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# Rootstock influence on the performance of field-grown grapevines *(Vitis vinifera)* during autumn-winter harvest

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## ABSTRACT

The production of high-quality wines elaborated from grapes harvested during the dry winter has considerably increased in the Brazilian southeast, mainly with the Syrah cultivar. However, the double pruning management used to induce winter harvest has reduced the vigour and yield of some classic cultivars such as Merlot and Cabernet-Sauvignon. In viticulture, rootstocks became a useful tool to manipulate vegetative and reproductive vigour. This study aimed to determine the effect of eight rootstocks on vigour, yield and grape quality of Merlot, Cabernet-Sauvignon, and Syrah grapevines grown during the autumn-winter season in the south of Minas Gerais under double pruning management. The treatments were evaluated from 2018 to 2022 seasons, in a 3 × 8 factorial arrangement, with three cultivars and eight rootstocks. The yield and grape quality of Syrah, Cabernet-Sauvignon, and Merlot grapevines under double pruning management could be improved using medium to high vigorous rootstocks. To all cultivars, the combination with IAC 766, SO4, RUPESTRIS, GRAVESAC, 101-14, and KOBER increased yield with no impairment in grape maturation. IAC 572 can be used under double-pruning management for Merlot and Cabernet-Sauvignon when long spur pruned. However, grafting must be carefully recommended, because this rootstock is extremely vigorous and can affect the balance between vegetative and reproductive vigour.

**KEYWORDS:** vegetative vigour, grape quality, double pruning management, vine balance, yield

## INTRODUCTION

In the vineyards of the Brazilian southeast, the double pruning strategy has enhanced wine quality by shifting the harvest period from the warm and wet summer to the dry and mild winter (Favero *et al.*, 2011). Under this management, the grapevines are initially spur pruned at the end of winter (August or September) to induce the vegetative cycle, where all clusters are removed. The reproductive cycle is started after the second spur pruning, performed in January (or February), allowing for grape harvest during the winter (July or August) (Favero *et al.*, 2011, Regina *et al.*, 2011; Mota *et al.*, 2010). These authors demonstrated that the quality of fine wine (from *Vitis vinifera* L. cultivars) has significantly improved due to increased sugar and phenolic compounds in grapes, which are favoured by low rainfall and high thermal amplitude of the autumn-winter season. The winter harvest has contributed to the expansion of fine wine-producing regions in Brazil (Brant *et al.*, 2021).

However, two growing cycles demand considerable energy from the vines, and a search for rootstocks that provide the necessary vigour to support two annual pruning seasons is required. This is particularly important to grow varieties such as Cabernet-Sauvignon and Merlot, which have exhibited low yield during the winter cycle production under subtropical conditions, as compared to Syrah and Sauvignon blanc (Regina *et al.*, 2011). This low performance of Cabernet-Sauvignon and Merlot may be correlated with a poor fruit set caused by low temperatures in cool regions (Dry *et al.*, 2010; Kidman *et al.*, 2013). Over the past 15 years, Syrah and Sauvignon blanc have been the primary cultivars used to produce winter wines, as they have demonstrated the best agronomic and oenological potential in double-pruned vineyards grafted onto medium vigour rootstocks such as 1103 P.

Grafting is widely used in viticulture and significantly contributes to the vegetative growth and yield of the scion under many cultivation conditions. Several studies have shown the rootstock effect on vigour (Jones *et al.*, 2009; Kidman *et al.*, 2013), ecophysiology (Santarosa *et al.*, 2016; Pou *et al.*, 2022; Buesa *et al.*, 2023), grape quality (Satisha *et al.*, 2010; Chou & Li, 2014; Dias *et al.*, 2017) and wine quality (Köse *et al.*, 2014; Miele & Rizzon, 2017; Wang *et al.*, 2019) in vineyards under traditional management with only one annual pruning. However, there are limited studies on rootstocks conducted under double pruning management, with most focusing on Syrah (Dias *et al.*, 2012; Dias *et al.*, 2017) and Cabernet-Sauvignon at high altitudes (Souza *et al.*, 2015).

There is a pressing need for more information regarding scion-rootstock interactions during the winter growing season in vineyards subjected to double-pruning practices. Furthermore, there is currently no information available on the rootstock effect on Merlot performance under double-pruning management. Using vigorous rootstock could be a viable strategy to enhance the yield of Merlot and Cabernet-Sauvignon, thereby increasing grape variability for winter wine production in the Brazilian southeast. Although some rootstocks have improved the vigour of Syrah and Cabernet-Sauvignon during the winter season, these results were obtained from very young vines (Dias *et al.*, 2017) and in a high-altitude region (Souza *et al.*, 2015), respectively. More combinations of scion/rootstocks must be investigated to improve the vine balance of these cultivars grown under double-pruning management.

In this study, the rootstock effects were evaluated over four seasons. Vines were short spur pruned (two spur nodes) during two seasons (2018-2020) and long spur pruned (three or four spur nodes) during the last two seasons (2021-2022). It is well established that the differences in crop level can be induced by variability in bud fruitfulness along the cane (Sánchez & Dokoozlian, 2005). Furthermore, these findings could contribute to enhancing the understanding of scion-rootstock interaction under low temperature, as most studies on rootstock effects in the literature were carried out during hot summer conditions.

This study investigated the effect of eight rootstocks on agronomic responses and grape quality of Merlot, Cabernet-Sauvignon and Syrah vines grown during autumn-winter in a double-pruned vineyard under subtropical conditions in Brazil.

## MATERIALS AND METHODS

### 1. Location, plant material, and experimental design

The experimental site was in a commercial vineyard, in Andradas, at Casa Geraldo winery, in the south of the state of Minas Gerais, Brazil (22° 05' 28" S 46° 33' 50" W, 913 m above sea level). The vineyard was planted in November of 2015, and the experiment was carried out during the 2018 to 2022 seasons.

Merlot, Cabernet-Sauvignon and Syrah were grafted onto eight rootstocks from different origins and within a variable range of vigour described in Table 1.

The treatments consisted of a 3 × 8 factorial arrangement, with three grape cultivars and eight rootstock combinations, and the experimental plot consisted of 10 vines, totalling 240 vines. The experimental area was homogeneous and treatments were arranged in a completely randomised design in four rows of 60 m. Non-irrigated vines were trained in vertical shoot position, north-south orientated, harrowed with three wire strands, spaced 2.50 m × 1.00 m. From 2018 to 2019, at yield pruning (January), the vines were short pruned with two buds per spur (short spur—SS), and to increase bud fruitfulness, from the 2021 and 2022 seasons, depending on the length of internode vines were spur pruned with three or four nodes per spur (long spur—LS). Data from the 2020 season were discarded due to high rainfall during the flowering stage, which drastically reduced yield and quality parameters.

**TABLE 1.** Rootstocks evaluated in this study.

| Rootstock | Abbreviation | Parentage | Vigour conferred | References |
|---|---|---|---|---|
| IAC 766 | IAC766 | Ripária do Traviú × *Vitis caribaea* | Medium to vigorous | Pommer (2000) |
| IAC 572 | IAC572 | *Vitis caribaea* × 101-14 Mgt | Highly vigorous | Pommer (2000) |
| 1103 Paulsen | 1103 P | *Vitis berlandieri* × *Vitis rupestris* | Medium to vigorous | Ferlito *et al.* (2020)<br>Bettiga (2003) |
| Selection Oppenheim 4 | SO4 | *Vitis berlandieri* × *Vitis riparia* | Medium | Pavloušek (2014)<br>Bettiga (2003) |
| Kober 5BB | KOBER | *Vitis berlandieri* × *Vitis riparia* | Medium | Pavloušek (2014)<br>Bettiga (2003) |
| Rupestris du Lot | RUPESTRIS | *Vitis rupestris* | Medium to vigorous | Christensen (2003) |
| Gravesac | GRAVESAC | 161-49 (*Vitis riparia* × *Vitis berlandieri*) × 3309 (*Vitis riparia* × *Vitis rupestris*) | Medium | Galet (1999) |
| 101-14 Millardet et de Grasset | 101-14 | *Vitis riparia* × *Vitis rupestris* | Weak to medium | Christensen (2003)<br>Bettiga (2003) |

The double pruning management was applied according to the methodology described by Favero *et al.* (2011), which the first pruning was done in August to promote the vegetative cycle for latent bud formation (all cluster were removed) and the second (yield pruning) was done in January in lignified shoots (reproductive cycle) for grape production during the autumn-winter. In both cycles, the buds were sprayed after pruning with hydrogen cyanamide at 5 % to stimulate and standardise the budburst. Pest control sprays and vineyard management were applied in accordance with conventional vineyard practices. Information regarding climate conditions (precipitation, maximum and minimum temperature) recorded during the seasons is presented in Figure 1.

## 2. Vine vigour evaluations

During the vegetative and reproductive cycles, agronomical and ecophysiological parameters were evaluated to characterise vine performance. The vegetative and reproductive vigour were estimated by pruning weight and shoot fruitfulness evaluations, respectively. The pruning weight (fresh weight of pruned shoots) was measured in August (winter pruning weight), after the reproductive cycle, in six vines per treatment. The vine balance was evaluated by the Ravaz index (yield/winter pruning weight ratio) using six vines per treatment. Shoot fruitfulness was calculated during full bloom (at BBCH 65) by dividing the number of inflorescences by the number of shoots, using four vines for each treatment.

