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Shoot topping of 'Niagara Rosada' grapevine grafted onto different rootstocks

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Abstract

Vineyard cultural practices may influence amount of sugars, acids, phenolic compounds and antioxidant activity of grapes. Thus, the purpose of this study was to evaluate the effect of different shoot topping levels on the chemical characteristics of the grapes during its maturation, yield and the physicochemical characteristics and phenolic compounds of 'Niagara Rosada' grapevine grafted onto different rootstocks. The experiment was conducted in a 2-year-old vineyard of Niagara Rosada cultivar in Southeast of Brazil. The vines were trained on a unilateral cordon system in a vertical shoot positioning. The manual shoot topping was carried by leaving 6, 7, 8 or 9 leaves above the last cluster. Vines were topped 60 days after pruning, when the vines were in the beginning of bunch closure. All vines were grafted onto 'IAC 766' and 'IAC 572' rootstocks. Fruit maturation curve, yield and physicochemical grapes characteristics were analyzed to determine the effects of shoot topping levels and rootstocks. The rootstocks and the different shoot topping levels did not influence the yield, productivity and cycle duration of 'Niagara Rosada' grapevines. Larger and heavier clusters and berries were harvested from vines grafted on 'IAC 766' rootstock. However, grapes harvested from grapevines grafted on 'IAC 572' rootstock presented higher total anthocyanins content. The shoot topping carried out with 7 or 8 leaves above the latest cluster provided higher sugars, total polyphenols content and antioxidant activity.

Keywords: canopy management, green pruning, leaf removal, phenolic compounds, *Vitis labrusca* L.
Abbreviations: DAP_days after pruning; LAC_leaves above the last cluster; SS_soluble solids;
TA_titratable acidity

Introduction

Part of the success of viticulture in Brazil is due to the grapevine responses to certain handling practices, such as irrigation, nutrition and also pest and disease control (Dardeniz et al., 2008). However, there is still a great diversity of handling practices on grapevine shoot toppings that needs to be further explored in order to contribute to an enhanced production and, above all, grapes quality. The study of vineyard cultural practices that may influence the content of sugars, acids, phenolic compounds and antioxidant activity of grapes is what we desired (Mota et al., 2010).

Among the main practices that aim to modify the vegetative canopy of the vineyards, different green pruning modalities, such as sprouting, shoot topping and defoliation can highlight. Regarding shoot toppings, its purpose is to limit the vegetative growth of vines by removing the final part from herbaceous branches and a variable number of young underlying leaves (Miele and Mandelli, 2012). This is a common practice in grapevine cultivars in Brazil (*Vitis vinifera* L.), especially in grapes for winemaking purposes (Brighenti et al., 2010). However, there is little information about effect of this practice on the production and quality of table grapes, especially on Niagara Rosada cultivar (*V. labrusca* L. x *V. vinifera* L.). 'Niagara Rosada' is a very important grape in Brazil, as an alternative for small producers to aim their production for local markets supply.

Studies have shown that the effect of shoot topping on grapevines may vary, mainly, regarding the cultivar, season of enforcement and its intensity (Dardeniz et al., 2008). This practice may be carried out by keeping a different number of leaves per shoot, which can vary from the shoot elimination up until the maintenance of only two leaves above the last cluster. This should be a rigorous choice, since if performed improperly it can affect negatively the vine development (Miele and Mandelli, 2012).

The agronomic and physiological characteristics of grapevines, such as yield, vigor, size of clusters and berries, sugar and fruits acidity content and other important compounds for their quality can also be influenced by rootstocks (Dias et al., 2012; Pozzan et al., 2012; Bruna and Back, 2015). The choice of rootstock in viticulture depends on the soil conditions of each producing region and, within a region, it can undergo many variations. This makes it a difficult choice and research papers should be repeatedly done for each specific growing site (Leão et al., 2011).

Several studies have been carried out aiming to assess the 'Niagara Rosada' grapevines on different rootstocks, making its influence over vegetative growth, productivity, plants nutritional aspects and the clusters characteristics very clear (Alvarenga et al., 2002; Mota et al., 2009; Tecchio et al., 2011; Bruna and Back, 2015). However, there are few studies on the effect of rootstocks on phenolic compounds content and grapes antioxidant activity.

The knowledge about the vigor that the rootstock provides to the canopy is of fundamental importance for the ideal combination choice between canopy and rootstock, and for the production techniques to be adopted. Adequate vigor, without excessing or lacking productivity, must be seek by adopting suitable production techniques for each combination, such as pruning, green pruning and special winter pruning with a higher number of buds on more vigorous plants (Alvarenga et al., 2002; Bruna and Back, 2015).

Despite the importance of 'Niagara Rosada' grapevines for Brazilian viticulture, most of the studies involving the rootstock effect on this cultivar are related only to the agronomic characteristics. There is also very little research on shoot topping effects, both on the production characteristics and post-harvest quality of this grape. In this context, the purpose of this study was to evaluate the effect of different shoots topping levels on the chemical characteristics of the grapes during its maturation, yield and the physicochemical characteristics and phenolic compounds of 'Niagara Rosada' grapevine grafted onto different rootstocks.

Results

Fruit maturation curve

There was no significant interaction between shoot topping levels and days after pruning (DAP) on the evolution of the chemical characteristics of 'Niagara Rosada' grape in both rootstocks. After analyzing the isolated effect of time, then quadratic regression models were adjusted in order to express the increase in pH, soluble solids content and maturation index of the grape must. Then the same quadratic regression model was adjusted to express the reduction in the must titratable acidity (Fig 1). At the maximum maturation point at 138 DAP, the solids content and titratable acidity of 'Niagara Rosada' grapes on 'IAC 766' and 'IAC 572' rootstocks were 16.62 and 15.93 °Brix, and 0.56 and 0.61% tartaric acid, respectively.

There was a significant difference among the shoot levels only regarding the grape must titratable acidity of 'Niagara Rosada' grafted on 'IAC 766' (Table 1). The lowest value was found in grape must whose grapevines were topped with 8 leaves above the last cluster (LAC) (0.90%), and the highest value when we carried out the shoot topping maintaining 9 LAC (1.05%). The titratable acidity average value we found when vines were grafted on 'IAC 572' was of 1.03%.

