3. On the contrary, in MP1, the number of buds per spur increased from the second season, after which they remained stable. In MP3 treatment, the decrease in spur length from the second season was because sprouting occurred mainly in buds located at the distal nodes, closer to the cutting height, which was maintained over successive seasons (Figure 1). This finding is supported by Poni *et al*. (2004) who mechanically pruned vines at 10 cm height from the cordon, observing lower budburst of basal buds compared to vines mechanically pruned as close as possible to the cordon. In the same sense, Peppi *et al.* (2007) observed that bud number per spur decreased in large box pruning at 25 cm height from the cordon, while buds per spur were unaffected in small box pruning at 12 cm height from the cordon. In large mechanical box pruning plants, the buds per spur gradually decrease, showing a subsequent stabilisation over successive seasons. This means that mechanised pruning intensity will have less influence on bud load per plant through the years.

Budburst decreased when the number of retained buds after pruning increased. This compensation effect, previously described in grapevine by many other authors (e.g., Poni *et al*., 2004; Peppi *et al*., 2017), has been called the physiologically self-regulating budburst mechanism (Keller *et al*., 2004; Intrieri *et al*., 2011).

These findings support the hypothesis that budburst is influenced by assimilated competition among buds (Wang *et al*., 2020), and budbreak is strongly influenced by carbohydrates availability for the buds during the budburst period (Elwafa and Thoraua, 2018). Distal buds act as auxin and gibberellin sources, causing a translocation of carbohydrates from basal to distal buds and thus promoting their development. In addition, the distal buds translocate abscisic acid o the basal bud (Rizk and El-Kenawy, 2006). In this way, box pruning size and its influence on bud load were largely driven by a self-regulatory mechanism that compensates for budburst percentage, which finally controls the yield through the number of shoots developed (Poni *et al*., 2004).

Plant vigour, expressed as pruning weight and shoot length, was affected by mechanised box pruning intensity and season, whereas leaf area was only affected by the season. The smallest box pruning showed higher pruning weight and longer shoots than the largest box pruning, while both variables and leaf area decreased in the second season (Table 2). Similarly, in previous studies, increasing pruning intensity led to an increase in vegetative growth, related to fewer retained buds and lower competition with reproductive sinks (De Toda and Sancha, 1999; Greven *et al*., 2014).

Our previous analysis focused on shoot development and their characteristics in relation to retained buds after mechanical pruning, which allows us to explain the observed results on yield (Table 2). In the first year, the MP3 treatment showed longer spurs, and higher number of fruitful shoots than MP2 and MP1, and presented more fruitful shoots than MP1 only in the second year (Figure 2). Accordingly, McLoughlin *et al.* (2011) observed that spur length affected the number of fruitful shoots and proposed that distal node positions present higher fertility than basal positions. Despite the fact that in the second season MP3 showed 42 % more clusters than MP1, this was not related to a significant increase in yield since MP1 increased berry weight by 19 % and cluster weight by 25 % relative to MP3. Yield compensation due to cluster weight was also observed by Gatti *et al.* (2011) and Greven *et al.* (2014). These processes of yield compensation must be considered in the management strategies to maintain high yield potential in vineyards trained in free-canopy systems. Providing conditions of non-limiting assimilate supply, favouring reserve accumulation through adequate vigour management and obtaining an optimal leaf-to-fruit ratio might attenuate the decrease in budburst percentage and berry/or cluster weight (Botelho *et al.,* 2020). Concerning the LA/Y ratio, MP3 plants showed a value 3.6-fold higher than MP1 in the first season, with no differences during the second season. In addition, the LA/Y ratio (or source-sink relationship) decreased in both treatments during the second season, but no differences between treatments and seasons were found concerning TSS. This could indicate that yield and TSS concentration in the berry were not limited by the source (i.e., leaf area), even during the second season when a higher decrease in the LA/Y ratio was observed. However, the RI highly increased in MP3 plants (14.5) in the second season, indicating an overcropping situation which could be related to the slightly lower TSS value observed in the second season (p-value = 0.07). On one hand, the range of optimal values for free-canopy training systems seems to be closer to those proposed previously for divided canopy systems (Kliewer and Dokoozlian 2005). On the other, the RI was easily determined over the whole plant, while the LA was measured over a sample of leaves and then scaled up to the whole plant. Therefore, the accuracy of our methodology seems to be stronger for the RI than for LA. Moreover, it has been proposed that the RI could be a better indicator of the source–sink relationship and the sustainability in the mid-term, whereas the LA/Y would be more related to short-term effects within the season (Keller, 2020). Consequently, more research must be conducted to determine the optimal ranges of balance indexes for free-canopy training systems, and more accurate and easier methods to measure exposed leaf area must be developed (Ahumada *et al.,* 2021).

CONCLUSION

Even though mechanical pruning intensity and box size were maintained over the years, box pruning resulted in an increase in retained buds with each successive pruning. In contrast, manual spur pruning allows one to retain the same bud number over the seasons. The increase in retained buds with mechanical pruning led to a higher number of clusters and yield compared to manual pruning.

After the first season, mechanised pruning intensity modified the number of retained buds, but differences between treatments were reduced with subsequent pruning. As a result, after two seasons with mechanical pruning, no differences were observed between the pruning intensities due to different compensation mechanisms. First, the increase in retained buds was partially compensated by a decrease in budburst percentage, resulting in similar shoot numbers among treatments. However, larger box sizes retained longer spurs carrying buds with higher fertility, increasing, therefore, the final cluster number. Second, this increase in the number of bunches was partially compensated by a decrease in berry weight. Based on these observations, when manual pruning is converted to mechanical box pruning, it seems more appropriate to start with a small box size that favours a high percentage of bud sprouting located in different positions of the spurs and the cordon, increasing the number of buds retained in the following years. Fully mechanised systems have increased their use these last years, but there is still scarce information concerning these training systems. Our findings regarding manual and mechanical pruning show that opportunities exist to manage fully mechanised pruning systems without compromising yield and quality. In addition, grapevine cultivars could markedly influence the yield responses to mechanical pruning intensity for which few are currently available. Furthermore, canopy management practices such as shoot trimming, leaf to fruit ratio or irrigation and nutrition requirements still need to be fine-tuned. Therefore, further studies on box mechanical pruning would include other cultivars and consideration of bud development and balance indexes.

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