## 3. Yield components and grape composition

At harvest, the number and weight of bunches per vine were measured. Berry mass was calculated from four replicates of 150 berries per treatment. Chemical analyses (soluble solids, pH and titratable acidity) were performed on the juice of pressed berries (four replicates of 100 berries per treatment). Soluble solids (°Brix) were determined using a portable digital refractometer (PAL 1 model, ATAGO). The pH of undiluted juice of each sample was determined using a Micronal pH meter B474 calibrated with standards 4.0 and 7.0, and titratable acidity was determined by titration with 0.1 M NaOH to a phenolphthalein end point at pH 8.2 and expressed as g $L^{-1}$ of tartaric acid.

Phenolic maturation was determined using four replicates of 60 berries per treatment. The skin was removed, dried at room temperature, weighed, crushed with liquid nitrogen and stored at –80 °C until analysis. For the determination of phenolic compounds, 150 mg samples of the crushed skin were homogenised in an Ultra Turrax homogeniser (IKA T-18 basic) in an acidified methanol solution (1 % HCl). The samples were kept overnight at 7 °C in the dark, centrifuged at 8,000 rpm for 15 minutes until total removal of pigments. The supernatant was transferred to 50 mL volumetric flasks. Anthocyanins in berry skins were determined by the pH differential method (Giusti & Wrolstad, 2001) and total phenolics were determined by the Folin–Ciocalteau method based on a standard curve of gallic acid (Amerine & Ough, 1980).

## 4. Statistical analysis

The statistical analyses were performed using R software (R Core Team, 2023). A priori, non-normal data were submitted to a Box–Cox transformation, using the *MASS* package. A two-way ANOVA was carried out for each spur pruning treatment, comparing all variables, as well as possible interactions among treatments by the Scott–Knott's test ($p < 0.05$) for rootstocks and the Tukey's test ($p < 0.05$) for cultivars, using the *ExpDes* package. A principal component analysis (PCA) and clustering analysis (CA) were performed for all variables, using the *factoextra* package, and Pearson's correlation was performed using the *corrplot* package.

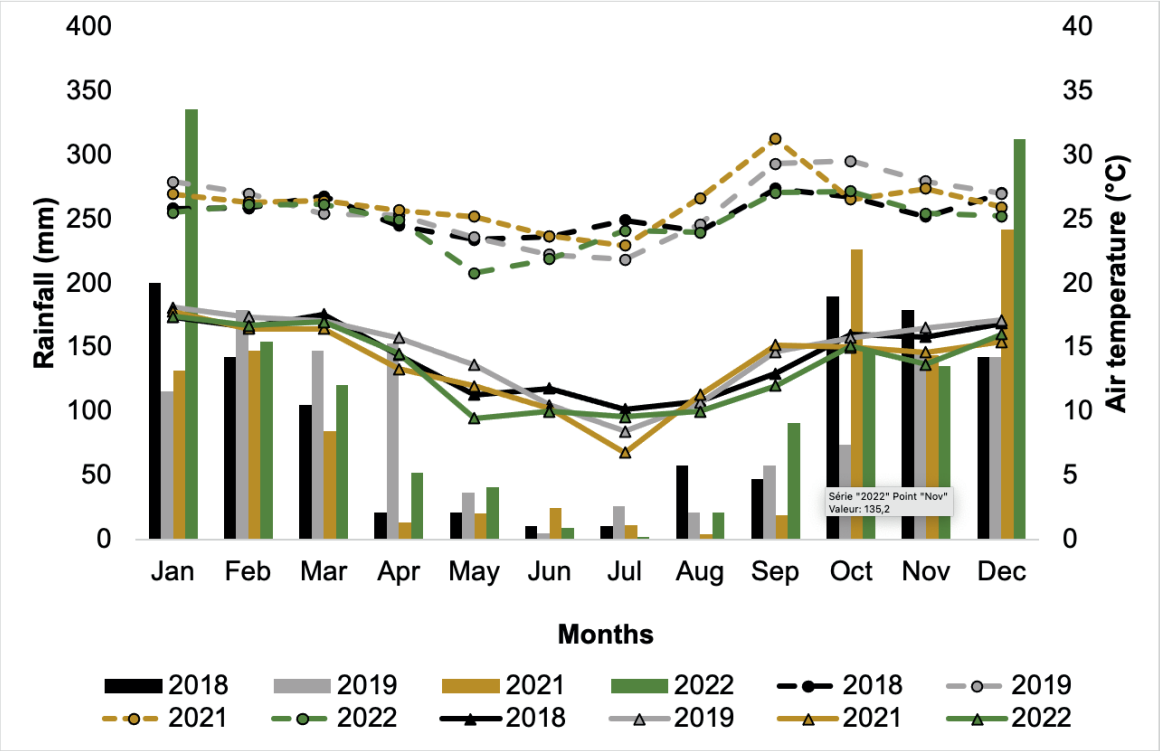



**FIGURE 1.** Average monthly rainfall (mm) and minimum (Tmin) and maximum (Tmax) air temperature (°C) during 2018-2022 in Andradas, Minas Gerais, Brazil.

## RESULTS

### 1. Short spur pruning

There was a significant interaction between factors affecting vegetative vigour, as evidenced by pruning weight (Figure 2A). The pruning weight of Syrah was significantly enhanced by IAC 766, IAC 572, and RUPESTRIS, while it was reduced by SO4, KOBER, 101-14 and 1103 P. Conversely, Merlot grafted onto IAC 766 and RUPESTRIS exhibited the highest pruning weight values. In Cabernet-Sauvignon, the highest pruning weight was induced by IAC 766, IAC 572, SO4, RUPESTRIS, GRAVESAC and the lowest was observed with 1103 P, KOBER, and 101-14. Merlot demonstrated the lowest vigour compared to Syrah, which consistently exhibited the highest pruning weight across all rootstocks, except with SO4, which induced similar vigour to Cabernet-Sauvignon (Figure 2A).

No interaction was observed between cultivars and rootstocks regarding the Ravaz index and shoot fruitfulness (Figures 2B and 2C). IAC 572 induced the lowest Ravaz index (~2.77) compared to other rootstocks (~3.5) (Figure 2B). Syrah showed the highest Ravaz index (5.38), with intermediate values for Merlot (2.68) and the lowest for Cabernet-Sauvignon (1.95). Only vines grafted onto 1103 P, IAC 572 and 101-14 presented shoot fruitfulness lower than 1 (Figure 2B). Shoot fruitfulness was higher in Syrah than in other cultivars (Figure 2C).

Yield components were affected by treatments, but only isolated effects of cultivars and rootstocks were noted (Figure 3). The weight of clusters was greater for all cultivars when grafted onto IAC 766, SO4, KOBER, RUPESTRIS and GRAVESAC, averaging 120 g per cluster, which is 20 % higher than those grafted onto 1103 P, IAC 572 and 101-14 (Figure 3A). The lowest yield (0.98 kg) was recorded for vines grafted onto 1103 P and IAC 572, which was statistically different from other rootstocks (1.15 kg) (Figure 3C).

Syrah was the most productive cultivar, yielding 2.5 kg per vine, followed by Merlot (0.96 kg per vine) and Cabernet-Sauvignon (0.92 kg per vine) (Figure 3C). This was significantly influenced by the number of clusters (~20 clusters), which showed a marked difference compared to Cabernet-Sauvignon and Merlot (10 and 8 clusters, respectively) (Figure 3B). Syrah and Cabernet-Sauvignon presented the greatest (130 g) and the lowest (90 g) weight of clusters, respectively (Figure 3A).

Rootstocks influenced the physicochemical quality of berries, with interaction among treatments found only for berry mass and soluble solids (Figures 4A and 4B). Greater berry mass was observed when Cabernet-Sauvignon was grafted onto IAC 766, SO4, KOBER, RUPESTRIS and GRAVESAC (~1.10 g) and when Merlot was grafted onto IAC 766, IAC 572, RUPESTRIS and GRAVESAC (~1.15 g). The berry mass (1.15 g) of Syrah was unaffected by rootstocks (Figure 4A). RUPESTRIS induced higher soluble solids (23 °Brix) in Syrah berries than other rootstocks (21.5 °Brix) (Figure 4B). Rootstocks did not affect Cabernet-Sauvignon sugar content (mean of 22.1 °Brix), whereas Merlot grafted onto IAC 572 exhibited the lowest value for this parameter (20.3 °Brix) compared to other rootstocks (21.6 °Brix) (Figure 4B).