Yield and physicochemical characteristics of grapes

There was no significant interaction between shoot topping levels and rootstocks regarding all productive and physicochemical characteristics of 'Niagara Rosada' grape (Tables 2 and 3). Thus, these factors were analyzed separately.

The shoot topping had no influence over the productive characteristics of the vines and the physical characteristics of the grapes. The values obtained on yield, productivity and number of clusters per plant were 8.25 clusters, 1.53 kg per plant and 9.54 t ha^{-1}, respectively. There was effect the rootstocks over the fresh mass and width of clusters (Table 2), and over the fresh mass, length and width of berries (Table 3), with the highest values found in grapevines grafted on 'IAC 766'.

Regarding the chemical characteristics of 'Niagara Rosada' grape must, there was no significant effect from 'IAC 766' and 'IAC 572' rootstocks on pH, soluble solids content, titratable acidity and maturation index, with respective values of 3.22; 16.6 °Brix, 0.65% tartaric acid and 25.57. However, when it was observed the effect from different shoot topping levels on these characteristics, it is generally verified that a grape must from grapevines that shoots were topped with 8 LAC presents a higher soluble solids content (17.2 °Brix) and lower titratable acidity (0.63% tartaric acid).

Reducing sugars, total phenolic compounds and antioxidant activity of grapes

'Niagara Rosada' grapes harvested from vines grafted on 'IAC 572' rootstock contain higher reducing sugars and total monomeric anthocyanins. However, there was no effect from rootstocks on the grapes total flavonoids content, total phenolic compounds and antioxidant activity. All of these compounds were significantly influenced by the shoot topping applied (Table 4).

In general, it was found a higher content of reducing sugars, total monomeric anthocyanins, total flavonoids and total polyphenols on grapes whose vines were topped with 7 or 8 LAC. Consequently, these levels also developed a higher *in vitro* antioxidant activity of the 'Niagara Rosada' grape. These compounds content was lower when the shoot topping was performed with 6 LAC.

Discussion

The maturation evolution of 'Niagara Rosada' grapes during this study were in accordance to the standard parameters described in the literature (Sato et al., 2009), what in turn means that, in between the berries change of color and their complete maturation there was an increase in pH, soluble solids and maturation index. During the same period, titratable acidity decreased (Fig 1).

Although the grapes from grapevines grafted on the 'IAC 572' rootstock present a higher soluble solids content and a higher titratable acidity content throughout the whole maturation period, the ideal harvesting time for 'Niagara Rosada' grapes grafted on both rootstock varieties occurred at the same time, that is, 138 days after pruning. A similar result was found in a study conducted along another Brazilian region (Minas Gerais, Brazil) in which the 'IAC 766' and 'IAC 572' rootstocks did not influence the time required for the maturation of 'Niagara Rosada' grapes (Alvarenga et al., 2002). The knowledge about grapes maturation curve is an important tool for the grape grower, since it is possible to

Table 1. pH, soluble solids (SS), titratable acidity (TA) and maturation index (SS/TA) throughout the maturation period of 'Niagara Rosada' grapes over different shoot topping levels. Botucatu, São Paulo, Brazil, 2015/2016.

Shoot topping level (LAC)†	'IAC 766' rootstock				'IAC 572' rootstock			
	pH	SS (°Brix)	TA (%)	SS/TA	pH	SS (°Brix)	TA (%)	SS/TA
6	3.11±0.26a	14.4±2.39a	0.95±0.4ab	19.5±11.1a	3.12±0.27a	13.8±2.29a	0.97±0.44a	17.8±8.92a
7	3.10±0.26a	14.7±2.46a	0.99±0.5ab	19.0±10.1a	3.08±0.27a	14.1±2.43a	1.01±0.44a	17.6±9.15a
8	3.14±0.27a	14.9±2.24a	0.90±0.3b	19.9±9.4a	3.11±0.24a	14.0±2.36a	1.04±0.43a	16.8±7.99a
9	3.06±0.23a	14.0±2.23a	1.05±0.4a	16.4±8.0a	3.08±0.25a	13.6±2.27a	1.08±0.45a	15.4±7.64a
CV (%)	2.50	7.11	10.8	19.4	4.12	8.55	14.9	21.0

Values are the mean (±SD) of four different lots of berries. Values followed by different letters in the same column differ significantly (Tukey test, P<0.05). CV: coefficient of variation. †LAC: leaves above the last cluster.

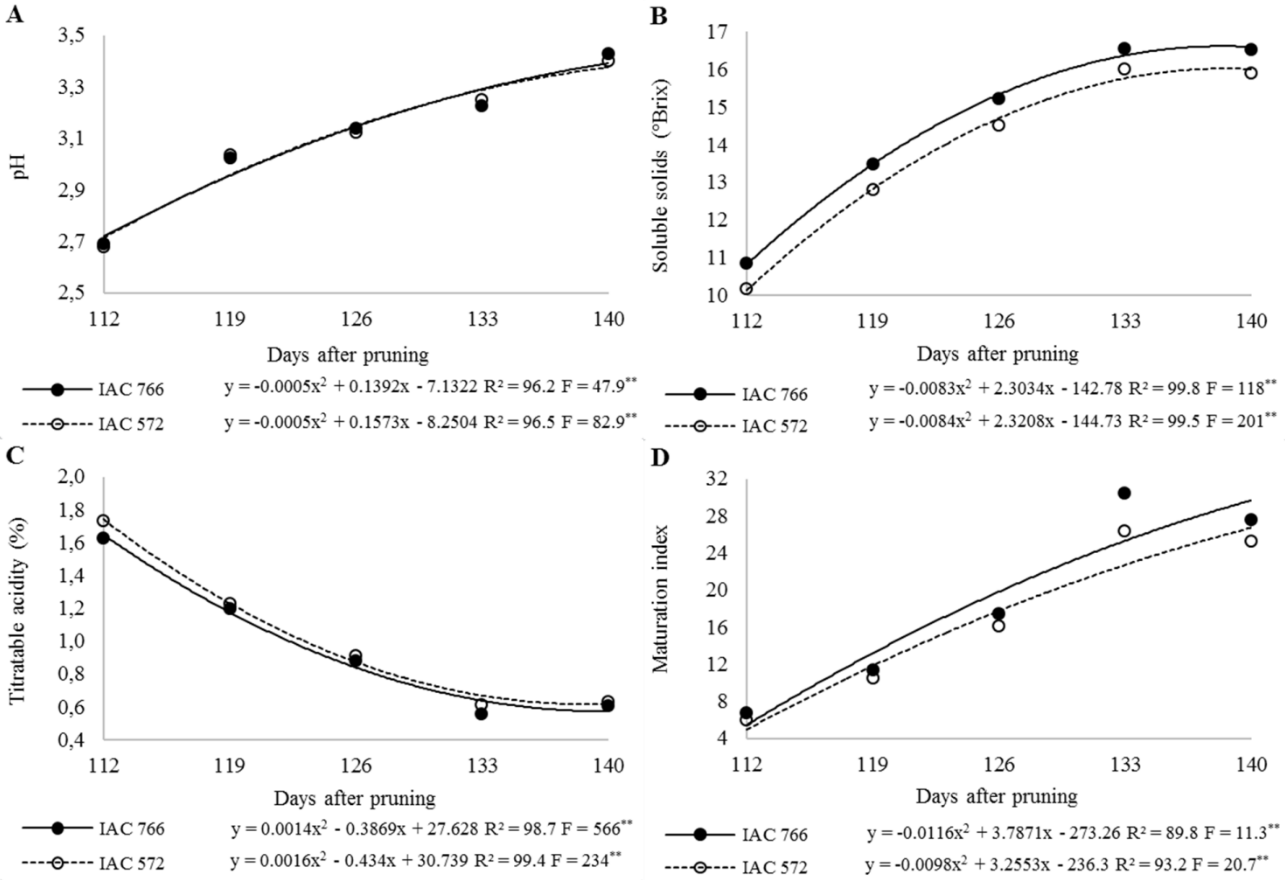

Fig 1. Fruit maturation curve of 'Niagara Rosada' grapes grafted on 'IAC 766' and 'IAC 572' rootstocks over different shoot topping levels. Botucatu, São Paulo, Brazil, 2015/2016. A: pH; B: soluble solids content; C: titratable acidity; D: maturation index.

Table 2. Productive and physical characteristics of 'Niagara Rosada' grapes grafted on 'IAC 766' and 'IAC 572' rootstocks over different shoot topping levels. Botucatu, São Paulo, Brazil, 2015/2016.

Shoot topping level (LAC)†	NCV	YV (kg)	PDT (t ha^{-1})	CM (g)	CL (cm)	CW (cm)	BPC
6	7.95±2.65a	1.58±0.67a	9.86±4.20a	246.2±58.4a	14.97±3.20a	7.46±0.66a	53.92±9.90a
7	8.15±1.42a	1.44±0.40a	9.02±2.48a	237.0±35.9a	12.61±1.14a	7.33±0.48a	50.18±6.71a
8	8.66±2.42a	1.57±0.55a	9.81±3.45a	243.5±38.3a	12.80±1.01a	7.42±0.39a	52.91±8.05a
9	8.24±3.35a	1.51±0.68a	9.46±4.26a	242.5±52.2a	12.67±1.63a	7.46±0.59a	51.02±9.43a
Rootstock							
IAC 766	9.06±2.33a	1.66±0.55a	10.35±3.43a	259.8±46.4a	13.02±1.04a	7.64±0.55a	53.53±9.74a
IAC 572	7.43±2.34a	1.40±0.56a	8.72±3.49a	224.8±37.0b	13.51±5.23a	7.19±0.39b	50.49±9.01a
CV (%)	27.20	38.34	38.28	18.51	27.29	6.96	18.01

Values are the mean (±SD) of four different vines experimental blocks. Values followed by different letters in the same column differ significantly (Tukey test, P<0.05). CV: coefficient of variation. †LAC: leaves above the last cluster. NCV, number of clusters per vine; YV, yield per vine; PDT, productivity; CM, cluster mass; CL, cluster length; CW, cluster width; BPC, number of berries per cluster.

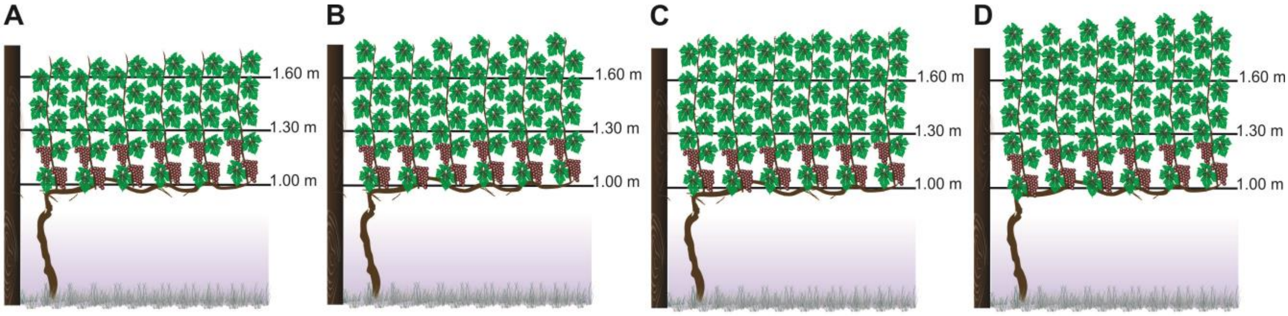

Fig 2. Graphic representation of different shoot topping levels performed on 'Niagara Rosada' grapevines trained on a unilateral cordon system (1 m above soil) in a vertical shoot positioning by means of iron wires. Manual shoot topping carried out maintaining 6 (A), 7 (B), 8 (C) and 9 (D) leaves above the last cluster. Botucatu, São Paulo, Brazil, 2015/2016.

Table 3. Physicochemical characteristics of 'Niagara Rosada' grapes grafted on 'IAC 766' and 'IAC 572' rootstocks over different shoot topping levels. Botucatu, São Paulo, Brazil, 2015/2016.