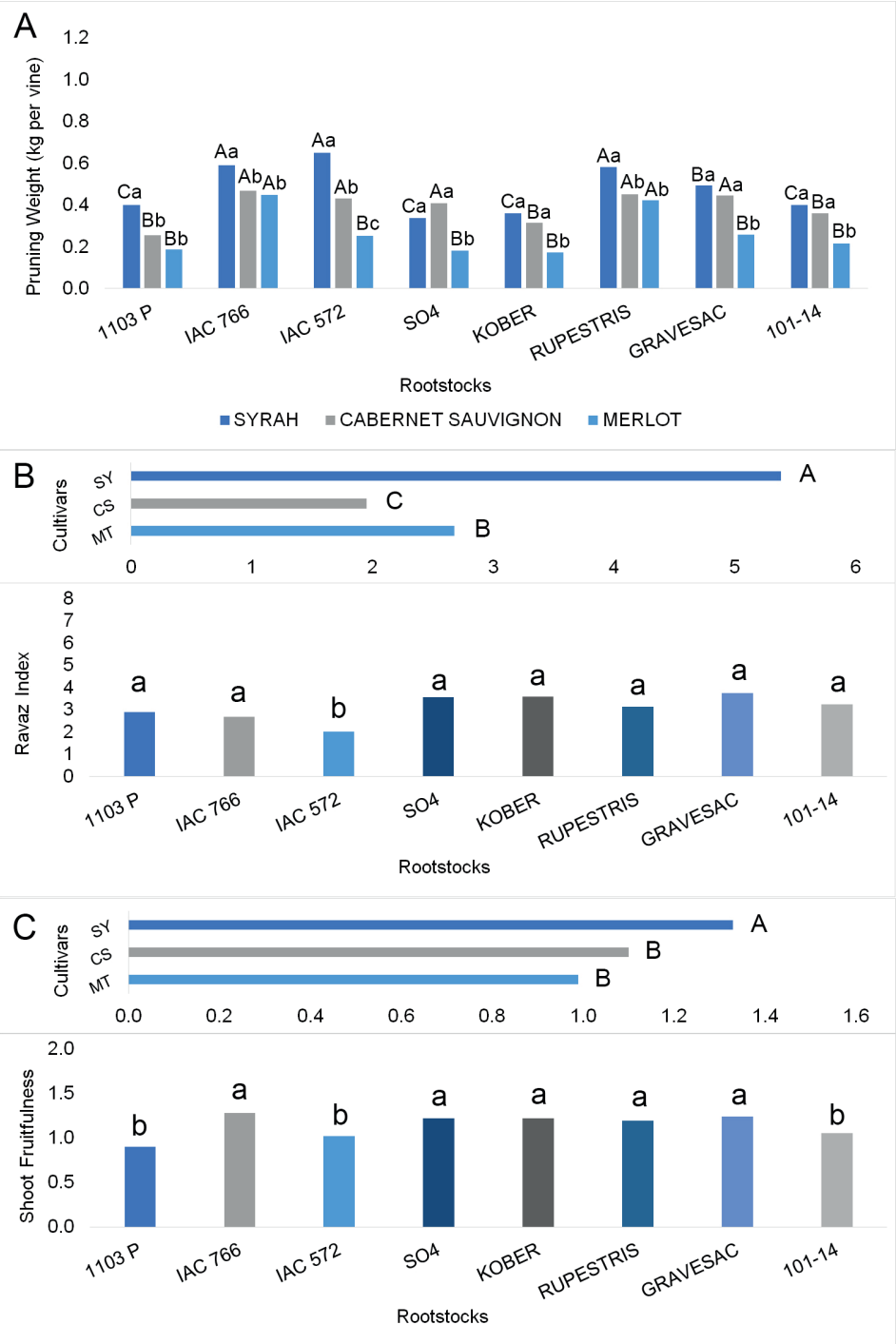



**FIGURE 2.** Pruning weight (A), Ravaz index (B), and shoot fruitfulness (C) of short spur pruned Syrah, Cabernet-Sauvignon, and Merlot vines, grafted onto different rootstocks. Means followed by the same letters, uppercase among cultivars and lowercase among rootstocks, are not statistically different ($p < 0.05$). Values are the averages of the 2018 and 2019 seasons.

The acidity of berries from all cultivars was not influenced by rootstocks, reaching values around 5 g of tartaric acid (Figure 4C). On the other hand, berries from all cultivars grafted onto SO4, IAC 766, KOBER, RUPESTRIS and GRAVESAC showed lower values (around 3.65) of pH than when grafted onto 101-14, 1103 P and IAC 572 (3.8) (Figure 4D). Among cultivars, Syrah presented the highest pH (4.01) and lowest acidity (4.08 g of tartaric acid), whereas Cabernet-Sauvignon presented the highest acidity (5.61 g of tartaric acid) and Merlot the lowest pH (3.52) (Figures 4C and 4D). Rootstocks presented similar anthocyanins and total phenolic content, averaging 0.78 and 2.52 mg per g of berry, respectively. Syrah berries had the highest content for both parameters (0.86 and 2.74 mg per g of berry), while Cabernet-Sauvignon showed higher anthocyanin levels than Merlot, with total phenols being similar in both cultivars (Figures 4E and 4F).

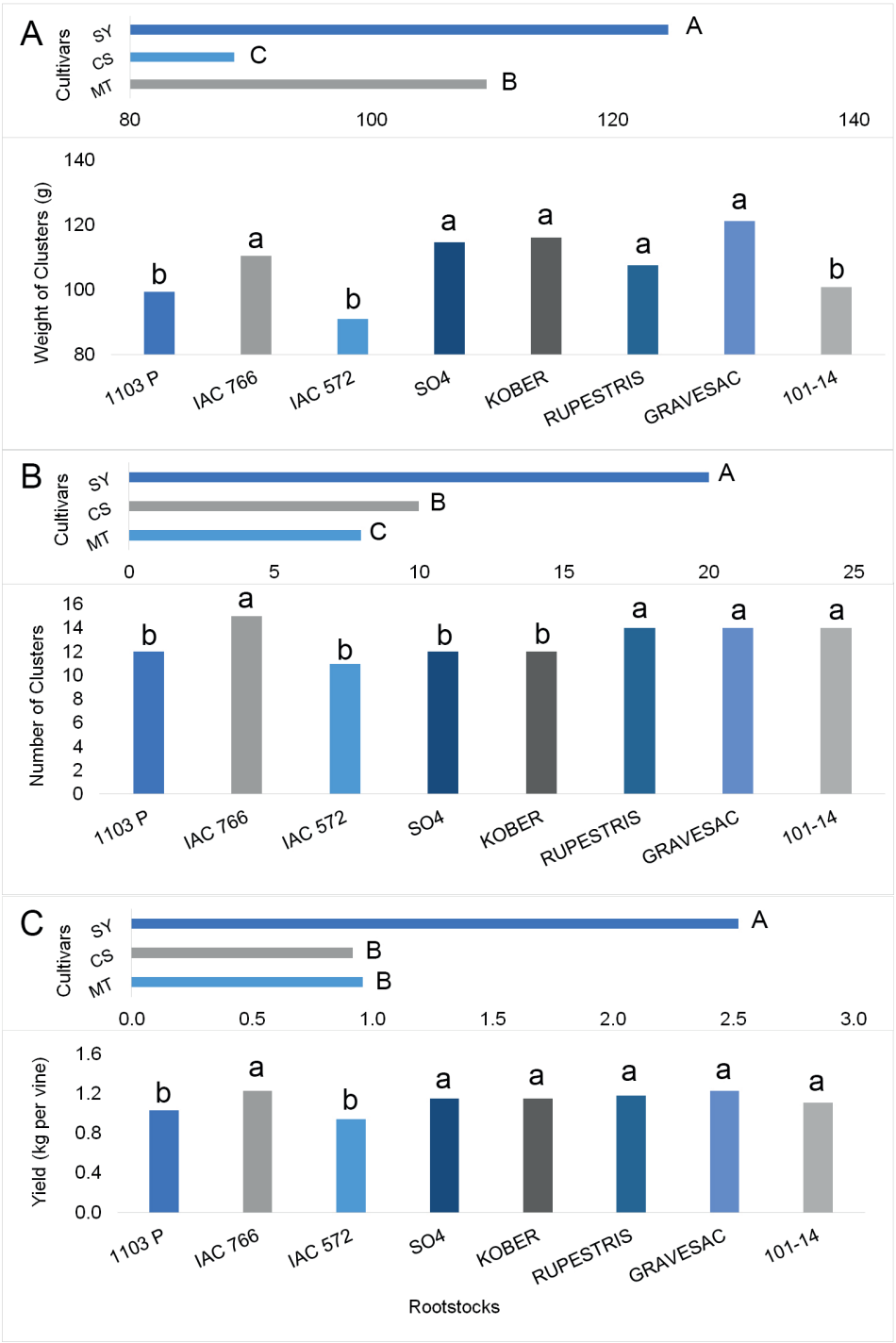



**FIGURE 3.** Weight of clusters (A), number of clusters (B), and yield (C) of short spur pruned Syrah, Cabernet-Sauvignon, and Merlot vines grafted onto different rootstocks. Means followed by the same letters are not statistically different ($p < 0.05$). Values are the averages of the 2018 and 2019 seasons.

Principal Component Analysis (PCA) was performed on the correlation matrix derived from the 12 parameters evaluated for eight rootstocks for each cultivar. The PCA biplot provides a visual overview of how different parameters were influenced by rootstocks, allowing for the grouping of treatments that reveal similarities among variables. The first two principal components (PCs) accounted for Syrah (60.24 %), Cabernet-Sauvignon (77.65 %) and Merlot (79.24 %) of the total variance. In most cases, vigour and yield parameters were associated with PC 1, while grape quality parameters were linked to PC 2, as indicated by coloured arrows in the biplot (Figures 5A, 5C and 5E).

The clustering analysis categorised samples into three to four clusters. Medium to high vigorous rootstocks such as IAC 766, SO4, RUPESTRIS, GRAVESAC and KOBER enhanced yield without impairment in technological grape parameters characterised by higher soluble solids, greater titratable acidity and lower pH across all cultivars.