Shoot topping level (LAC)[1]	BM (g)	BL (cm)	BW (cm)	pH	SS (°Brix)	TA (%)	MI (SS/TA)
6	4.47±0.36a	2.17±0.07a	1.90±0.06a	3.24±0.06ab	15.8±1.0b	0.65±0.05ab	24.7±2.3b
7	4.58±0.40a	2.16±0.07a	1.92±0.06a	3.18±0.08b	17.0±0.8a	0.68±0.01a	24.9±1.3b
8	4.47±0.22a	2.18±0.07a	1.90±0.03a	3.26±0.06a	17.2±0.8a	0.63±0.05b	27.7±2.3a
9	4.62±0.22a	2.18±0.05a	1.91±0.04a	3.19±0.07ab	16.5±0.7ab	0.66±0.04ab	25.1±2.1ab
Rootstock							
IAC 766	4.72±0.23a	2.21±0.04a	1.92±0.05a	3.20±0.07a	16.5±1.1a	0.66±0.04a	25.3±2.9a
IAC 572	4.35±0.25b	2.14±0.06b	1.89±0.04b	3.23±0.07a	16.8±0.8a	0.65±0.04a	25.9±1.6a
CV (%)	4.87	2.48	1.98	1.90	4.26	5.85	7.68

Values are the mean (±SD) of four different vines experimental blocks. Values followed by different letters in the same column differ significantly (Tukey test, *P*<0.05). CV: coefficient of variation. †LAC: leaves above the last cluster. BM, berries mass; BL, berries length; BW, berries width; SS, soluble solids; TA, titratable acidity, expressed as tartaric acid percentage; MI, maturation index (SS/TA ratio).

Table 4. Reducing sugars, phenolics compounds and *in vitro* antioxidant activity of 'Niagara Rosada' grapes grafted on 'IAC 766' and 'IAC 572' rootstocks over different shoot topping levels. Botucatu, São Paulo, Brazil, 2015/2016.

Shoot topping level (LAC)†	RS‡ (%)	TMA§ (mg 100 g^{-1})	TFL¶ (mg 100 g^{-1})	TPC†† (mg 100 g^{-1})	AOX‡‡ (mg g^{-1})
6	11.41±1.08c	4.89±1.01b	20.95±1.17b	106.1±5.4b	2.53±0.11b
7	13.84±1.73ab	5.73±0.63ab	23.92±1.53a	123.9±3.7a	2.98±0.15a
8	15.37±1.40a	5.94±0.70a	24.00±2.51a	124.5±10.0a	2.97±0.30a
9	13.62±1.73b	5.22±1.48ab	21.56±1.35ab	111.7±10.6b	2.80±0.15ab
Rootstock					
IAC 766	13.00±2.13b	4.83±1.06b	22.57±1.90a	116.1±11.1a	2.77±0.22a
IAC 572	14.13±1.82a	6.05±0.59a	22.65±2.42a	117.0±11.4a	2.88±0.29a
CV (%)	8.50	13.45	7.77	6.92	7.17

Values are the mean (±SD) of four different lots of berries. Values followed by different letters in the same column differ significantly (Tukey test, *P*<0.05). CV: coefficient of variation. †LAC: leaves above cluster. ‡Reducing sugar, measures with Somogy-Nelson reagent and expressed as glucose percentage. §Total monomeric anthocyanins quantified by the technic of difference of pH and expressed as equivalent to cyanidin 3-glucoside. ¶Total flavonoids expressed as mg 100^{-1} g equivalent to catechin. ††Total phenolics compounds measured with Folin-Ciocalteau expressed as mg 100^{-1} g equivalent to gallic acid. ‡‡Antioxidant activity determined with DPPH radical scavenging method and expressed mg equivalents of Trolox g^{-1}.

estimate the best time to harvest and attain better quality grapes by the expected time (Sato et al., 2009).

The sugars content found in the grape berry is related to the sucrose produced in the mesophyll of mature leaves through photosynthesis (Conde et al., 2007). In spite of the difference in the number of leaves kept at each shoot topping level, which consequently, presented the treatments different levels of leaf area, they had no influence over the soluble solids content during maturation.

There was no effect regarding the shoot topping levels on yield and, consequently, on the productivity of 'Niagara Rosada' grapevines (Table 2). This effect can be attributed to the fact that shoot topping has no influence on other characteristics that present high correlation with the yield, such as the number of clusters per plant (r = 0.91; p < 0.01), the mass of clusters (r = 0.73; p < 0.01) and the number of berries per cluster (r = 0.71; p < 0.01). Other studies have also shown that different shoot topping levels did not affect the grapevine production (Poni and Giachino, 2000; Leão et al., 2016).

Due to the shoot topping having been applied after the definition of the number of bunches and the inflorescences/clusters had not been removed, it is unlikely that it could have influenced the number of clusters per plant in the current cycle. However, in some cases, this characteristic may increase significantly as a result of shoot topping in later cycles. The use of this practice in a productive cycle can affect the beginnings of inflorescence and, thus, the number of clusters during the next cycle (Collins and Dry, 2009).

Certainly, the number of berries per bunch that were found in this study (Table 2) has not differed either due to the fact that the shoot topping was carried out on 'Niagara Rosada' grapevines after the critical period for berry drop after fruitset, that happens from full flowering up until two weeks after this stage (Nicolosi et al., 2012). Thus, regarding the number of clusters, there might be an influence on the next productive cycle number of flowers, as a result of the effects on the early stages of shoots inflorescence from the current cycle (Dunn and Martin, 2007).

There was no effect regarding the different shoot topping levels on the physical characteristics of the 'Niagara Rosada' clusters and berries. These results were similar to those found by other authors. The cluster fresh mass, length and width from the 'Karasakiz' (*V. vinifera* L.) were not influenced by the grapevine shoot topping (Dardeniz et al., 2008). In the same way, shoot topping showed no effect over the cluster fresh mass from 'Merlot' (*V. vinifera* L.) (Mota et al., 2010) and over the berries fresh mass from 'Chardonay' (*V. vinifera* L.) as well (Collins and Dry, 2009).

The shoot topping effects occurred, mainly, on the grape chemical characteristics (Table 3). Throughout of this study there was an increase in the soluble solids content and a decrease in titratable acidity as the number of leaves per shoot increased from 6 to 8 LAC. Other studies have also demonstrated that the soluble solids content and grape maturation index increase as the number of remaining leaves per cluster on the grapevines is kept high, whilst there is a reduction in titratable acidity at that same level (Dardeniz et al., 2008). However, it was found that when the number of leaves exceeds that in which would be sufficient to nourish the clusters, the berries chemical characteristics can be negatively affected, which, in this study, occurred when it was kept 9 LAC. This effect was also described by Brighenti et al. (2010) in the soluble solids content and total anthocyanin content of the 'Merlot' grape (*V. vinifera* L.).

The low number of leaves per shoot on the grapevines, due to either the shoot topping high intensity or to other practices, such as defoliation, can greatly reduce the photosynthetic area of the plant. This promotes a decrease in the carbohydrates contribution to the clusters and, consequently, compromises the quality of the fruits (Brighenti et al., 2010). The maintenance of only 6 LAC on each 'Niagara Rosada' shoot may have contributed to this effect. The lowest photosynthetic area in this condition certainly reduced the photoassimilates ability of translocating to the clusters, affecting negatively the sugar content, one of the main catalysts for secondary metabolites synthesis, such as total anthocyanins, total flavonoids, total phenolic compounds and, consequently, the grapes antioxidant activity.

Likewise, the decrease in grapes phenolic content when it was carried out the shoot topping maintaining 9 LAC (Table 4) may have been caused by the higher vigor of these shoots. In vigorous shoots there is higher attraction of nutritive substances towards the vegetative apex, which reduces these substances translocation to the clusters, causing damages to both their technological and phenolic maturation (Fregoni, 1998). In addition, although more vigorous shoots produce more carbohydrates, their intake is also higher as a result of their higher respiratory activity. Regarding average vigor shoots, however, there is a better balance between photosynthesis and respiration. Thus, plants with better photosynthetic balance accumulate a higher carbohydrates concentration on their berries, which are precursors for anthocyanins (Brighenti et al., 2010; Fregoni, 1998), since they are by-products for the composition of pentose phosphate compounds.

In addition to the higher vigor, shoot topping with 9 LAC may have promoted a decrease in the grapes' content of sugars, phenolic compounds and antioxidant activity due to the grapevines support system. Along low back support systems, the branch on which the shoot topping is performed with 9 LAC surpasses the upper wire in about 30 cm (Fig2D). This allows the branch to begin bending down, causing the lower leaves to shade and, as a consequence, the decrease on the photosynthetic rates and the production of photoassimilates by the plant (Poni and Giachino, 2000).

The effect of shoot topping may vary not only according to the intensity in which it is carried out but also due to other factors such as the cultivar, application season and interaction with climate and soil conditions. Mota et al. (2010) have observed that the shoot topping of 'Merlot' (*V. vinifera* L.) provided an increase in the grape anthocyanins and total phenolic compounds content, however, under the same conditions there was no effect from the these compounds treatments over 'Cabernet Sauvignon' (*V. vinifera* L.).

Positive correlations were found among total anthocyanins (r = 0.37; p < 0.05), flavonoids (r = 0.76; p < 0.01) and polyphenols content (r = 0.72; p < 0.01), and antioxidant activity *in vitro*. Other studies also verified that the grapes antioxidant activity depends on their phenolic composition (Burin et al., 2014; Rockenbach et al., 2011). However, it has been observed that the absence of correlation and negative correlation can occur between the content of polyphenols and the antioxidant activity (Nixford and Hermanosín-Gutiérrez, 2010).

There were effect of 'IAC 766' and 'IAC 572' rootstocks, mainly on the physical characteristics of 'Niagara Rosada' grapes clusters and berries. The 'IAC 572' rootstock is considered to be more vigorous than the 'IAC 766' (Maia and Camargo, 2012), yet it was found a higher amount of mass and wider clusters on the 'Niagara Rosada' grapevines grafted on the 'IAC 766', as well as a higher amount of mass on berries that were also longer and wider. These results are similar to those observed by Bruna and Back (2015) when studying the effect of these same rootstocks on the physical characteristics of 'Niagara Rosada' grapes. On the other hand, other authors present a higher amount of mass on clusters from 'Niagara Rosada' grapes grafted on 'IAC 572' rootstock compared to those grafted on 'IAC 766' (Alvarenga et al., 2002).

The difference in vigor between rootstocks had no significant influence over the number of clusters, yield and grapevines productivity, as it was observed in other studies (Alvarenga et al., 2002; Tecchio et al., 2014; Bruna and Back, 2015). The highest vigor from 'IAC 572' rootstocks when compared to 'IAC 766' favors the production of 'Niagara

Rosada' (Mota et al., 2009). However, other authors obtained higher productivity with the 'IAC 766' rootstock compared to the 'IAC 572' rootstock (Tecchio et al., 2011), evidencing the environmental conditions influence on the interaction between canopy and rootstock.

Regarding the chemical characteristics of the must, the rootstocks presented no influence on pH, soluble solids content, titratable acidity and grape maturation index (Table 3). Some studies have presented different results using the same rootstocks, meaning that, higher soluble solids content and lower titratable acidity of 'Niagara Rosada' grapes were found when grapes were grafted on the 'IAC 572' rootstock (Alvarenga et al., 2002; Mota et al., 2009). As it was the case regarding productive characteristics, the rootstocks effect on the grape chemical composition may be related to factors such as vigor, water and nutrient absorption capacity, resistance to diseases and interaction with the canopy. Thus, this effect can directly influence the plants primary and secondary metabolites and, consequently, the quality of the grapes (Dias et al., 2012; Lee and Steenwerth, 2013; Tecchio et al., 2014).