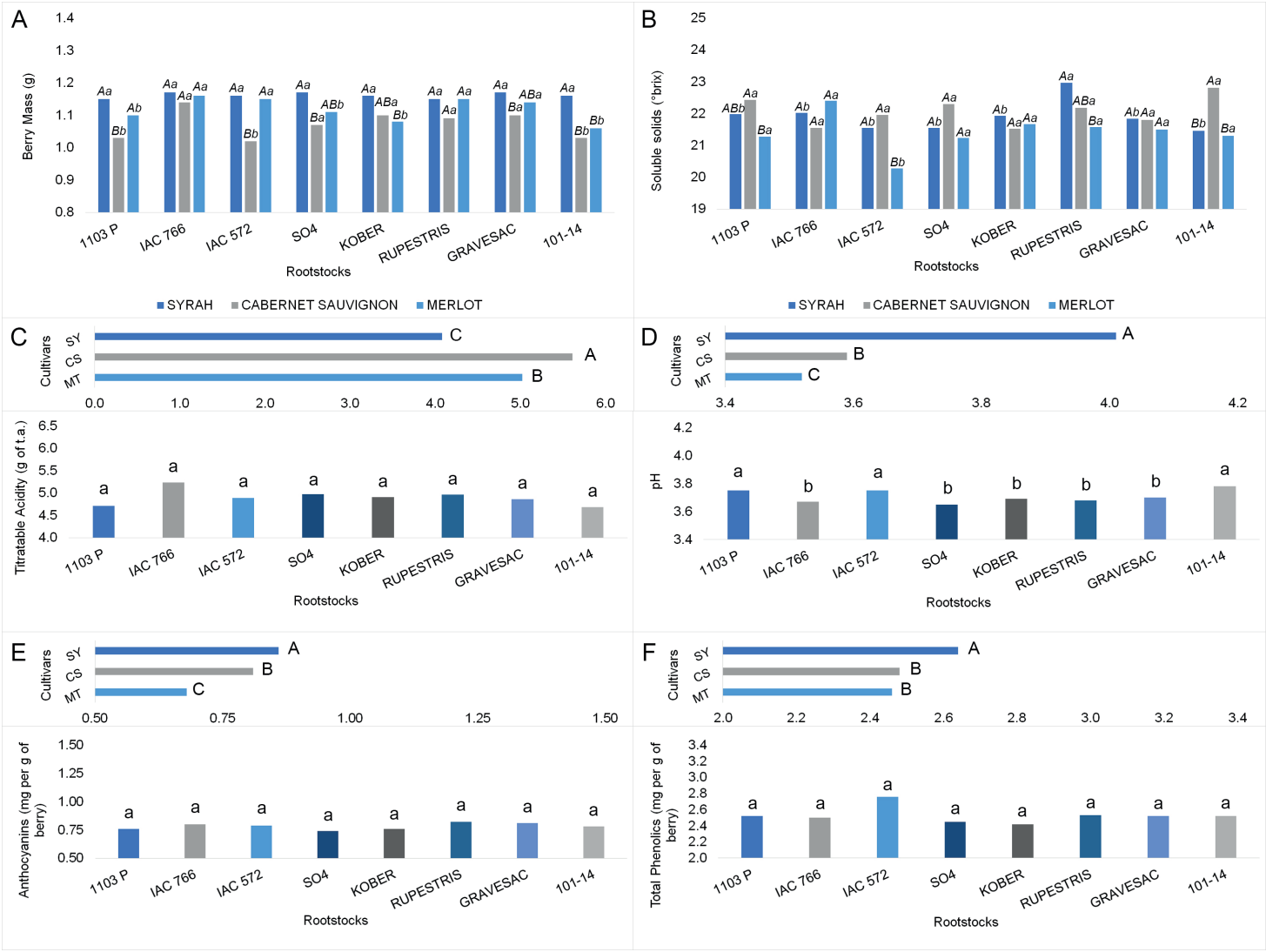



**FIGURE 4.** Berry mass (A), soluble solids (B), titratable acidity (C), pH (D), anthocyanins (E), and total phenolics (F) of short spur pruned Syrah, Cabernet-Sauvignon, and Merlot vines grafted onto different rootstocks. Means followed by the same letters, uppercase among cultivars and lowercase among rootstocks, are not statistically different ($p < 0.05$). Values are the averages of the 2018 and 2019 seasons.

In contrast, vines grafted onto 101-14 and 1103 P exhibited higher pH and greater anthocyanin and total phenolic contents, but the lowest vegetative and productive parameters. IAC 572 demonstrated greater vegetative vigour (pruning weight) but negatively impacted the remaining parameters (Figures 5B, 5D and 5F).

## 2. Long spur pruning

Under long spur pruning, vegetative vigour exhibited a response to scion/rootstock combinations that was similar to that of short spur pruned vines, as indicated by the interaction among treatments. Pruning weight was higher for all cultivars grafted onto IAC 572, IAC 766, GRAVESAC and RUPESTRIS while KOBER, SO4, 101-14 and 1103 P induced the lowest vigour (Figure 6A). Syrah and Cabernet-Sauvignon displayed higher vegetative vigour across all rootstocks, except for 1103 P, IAC 766 and RUPESTRIS. Greater variability of pruning weight among cultivars was found when vines were grafted onto IAC 572, ranging from 0.52 kg (Merlot) to 1.02 kg per vine (Syrah) (Figure 6A).

No interactions were found between cultivars and rootstocks regarding shoot fruitfulness and Ravaz index (Figures 6B and 6C). SO4 exhibited the highest value for the Ravaz index of 7.37, followed by KOBER (5.77), while the remaining rootstocks showed values around 4 (Figure 6B). Vines grafted onto SO4 demonstrated the highest shoot fruitfulness values (1.72), while other rootstocks induced values around 1.34 (Figure 6C). Cultivars showed differences in the Ravaz index; the highest values were recorded in Merlot (5.9), followed by Syrah (4.9) and Cabernet-Sauvignon (3.5) (Figure 6B). Merlot and Cabernet-Sauvignon presented greater shoot fruitfulness (1.5) than Syrah (1.2) (Figure 6C).

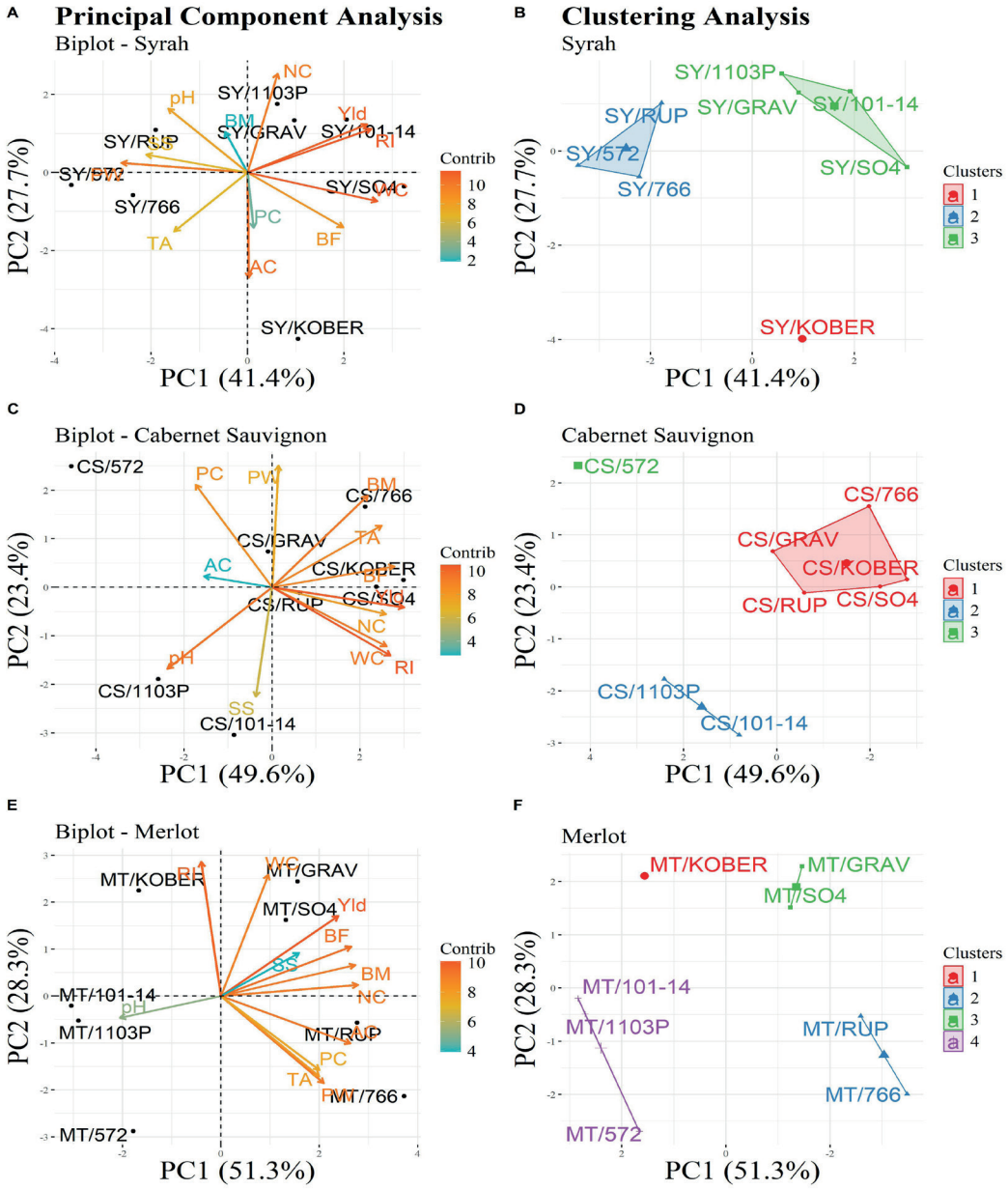



**FIGURE 5.** Principal component and clustering analysis performed with 12 parameters determined on Syrah (A, B), Cabernet-Sauvignon (C, D), and Merlot (E, F) onto different rootstocks under short spur pruning. AC = anthocyanin content, BF = shoot fruitfulness, BM = berry mass, NC = number of clusters, PC = phenolic content, pH = pH, PW = pruning weight, RI = Ravaz index, SS = soluble solids, TA = titratable acidity, WC = weight of cluster, Yld = yield.

Significant interactions were observed between factors regarding the number and weight of clusters and yield (Figure 7). In Syrah vines, the heaviest clusters were induced by SO4, while no differences among other rootstocks (Figure 7A). Merlot vines grafted onto SO4 also exhibited the highest cluster weight, followed by IAC 766, IAC 572, KOBER, RUPESTRIS, which induced intermediate values, while 1103 P and 101-14 induced the lowest values. In Cabernet-Sauvignon, the weight of the cluster increased with IAC 766, IAC 572 and GRAVESAC (Figure 7A). The cluster weight of Merlot was consistently higher than Syrah and Cabernet-Sauvignon in all rootstocks except for 101-14, which induced similar values between Syrah and Merlot.