Although the rootstocks did not influence the soluble solids content of the 'Niagara Rosada' grapes must, grapes from grapevines grafted on 'IAC 572' rootstock contain a higher reducing sugars content (Table 4). Glucose and fructose are the main grape source of sugars (Conde et al., 2007) and are the larger part of the soluble solids content as well, although sucrose and other carbohydrates are also presented in small quantities (González-Fernández et al., 2012). Possibly, these other compounds may be present in higher concentrations on grape must obtained from grapevines grafted on 'IAC 766' rootstock, thus altering the acquired results.

The higher concentration of reducing sugars in the 'Niagara Rosada' grapes must when grafted on the 'IAC 572' rootstock may have been one of the factors responsible for the higher content of anthocyanins we found on this grape (Table 4), considering the positive correlation between these compounds (r = 0.56; p < 0.01). The accumulation of sugars works as a metabolic substrate for the phenolic compounds synthesis, among them, anthocyanins and others related to grape aroma (Abe et al., 2007; Pozzan et al., 2012). The highest total anthocyanins content we observed on 'Niagara Rosada' grapes over 'IAC 572' rootstock may also be related to a smaller size of berries (r = -0.41; p < 0.05), once smaller berries favor the accumulation of solutes, such as sugars, acids, anthocyanins and other phenolic compounds (Conde et al., 2007). However, results of this study differ from other authors who found higher anthocyanins content on 'Niagara Rosada' grapes when grafted on 'IAC 766' rootstock compared to those grafted on 'IAC 572' (Mota et al., 2009).

The 'IAC 766' and 'IAC 572' rootstocks had no effects over total flavonoid content, total phenolic compounds and antioxidant activity of 'Niagara Rosada' grapes (Table 4). In Minas Gerais, Brazil, the 'IAC 766' and '196-17' rootstocks did not influence the total phenolic compounds, total anthocyanins and antioxidant capacity of the 'Niagara Rosada' grapes, however, the total flavonoid content was higher on the 'IAC 766' rootstock (Abe et al., 2007). In general, higher phenolic compounds levels in grapes may be related to the use of less vigorous rootstocks (Dias et al., 2012), however, it should be taken into account that these compounds content in grapes vary not only in function of the isolated rootstock effect, but also due to the effect of the interaction between rootstock genotypes and canopy cultivars.

Materials and methods

Grapevines and growing conditions

The experiment was conducted in a 2-year-old vineyard of Niagara Rosada (*Vitis labrusca* L. x *Vitis vinifera* L.) cultivar. Niagara Rosada is a grape cultivar intended for *in natura* consumption much appreciated by the Brazilian consumer, mainly in the South and Southeast regions of Brazil, where this cultivar is widely cultivated. The experiment was carried out in Botucatu, São Paulo, Brazil (22° 55′ 55″ S, 48° 26′ 22″ W, altitude 810 m), from July 2015 to January 2016. According to the Köppen classification, the climate is type *Cwa*, *i.e.*, subtropical climate with an average temperature in 20.7 °C, and the average annual rainfall is 1359 mm, with a tendency for concentrated rainfall in the summer months. The soil of the area was classified as UDULT according to previously published criteria (Embrapa, 2006).

The vines were planted with 2.0 m spacing between rows and 0.8 m within plants, for a total of 6250 vines ha^{-1}. The vines were trained on a unilateral cordon system (1 m above soil) in a vertical shoot positioning by means of iron wires. The pruning was performed leaving one bud per spur, and subsequently, 5% hydrogen cyanamide was applied to the buds to induce and standardize sprouting. When berries begin to color (*veraison*), *i.e.*, phenological stage 35 according Eichhorn and Lorenz (1984), vines were protected with anti-hail screens, aiming protection against hailstorms, and attack by birds and bees.

Treatments and experimental design

The experimental design was a randomized block design in a 4 x 2 factorial scheme, with four levels of shoot topping and two rootstocks. Shoot topping was performed according to Brighenti et al. (2010) methodology with some modifications. The manual shoot topping was carried out by leaving 6, 7, 8 or 9 leaves above the last cluster (LAC), as shown in Fig 2. The shoots were topped 60 days after winter pruning, when the vines were in the beginning of bunch closure, *i.e.*, phenological stage 32 according Eichhorn and Lorenz (1984). All grapevines were grafted onto two rootstocks: IAC 766 Campinas (106-8 Mgt x *Vitis caribaea*) and IAC 572 Jales (*V. caribaea* x 101-14 Mgt [*V. riparia x V. rupestris*]).

Traits measured

Fruit maturation curve

The fruits maturation curve was performed by determinations of soluble solids content (SS), titratable acidity (TA), maturation index (SS / TA ratio) and pH during different periods, starting at early ripening up until the fruit harvest. It was carried out samplings at 112, 119, 126, 133 and 140 days after pruning (DAP). The grapes were collected from 10 representative clusters per experimental plot, in which 6 berries were removed from every cluster. A randomized complete block design with subdivided plots was used, the plots themselves represented the different shoot topping levels, while the subplots represented the assessment time (days after pruning).

Yield and physicochemical characteristics

The harvest of the grapes was determined by monitoring the maturation curve, whilst, in between two samplings there was a SS and TA contents stabilization. After the harvest, 10 clusters from each plot were selected for physicochemical analysis.

The yield per vine (kg) and productivity (t ha^{-1}) were then estimated from the number of clusters per vine and their mass. In order to assess the physical characteristics of the clusters and berries it was used their mass (g), length (cm) and width (cm) collected data, and the number of berries per cluster. And for the berries chemical characteristics, it was assessed them according to determinations of soluble solids content (SS, expressed as °Brix), titratable acidity (TA, expressed as tartaric acid percentage), pH, maturation index (SS/TA ratio) and reducing sugar (glucose percentage).

The soluble solids content was determined by refractometry of grape must; the titratable acidity was determined by titration of 0.1 N NaOH to the equivalence point of pH = 8.2; lastly the pH was determined by direct reading of the must in potentiometer Tecnal®, model r^2i300 (Brasil, 2005). Reducing sugars, in turn, were determined by the Somogy-Nelson colorimetric method (Nelson, 1944) and the absorbance values at 535 nm were compared to a glucose calibration curve.