The number of clusters of Syrah vines was only reduced for 1103 P, while in Merlot vines, it was only increased by IAC 766. In Cabernet-Sauvignon vines, SO4, KOBER, RUPESTRIS, GRAVESAC and 101-14 induced higher cluster number than 1103 P, IAC 766 and IAC 572 (Figure 7B). Except for 1103 P, which did not induce differences among cultivars, Syrah vines always consistently showed the highest number of clusters compared to Merlot, while KOBER, RUPESTRIS and GRAVESAC did not induce differences between Cabernet-Sauvignon and Syrah. Merlot vines only showed higher cluster number than Cabernet-Sauvignon when grafted onto IAC 766 (Figure 7B).

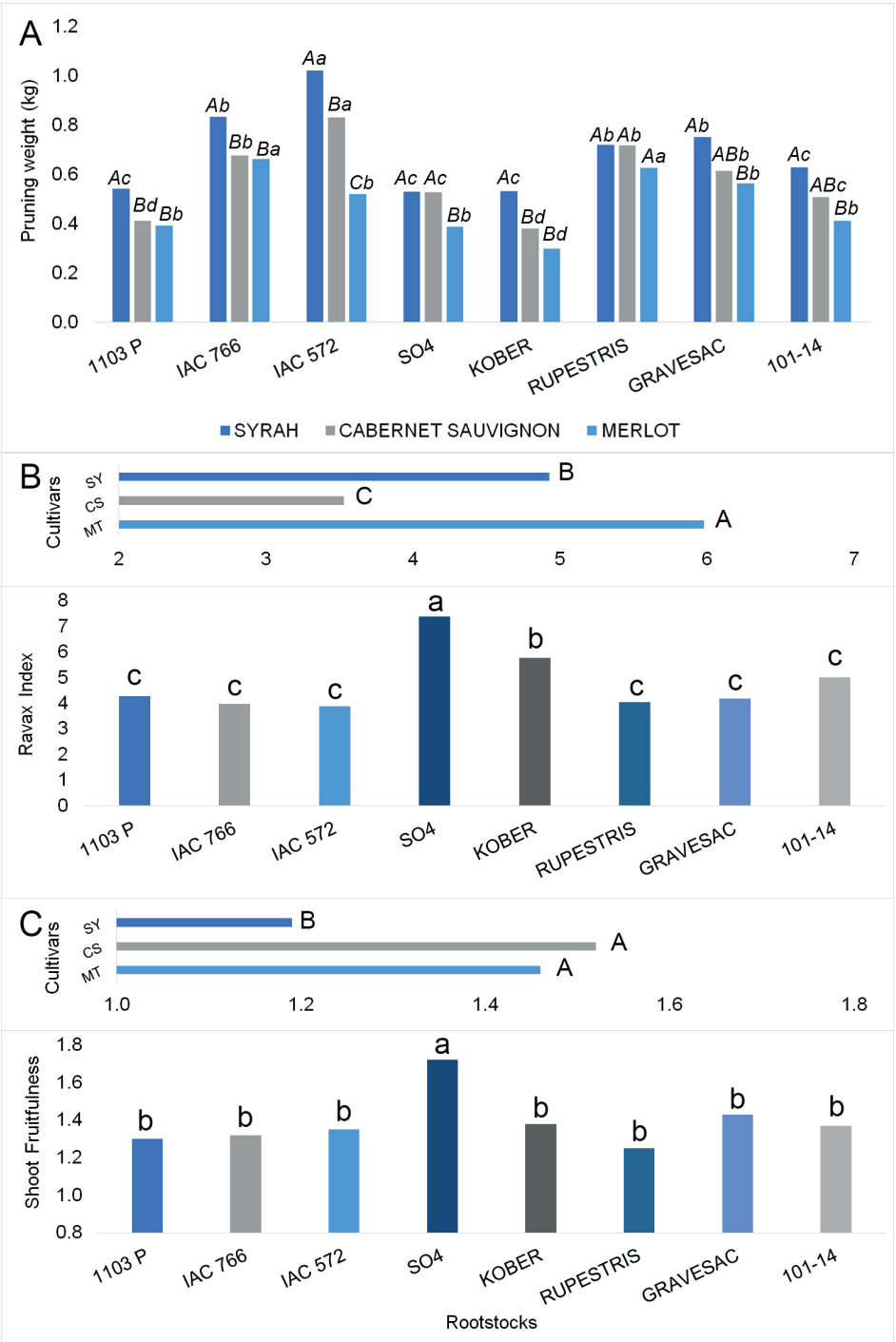



**FIGURE 6.** Pruning weight (A), Ravaz index (B), and shoot fruitfulness (C) of long spur pruned Syrah, Cabernet-Sauvignon, and Merlot vines, grafted onto different rootstocks. Means followed by the same letters, uppercase among cultivars and lowercase among rootstocks, are not statistically different ($p < 0.05$). Values are the means of the 2021 and 2022 seasons, except for shoot fruitfulness (only the 2021 season).

Merlot under 1103 P, 101-14 and KOBER and Syrah under 1103 P combination exhibited the lowest yield among rootstocks (around 2 kg per vine), whereas SO4, IAC 766, IAC 572, SO4 and RUPESTRIS induced the highest yield (> 2.5 kg per vine) in both cultivars. No differences among rootstocks were observed in Cabernet-Sauvignon vines, with yield ranging from 1.5 to 2 kg per vine (Figure 7C). The yield of Syrah and Merlot grafted onto 1103 P, IAC 766, IAC 572, SO4, RUPESTRIS and GRAVESAC was higher than Cabernet-Sauvignon, while 101-14 and KOBER induced similar yield between Cabernet-Sauvignon and Merlot (Figure 7C).

Regarding grape composition, significant interactions between rootstocks and cultivars were found only for soluble solids and anthocyanins (Figure 8). Berry mass from all cultivars grafted onto SO4, IAC 572, IAC 766, and RUPESTRIS was higher (1.3 g) than berries from

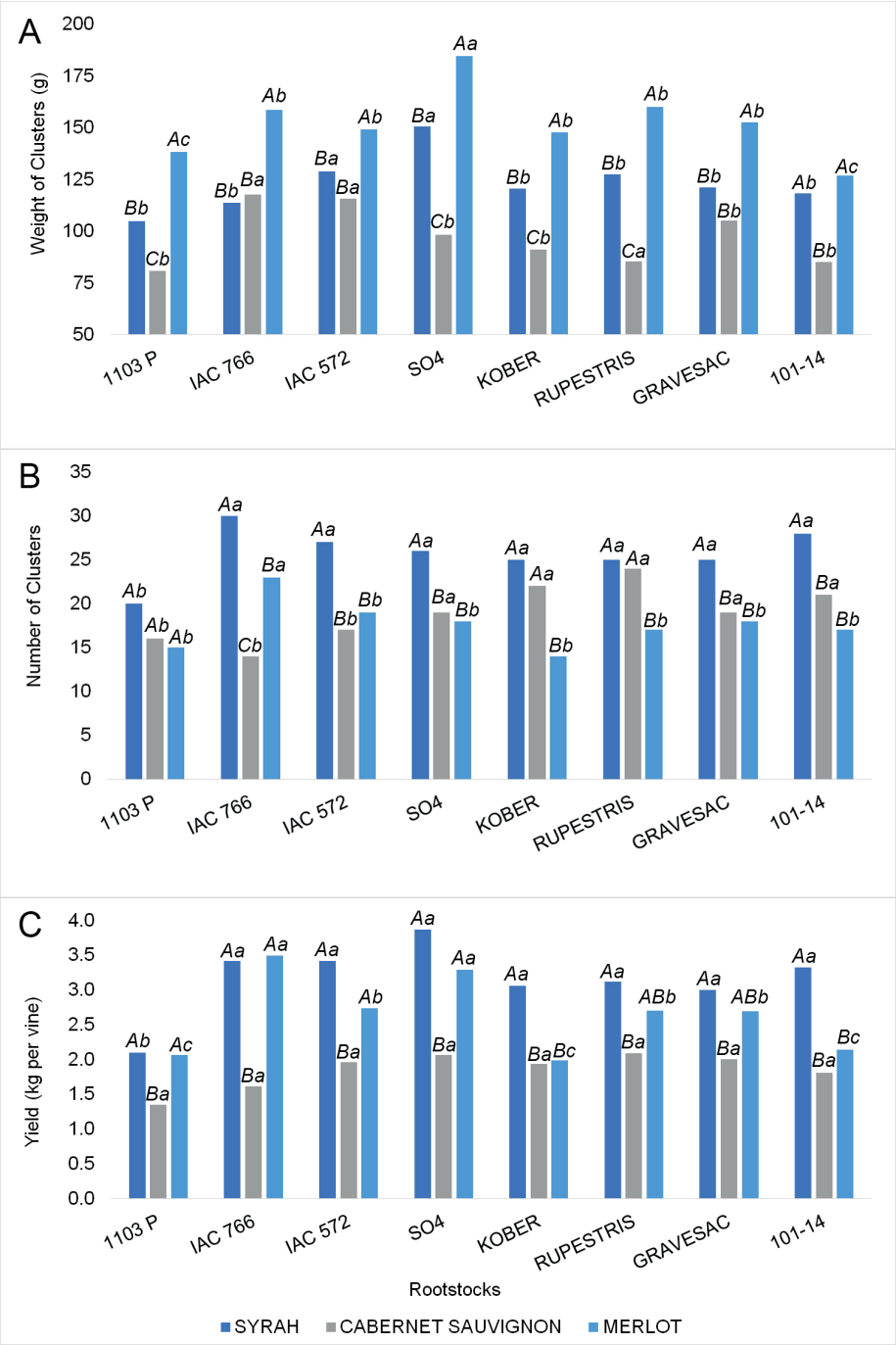



**FIGURE 7.** Weight of clusters (A), number of clusters (B), and yield (C) per vine of long spur pruned Syrah, Cabernet-Sauvignon, and Merlot vines grafted onto different rootstocks. Means followed by the same letters, uppercase among cultivars and lowercase among rootstocks, are not statistically different ($p < 0.05$). Values are the means of the 2021 and 2022 seasons.

vines grafted onto GRAVESAC, 1103 P, KOBER and 101-14 (1.2 g) (Figure 8A). Merlot and Syrah exhibited greater berry mass (1.3 g) than Cabernet-Sauvignon (1.1 g) in all rootstocks (Figure 8A).

No differences among cultivars were detected when vines were grafted onto 1103 P, IAC 766, IAC 572, KOBER and GRAVESAC, with mean soluble solids of 23.5 °Brix (Figure 8B). The highest content was verified on Cabernet-Sauvignon grafted onto RUPESTRIS and 101-14 (~24.1 °Brix) compared to Merlot and Syrah in the same rootstocks (~22.7 °Brix). Under SO4, the most significant differences were found between Cabernet-Sauvignon (22.9 °Brix) and Syrah (21.6 °Brix) (Figure 8B). In Syrah vines, the highest sugar content (> 23 °Brix) was induced by IAC 766, IAC 572 and GRAVESAC, whereas SO4 induced the lowest values (21.6 °Brix). Cabernet-Sauvignon grafted onto IAC 766, RUPESTRIS, GRAVESAC and 101-14 exhibited the highest soluble solids, while in Merlot, no differences were noted among rootstocks (~22.8 °Brix) (Figure 8B).

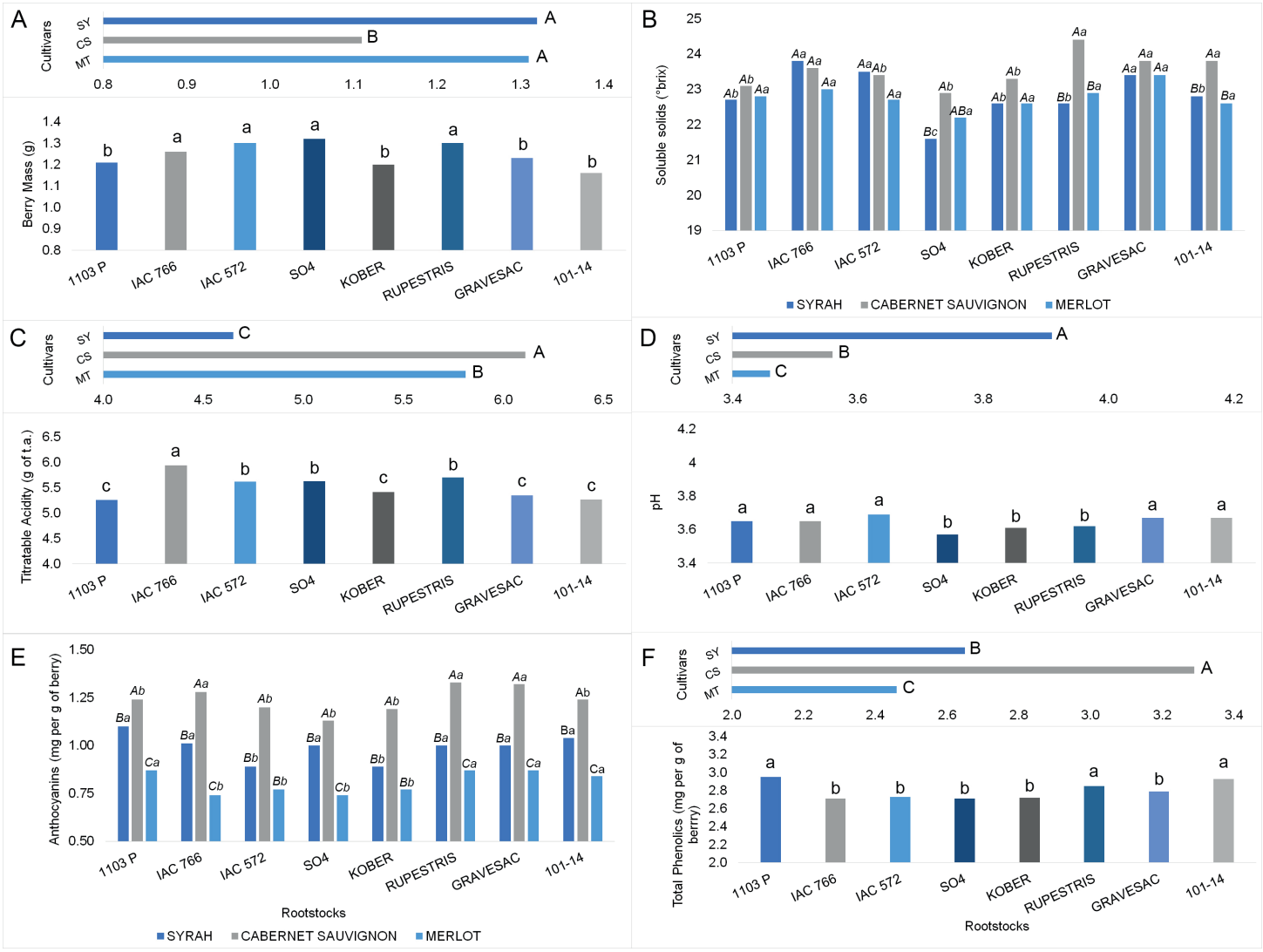



**FIGURE 8.** Berry mass (A), soluble solids (B), titratable acidity (C), pH (D), anthocyanins (E), and total phenolics (F) of long spur pruned Syrah, Cabernet-Sauvignon, and Merlot vines grafted onto different rootstocks. Means followed by the same letters, uppercase among cultivars and lowercase among rootstocks, are not statistically different ($p < 0.05$). Values are the means of the 2021 and 2022 seasons.

The lowest (4.65 g of tartaric acid) and the highest (6 g of tartaric acid) titratable acidity were recorded in Syrah and Cabernet-Sauvignon, respectively (Figure 8C). Berries from all cultivars grafted onto IAC 766 exhibited the highest titratable acidity values (6 g of tartaric acid), while 1103 P, KOBER, GRAVESAC and 101-14 induced the lowest values (~5.3 g tartaric acid) (Figure 8C). All rootstocks induced the lowest pH values, which is desirable for winemaking, particularly when grafted onto SO4, KOBER and RUPESTRIS (~3.6). Among cultivars, Syrah showed the highest pH (> 3.9) (Figure 8D).

The highest anthocyanin content was found in Cabernet-Sauvignon berries, while Merlot presented the lowest values regardless of the rootstocks. Among rootstocks, only KOBER and IAC 572 did not induce differences between Syrah and Merlot (Figure 8E). IAC 766, RUPESTRIS and GRAVESAC increased anthocyanins in Cabernet-Sauvignon berries, while the content was increased by 1103 P, RUPESTRIS, GRAVESAC, and 101-14 in berries of Merlot and was reduced by IAC 572 and KOBER in berries of Syrah (Figure 8E). Total phenolic content was increased when all cultivars were grafted onto 1103 P (2.95 mg per g of berry), 101-14 (2.93 mg per g of berry) and RUPESTRIS (2.85 mg per g of berry). Cabernet-Sauvignon exhibited the highest values (3.29 mg per g of berry), followed by Syrah (2.65 mg per g of berry) and Merlot (3.29 mg per g of berry), regardless of rootstocks (Figure 8F).

The first two principal components of the PCA performed with all 12 parameters accounted for 73.88 %, 62.28 % and 73.27 % of the total variance for Syrah, Cabernet-Sauvignon, and Merlot, respectively. Vigour and yield parameters were associated with PC 1, and grape quality parameters were linked to PC 2, specifically for Syrah and Merlot, whereas grape quality and vigour were related to PC 1 for Cabernet-Sauvignon, as indicated by coloured arrows in the biplot (Figures 9A, 9C and 9E).