Total phenolics content and in vitro antioxidant activity

In order to determine the total phenolics content and *in vitro* antioxidant activity, grapes samples were randomly collected from four blocks (4 x 100 g), by manually picking grape berries from different clusters from different spots (top, medium and bottom parts of the clusters).

The fresh grape samples (skin, flesh and seeds) were immerged in a solvent mixture 70:29:1 (v/v) of methanol, water and hydrochloric acid (Bubalo et al., 2016), with 1:2 (w/v) ratio sample/solvent. After that, we grounded the samples using a mixer for 1 min, incubated them for two hours at 8 °C and then centrifuged them at 3000 *g* for 7 min. The supernatant was separated and stored at -20 °C for further analyses.

The total monomeric anthocyanins content was determined using the pH-differential method (Giusti and Wrolstad, 2001). We diluted the grape extract samples with buffer solutions of KCl 25 mM pH 1.0 and CH_3COONa (0.4 M) pH 4.5, and we also carried out absorbance measurements at 520 and 700 nm on a spectrophotometer. The total monomeric anthocyanins content was expressed as cyanidin-3-glucoside equivalents in mg Kg^{-1} of grape.

The total phenolics content of grape was determined using the Folin-Ciocalteau colorimetric method (Singleton and Rossi Jr, 1965). The absorbance value obtained in tests at 765 nm on an UV–Vis SP 2000 BEL Photonics® spectrometer was compared with a calibration curve obtained for gallic acid and the results were expressed as mg 100^{-1} g gallic acid equivalents (GAE).

The total flavonoid content was determined using a colorimetric assay with aluminum chloride and then we expressed it as catechin equivalents (mg CE 100 g^{-1}) (Moreno-Montoro et al., 2015). The samples were prepared with solutions of $NaNO_2$ (0.5 g L^{-1}), $AlCl_3$ (1 g L^{-1}) and NaOH (1 M). We then stirred the mixture and took measures of the absorbance at 510 nm against water blanks.

A last the *in vitro* antioxidant activity of the grape was assessed using the DPPH radical scavenging method (Brand-Williams et al., 1995; Rossetto et al., 2009). The analytical standard Trolox was used to construct the calibration curves and the results were expressed as equivalents of Trolox per g of grape (mg TEAC g^{-1}). Absorbance measurements were performed on a UV–Vis SP 2000 BEL Photonics® spectrometer and the antioxidant activity of the grapes was assessed through the rate of decay in the absorbance at 517 nm. The DPPH radical (2.2-diphenyl-1-picrylhydrazyl) solution was prepared in methanol 80%. For each sample, the absorbance was determined at time t = 60 min after the addition of DPPH radical.

Statistical analyses

The data was submitted to analysis of variance, using the Tukey test ($p < 0.05$) in the plots and polynomial regression analysis in the subplots in order to determine the evolution of the grapes' maturation, afterwards we selected regression models based on the test F significance and the determination coefficient value ($R^2 \geq 0.70$). Regarding other assessed characteristics, the data were collected and subjected them to analysis of variance, and whenever this analysis indicated statistically significant treatment effects, subjected the data to the Tukey test ($p < 0.05$), aided by the SISVAR statistical program (Ferreira, 2011). The correlation analysis was also conducted ($p < 0.05$ and $p < 0.01$) to investigate the relation between the total phenolic compounds content and antioxidant activity, with the aid of the ASSISTAT statistical program (Silva and Azevedo, 2002).

Conclusions

The rootstocks and the different shoot topping levels did not influence the yield, productivity and cycle length of 'Niagara Rosada' grapevines, which required 138 days from pruning to harvest. Larger and heavier clusters and berries were harvested from vines grafted on 'IAC 766' rootstock. However, grapes harvested from grapevines grafted on 'IAC 572' rootstocks presented higher total anthocyanins content, which improves their external appearance, once this compound is present, mostly, along their skins. The different shoot topping levels did not influence the grape physical characteristics. The shoot topping carried out with 7 or 8 leaves above the last cluster provided a better balance in the grapevine source-drain relationship favoring the grape chemical characteristics, with higher sugars, total polyphenols content and antioxidant activity.

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References

Abe LT, Mota RV, Lajolo FM, Genovese MI (2007) Compostos fenólicos e capacidade antioxidante de cultivares de uvas *Vitis labrusca* L. e *Vitis vinifera* L. Ciênc Tecnol Aliment. 27(2):394-400.

Alvarenga ÂA, Regina MA, Fráguas JC, Chalfun NNJ, Silva AL (2002) Influência do porta-enxerto sobre o crescimento e produção da cultivar de videira Niágara Rosada (*Vitis labrusca* L. x *Vitis vinifera* L.), em condições de solo ácido. Ciênc Agrotec. Ed spe:1459-1464.

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 28(1):25-30.

Brasil (2005) Métodos físico-químicos para análise de alimentos. 1st edn Ministério da Saúde. Agência Nacional de Vigilância Sanitária, Brasília.

Brighenti AF, Rufato L, Kretzschmar AA, Madeira FC (2010) Desponte dos ramos da videira e seu efeito na qualidade dos frutos de "Merlot" sobre os porta-enxertos 'Paulsen 1103' e 'Couderc 3309'. Rev Bras Frutic. 32(1):19-26.

Bruna ED, Back ÁJ (2015) Comportamento da cultivar Niágara Rosada sobre diferentes porta-enxertos no sul de Santa Catarina, Brasil. Rev Bras Frutic. 34(5):924-933.

Bubalo MC, Ćurko N, Tomašević M, Ganić KK, Redovnikovic IR (2016) Green extraction of grape skin phenolics by using deep eutectic solvents. Food Chem. 200:159-166.

Burin VM, Ferreira-Lima NE, Panceri CP, Bordignon-Luiz MT (2014) Bioactive compounds and antioxidant activity of *Vitis vinifera* and *Vitis labrusca* grapes: Evaluation of different extraction methods. Microchem J. 114:155-163.

Collins C, Dry PR (2009) Response of fruitset and other yield components to shoot topping and 2-chlorethyltrimethyl-ammonium chloride application. Aust J Grape Wine Res. 15(3):256-267.

Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Gerós H (2007) Biochemical changes throughout grape berry development and fruit and wine quality. Food. 1(1):1-22.

Dardeniz A, Yıldırım I, Gökbayrak Z, Akçal A (2008) Influence of shoot topping on yield and quality of *Vitis vinifera* L. African J Biotechnol. 7(20):3628-3631.

Dias FAN, Mota RV, Fávero AC, Purgatto E, Shiga TM, Souza CR, Pimentel RMA, Regina MA (2012) Videira 'Syrah' sobre diferentes porta-enxertos em ciclo de inverno no sul de Minas Gerais. Pesqui Agropec Bras. 47(2):208-215.

Dunn GM, Martin SR (2007) A functional association in *Vitis vinifera* L. cv. Cabernet Sauvignon between the extent of primary branching and the number of flowers formed per inflorescence. Aust J Grape Wine Res. 13:95-100.

Eichhorn KW, Lorenz DH (1984) Phaenologische Entwicklungsstadien der Rebe. Eur Mediterr Plant Prot Organ. 14:295-298.

EMBRAPA (2006) Sistema Brasileiro de Classificação de Solos. Centro Nacional de Pesquisa de Solos. 4th edn EMBRAPA-SPI, Rio de Janeiro.

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Ciênc Agrotec. 35(6):1039-1042.

Fregoni M (1998) Viticoltura di qualitá, 1st ed. Verona, Itália.

Giusti MM, Wrolstad RE (2001) Characterization and measurement of anthocyanins by UV-Visible spectroscopy. Curr Protoc Food Anal Chem. F1.2.1-F1.2.13.

González-Fernández AB, Marcelo V, Valenciano JB, Rodríguez-Pérez JR (2012) Relationship between physical and chemical parameters for four commercial grape varieties from the Bierzo region (Spain). Sci Hortic. 147:111-117.

Leão PCS, Brandão EO, Gonçalves NPS (2011) Produção e qualidade de uvas de mesa 'Sugraone' sobre diferentes porta-enxertos no Submédio do Vale do São Francisco. Ciênc Rural. 41(9):1526-1531.

Leão PCS, Nunes BTG, Lima MAC (2016) Canopy management effects on 'Syrah' grapevines under tropical semi-arid conditions. Sci Agric. 73(3):209-216.

Lee J, Steenwerth KL (2013) 'Cabernet Sauvignon' grape anthocyanin increased by soil conservation practices. Sci Hortic. 159:128-133.

Maia JDG, Camargo UA (2012) O cultivo da videira Niágara no Brasil. 1st edn. Embrapa: Empresa Brasileira de Pesquisa Agropecuária, Brasília.

Miele A, Mandelli F (2012) Manejo do dossel vegetativo e seu efeito nos componentes de produção da videira Merlot. Rev Bras Frutic. 34(4):964-973.

Moreno-Montoro M, Olalla-herrera M, Gimenez-Martinez R, Navarro-Alarcon M, Rufián-Henares JA (2015) Phenolic compounds and antioxidant activity of Spanish commercial grape juices. J Food Compos Anal. 38:19-26.

Mota RV, Souza CR, Favero AC, Silva CPC, Carmo EL, Fonseca AR, Regina MA (2009) Produtividade e composição físico-química de bagas de cultivares de uva em distintos porta-enxertos. Pesqui Agropec Bras. 44(6):576-582.

Mota RV, Souza CR, Silva CPC, Freitas GF, Shiga TM, Purgatto E, Lajolo FM, Regina MA (2010) Biochemical and agronomical responses of grapevines to alteration of source-sink ratio by cluster thinning and shoot trimming. Bragantia. 69(1):17-25.

Nelson NA (1944) A photometric adaptation of Somogyi method for the determination of glucose. J Biol Chem. 135(1):136-175.

Nicolosi E, Continella A, Gentile A, Cicala A, Ferlito F (2012) Influence of early leaf removal on autochthonous and international grapevines in Sicily. Sci Hortic. 146:1-6.

Nixford SL, Hermanosín-Gutiérrez I (2010) Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. Anal Chim. 659:208-215.

Poni S, Giachino E (2000) Growth, photosynthesis and cropping of potted grapevines (*Vitis vinifera* L. cv. Cabernet Sauvignon). Aust J Grape Wine Res. 6(3):216-226.

Pozzan MSV, Braga GC, Salibe AB (2012) Teores de antocianinas, fenóis totais, taninos e ácido ascórbico em uva 'Bordô' sobre diferentes porta-enxertos. Rev Ceres. 59(5):701-708.

Rockenbach II, Gonzaga LV, Rizelio VM, Gonçalves AESS, Genovese MI, Fett R (2011) Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. Food Res Int. 44:897-901.

Rossetto MRM, Vianello F, Rocha SA, Lima GPP (2009) Antioxidant substances and pesticide in parts of beet organic and conventional manure. African J Plant Sci. 3(11):245-253.

Sato AJ, Silva BJ, Bertolucci R, Carielo M, Guiraud MC, Fonseca ICB, Roberto SR (2009) Evolução da maturação e características fisico-químicas de uvas da cultivar Isabel sobre diferentes porta-enxertos na Região Norte do Paraná. Semin Ciências Agrárias. 30(1):11-20.

Silva FS, Azevedo CAV (2002) Versão do programa computacional ASSISTAT para o sistema operacional Windows. Rev Bras Prod Agroindustriais. 4(1):71-78.

Singleton VL, Rossi Jr JA (1965) Colorimetry of total phenolics with phosphomolybidic-phosphotungstic acid reagents. Am J Enol Viticulticulture. 16:144-158.

Tecchio MA, Moura MF, Teixeira LAJ, Pires EJP, Leonel S (2014) Influence of rootstocks and pruning times on yield and on nutrient content and extraction in 'Niagara Rosada' grapevine. Pesqui Agropec Bras. 49(5):340–348.

Tecchio MA, Teixeira LAJ, Terra MM, Moura MF, Paioli-Pires EJ (2011) Extração de nutrientes pela videira 'Niagara Rosada' enxertada em diferentes porta-enxertos. Rev Bras Frutic. 33:736–742.