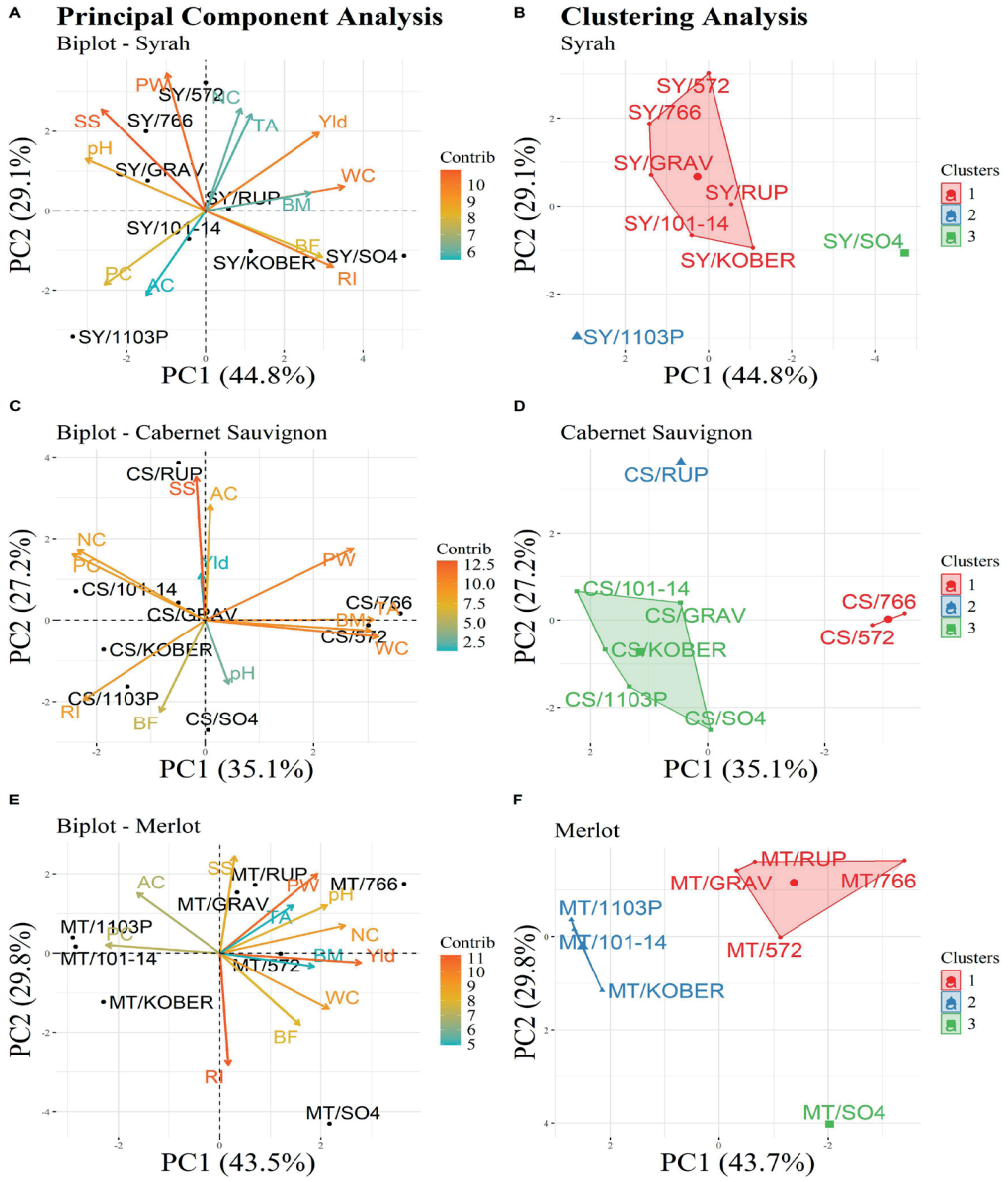



**FIGURE 9.** Principal component and clustering analysis performed with 12 parameters determined on Syrah (A, B), Cabernet-Sauvignon (C, D), and Merlot (E, F) onto different rootstocks under long spur pruning. AC = anthocyanin content, BF = shoot fruitfulness, BM = berry mass, NC = number of clusters, PC = phenolic content, pH = pH, PW = pruning weight, RI = Ravaz index, SS = soluble solids, TA = titratable acidity, WC = weight of cluster, Yld = yield.

The clustering analysis (CA) categorised rootstocks into three clusters for each cultivar. Under long spur pruning, the same range of rootstocks prevailed for Merlot vines when compared to spur pruning seasons, grouping IAC 766, SO4, RUPESTRIS, GRAVESAC and IAC 572, characterised by increased yield without impairment in technological grape parameters such as higher soluble solids and titratable acidity. Syrah and Merlot grafted onto 101-14 and 1103 P exhibited greater anthocyanins and total phenolics; however, this accumulation may result from lower yield, indicated by opposite arrows in the PCA. IAC 572 improved on yield parameters for all cultivars under long pruning, while SO4 remained distinct, mainly characterised by greater shoot fruitfulness and consequently greater yield. Regarding Cabernet-Sauvignon, higher yield did not compromise anthocyanins and sugar content when vines were grafted onto RUPESTRIS (green group, Figure 9D). Additionally, greater titratable acidity was observed when grafted onto IAC 766 and IAC 572 (blue group), and the red group presented greater total phenolics (Figures 9B, 9D and 9F).

## DISCUSSION

### 1. Rootstock effects on vigour and yield

This study reported how rootstocks affected ecophysiological traits, vegetative and reproductive development of non-irrigated field-grown grapevines during the winter growing cycle in a warm temperate zone defined as Cwa type, according to the Köppen climate classification (Alvares *et al.*, 2013).

In contrast to traditional wine growing regions, where grapevines are submitted to only one annual yield pruning and grapes ripe under high temperature and vapor pressure deficit of the summer, in this study, Syrah, Cabernet-Sauvignon, and Merlot grafted onto different rootstocks were pruned twice a year and the ripening period occurred under low temperature and evapotranspiration demand of the driest months of the year (from May to August). From initial vegetative growth (January) to berry development (April), the average of four seasons of mean monthly precipitation and maximum and minimum temperatures, predicted by NASA POWER, were higher (132 mm, 26 °C and 17 °C, respectively) than during the ripening period (20 mm, 24 °C and 10 °C).

Literature reports that high vigour may affect negatively yield and grape quality; several authors have demonstrated an inverse relationship between grapevine vigour, yield and the resulting composition of the must in terms of soluble solids, tannins and polyphenols (Vasconcelos *et al.*, 2009; Prajitna *et al.*, 2007; Bonilla *et al.*, 2015; Dobrei *et al.*, 2016). However, our results pointed the opposite way, where high vigour rootstocks such as IAC 766 had no negative effect on the yield of all cultivars, as well as SO4, RUPESTRIS, GRAVESAC, and KOBER. The response of the vines with these rootstocks to the double pruning management and the climatic conditions of subtropical regions can be explained by the fact that even when grapes ripen in the autumn/winter conditions, low rainfall and temperature did not affect grape composition, as no water stress was observed and suggesting the maintenance of sufficient photosynthesis. According to Champagnol (1984) and Campos *et al.* (2016), grapevines achieve maximum photosynthetic efficiency with light intensities between 700 and 1,100 µmol $m^{-2}$ $s^{-1}$, and during the ripening period, ambient sunlight was approximately 1,750 µmol $m^{-2}$ $s^{-1}$ (data not shown). In addition, the soil of the experiment site was a typic hapludult, characterised as a deep and clayey soil which retained available water during this period.

The yield improvement attributed to rootstocks is strongly correlated with the increase in the number of bunches ($r = 0.89$, 0.88 and 0.78, $p < 0.05$) rather than the increase in the weight of bunches ($r = 0.38$, 0.58 and 0.68, $p < 0.05$) for Cabernet-Sauvignon, Merlot, and Syrah, respectively. As for yield, high vigour did not compromise the mass of the berry, with above 1 g for all treatments, with a low variance, up to 1.4 g. This is very important since berry size is one of the factors which determines the quality of the wine grape (Conde *et al.*, 2007). This might be related to the vegetative cycle, which lasts around four to five months (September to February), associated with a post-harvest period of 20 to 30 days, allowing vines to accumulate reserves for the next reproductive cycle and balance productivity and grape quality. In contrast, under traditional management, vines have only a post-harvest period to accumulate reserves, which is known to be short (around two months), since climate tends to induce vine dormancy due to temperature reductions during fall and winter. Additionally, Keller *et al.* (2005) also observed that vines with greater pruning weight correlated positively with crop level (fruit weight per vine), and the authors report that large vines are capable of supporting heavier crops.

Syrah vines presented greater productivity (~12 t $ha^{-1}$) than Cabernet-Sauvignon and Merlot; however, rootstocks also induced significant productivity in both cultivars, averaging 7.5 and 10 t $ha^{-1}$, respectively. Syrah is a vigorous vine variety capable of yielding large quantities of grapes on a single vine; this behaviour was expected by its historically high yield under double pruning management, as observed in several studies (Favero *et al.*, 2011; Regina *et al.*, 2011; Dias *et al.*, 2012; Dias *et al.*, 2017). In fact, the effect of scion on rootstocks still requires evaluation; the scion depends on the rootstock for water and mineral nutrients, while the rootstock depends on the scion for photosynthetic assimilates (Kocsis *et al.*, 2012). In this study, interaction was only verified for pruning weight, soluble solids, yield and anthocyanin content, with the last two parameters observed only in the third and fourth seasons, likely influenced by spur pruning and vine age.

In this context, the selection of rootstocks under double-pruning management will be related to yield and specific characteristics induced by the rootstock. For instance, IAC 766, GRAVESAC, and RUPESTRIS improved the vigour of Syrah, Cabernet-Sauvignon and Merlot, as shown by pruning weight, while being classified as highly vigorous (Satisha *et al.*, 2010; Souza *et al.*, 2015; Silva *et al.*, 2018). These rootstocks improved yield without impairment in grape quality. Furthermore, IAC 766 is considered a tropical rootstock well adapted to Brazil's climatic conditions, while GRAVESAC is known to be a rootstock adapted to acidic soils, a very common characteristic in Brazilian soils (Pommer, 2000; Aguiar *et al.*, 2006; Audeguin *et al.*, 2024).

Another example of the rootstock effect is the SO4, which increased shoot fruitfulness across all cultivars, in some cases nearly reaching two clusters per branch. Vines grafted onto SO4 may induce a better carbohydrate balance, as evidenced by the increase in vegetative vigour with all rootstocks when long spur pruned, except with SO4, which remained steady. A favourable effect of SO4 was noted by Miele and Rizzon (2017) on shoot fruitfulness in Cabernet-Sauvignon vines under traditional management. In addition, in our study, SO4 also induced a greater and optimal Ravaz index (between 5 and 10), like KOBER and 101-14, while IAC 766, IAC 572, RUPESTRIS, and GRAVESAC presented values lower than 5, indicating undercropped vines. SO4 and KOBER share the same parentage between *V. riparia* × *V. berlandieri*, perhaps a suitable crossbreed that affords a better yield/vigour ratio.

Nonetheless, according to Kliewer and Dokoozlian (2005), an index higher than 10 indicates excessive yield, while below 5 indicates excessive vigour. However, this index is questionable when applied to double pruning management under subtropical climate conditions, since high vigour did not impact yield and grape quality as demonstrated.

In fact, under subtropical climates, vines tend to accumulate reserves, and canopy development leads to increased pruning weight, with yields exceeding 1.5 kg, which are optimal values for this parameter.

It is crucial to emphasise that, although 1103 P is a moderate vigour/yielding rootstock, as reported in several studies (Leão & Chaves, 2019; Clingeleffer *et al.*, 2021; Lo'ay *et al.*, 2021; Edwards *et al.*, 2022), and it possesses several agronomic characteristics of interests, under double pruning, low vigour, and productivity was verified when all cultivars were grafted onto it, particularly Cabernet-Sauvignon, even when high yield was stimulated through long spur pruning. These results align with studies by Dias *et al.* (2017) and Walker *et al.* (2019), which reported low yield with this rootstock. Moreover, in a scenario where other rootstocks like IAC 766, SO4, RUPESTRIS, and GRAVESAC can enhance yield without affecting grape quality, vine growers can pursue other rootstock options to increase yield, contrary to the current popularity of 1103 P in viticulture worldwide.

Conversely, when a long spur was implemented, the increase in shoot fertility and yield was stimulated by the rise in the number of shoots per vine. Syrah, Cabernet-Sauvignon, and Merlot raised shoots from 14, 11, and 7 to 18, 17, and 16, respectively. These values, combined with an increment of 0.3 in shoot fertility, raised the expected number of clusters from 14, 11, and 7 up to 23, 22, and 20 clusters for Syrah, Cabernet-Sauvignon, and Merlot, respectively. IAC 572 is commonly used to graft table grapes in Brazil, and grafting fine wine varieties requires further evaluation, focused on yield/vigour balance, as this rootstock is often employed to expedite vineyard establishment, reducing financial investment years until the first harvest.

IAC 572 is well recognised as the most invigorating rootstock in Brazil (Pommer, 2000; Camargo, 2003). The low productivity found in the first two seasons could be related to low shoot fruitfulness, probably caused by competition for carbohydrate reserves between active growing shoots and the development of the buds and flowering induction. According to Vasconcelos and Castagnoli (2000), competition between vegetative and reproductive growth causes a reduction in bud fertility. Yield reduction was also verified by Leão and Chaves (2019), where the Syrah/IAC 572 combination presented the lowest values among treatments.

## 2. Rootstock effect on grape composition

As with yield parameters, vigour had no significant impact on quality parameters. Although yield varied by 16 %, 12 % and 20 % among rootstocks for Syrah, Cabernet-Sauvignon, and Merlot, respectively, quality parameters for all rootstocks only fluctuated from 1.5 % to 3 % (soluble solids), 3.5 % to 4 % (acidity), 4.5 % to 7 % (anthocyanins), and 3 % to 4 % (total phenolics) under both pruning managements. Despite some differences among rootstocks, soluble solids exceeded 20 °Brix in all cultivar and rootstock combinations, with some cases reaching around 24 °Brix. Under long spur pruning, only the Syrah/SO4 combination did not achieve 22 °Brix, which may be associated with the yield amount (4 kg per vine) for this treatment. Grapes harvested during autumn-winter present better sugar content, due to ripening occurring in the driest months of the year, as found by Mota *et al.* (2010) and Favero *et al.* (2011), under double pruning management. Similar results were obtained by Keller *et al.* (2005), where there is no clear relationship between crop level and soluble solids or titratable acidity even when yield exceeds 10 kg per vine in Cabernet-Sauvignon, Riesling, and Chenin blanc.

For both spur pruning and most cultivars, it was verified in the PCA that IAC 766 and RUPESTRIS were often in the same grouping, characterised by inducing high vigour, yield, and titratable acidity. The higher titratable acidity provided by both rootstocks may be related to higher vigour that leads to a higher leaf area, preventing bunch exposure to sunlight, and temperature. Several studies (Staden *et al.*, 2005; Gouot *et al.*, 2019; Cabodevilla *et al.*, 2024) report that malic acid declines during ripening due to dilution and respiration. Viticultural practices, the prevailing climate and grape cluster environments may directly affect respiration, for instance protecting the bunches with a larger canopy. Another possibility is the effect of higher leaf area on faster sugar accumulation when compared to the other rootstocks, across seasons and cultivars. These rootstocks reached a high sugar content (> 22 °Brix) and were harvested 6 to 10 days earlier, which kept a higher acidity content.

On the other hand, cultivars grafted onto SO4 also presented high acidity, although SO4 was among the last rootstocks to be harvested, which could be related to SO4's ability to synthesise organic acids. According to Zhang *et al.* (2023), 110 R and SO4 increased NAD-MDH enzyme activity in grape clusters compared to self-rooted Cabernet-Sauvignon vines, and the impact of different rootstocks on enzyme activity was highly correlated with the impact on malic acid synthesis. Further studies are required to quantify the organic acid enzymes.

Organic acid degradation may also correlate with scion genotype, as each variety responds differently to temperature (Sweetman *et al.*, 2014). Total acidity was optimal in Cabernet-Sauvignon and Merlot grapes but low for Syrah. Syrah also presented higher pH, around 4, which is undesirable because wine pH is narrowly tied with microbiological and physiochemical stability, affecting microorganism's selection and some essential chemical reactions (Comuzzo & Battistutta, 2019), such as phenol levels and wine colour (Kontoudakis *et al.*, 2011; Forino *et al.*, 2020).

Syrah grapes appear to exhibit a different rate of sugar accumulation and organic acids degradation, impairing the acidity content and increasing the pH. This is likely due to berry shrivelling, a phenomenon commonly associated to this variety, caused by a progressive physiological ceasing of xylem/phloem vascular water transport to the berry and a responsiveness of the berry to imminent environmental factors that may increase water vapor deficit, berry transpiration and dehydration, and finally a loss of

functionality that affects physical and chemical ripening dynamics (Carlomagno *et al.*, 2018). In this sense, to increase sugar content, the maturation period is extended, compromising both titratable acidity and pH. In general, to produce quality red wines, the recommended characteristics are soluble solids above 20 °Brix, total acidity in the range of 6 to 7.5 $gL^{-1}$, and pH between 3.3 and 3.6 (Jackson, 2020). For most treatments, these parameter values were achieved.

Rootstocks could influence the levels of grape components and affect the synthesis of phenolic compounds in grape berries due to their strong effects on vegetative growth and reproductive parameters of the scion and their impacts on canopy microclimate and berry technological maturity (Main *et al.*, 2002; Cheng *et al.*, 2017; Olarte *et al.*, 2018). However, in this study, no significant correlation was verified among yield and soluble solids, acidity, anthocyanins and total phenolics (data not shown) for Merlot and Cabernet-Sauvignon. In Syrah, yield and phenolics showed a moderate negative Pearson's correlation (–0.5, $p < 0.05$), and linear correlation indicated a decay in phenolics only when yield exceeded 3 kg per vine ($y = –0.2 + 3.2797$; $R^2 = 0.6$).

These results may be related to crop level across seasons. During 2018 and 2019, the yield did not surpass 2.5 kg among cultivars, while in the 2021 and 2022 seasons, the Cabernet-Sauvignon and Merlot means among rootstocks were 1.8 and 2.6 kg per vine, respectively, whereas Syrah presented a mean of 3.2 kg per vine, with the highest yield when grafted with SO4. In addition, climatic conditions provided optimal conditions for grape maturation, evidenced by the absence of thermal or water stress, which could impact phenolic and anthocyanin accumulation. In the cluster analysis, 1103 P and 101-14, without exception, were grouped together, categorised by lower yielding and higher values of total phenols and anthocyanins. These results align with studies from Nikolau *et al.* (2021), Fayek *et al.* (2022a), and Fayek *et al.* (2022b), but the phenolic content was higher by only 2 % to 8 % compared to RUPESTRIS and IAC 766, which were found as invigorating rootstocks in this study. In addition, Wallis *et al.* (2013) report that improved vigour and nutrient uptake could, in turn, provide greater resources needed for the production of secondary metabolites such as phenolics. Dias *et al.* (2017) also found no clear separation between rootstocks over phenolic composition of Syrah under double pruning management, where IAC 766 and 1103 P presented similar phenolic content. In this sense, moderate to high vigorous rootstocks are suitable for double-pruning management linked to a grape bunch removal when necessary to avoid quality impairment.

Finally, the grape quality parameters identified in this study indicate that these varieties present a similar grape composition compared to values from important and recognised viticultural regions, such as Syrah in Australia (Antalick *et al.*, 2015) and Merlot under cool climates (Karoglan *et al.*, 2014). Parameters were even better than Cabernet-Sauvignon under traditional harvest conditions in highland regions of southern Brazil (Miele & Rizzon 2017; Marcon Filho *et al.*, 2019).

## CONCLUSION

The yield and grape quality of Syrah, Cabernet-Sauvignon, and Merlot grapevines under double pruning management could be improved using medium to high vigorous rootstocks. For all cultivars, the combination with IAC 766, SO4, RUPESTRIS, GRAVESAC, 101-14, and KOBER increased yield with no impairment in grape maturation. IAC 572 can be used under double-pruning management with Merlot and Cabernet-Sauvignon when long spur pruned. However, grafting must be carefully recommended, because this rootstock is extremely vigorous and can affect the balance between vegetative and reproductive vigour.

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