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The influence of the summer pruning on 'Fuji' apples storage under controlled atmosphere

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ABSTRACT. This study aimed to evaluate the effect of the summer pruning time in 'Fuji' apple trees on the quality of fruit at harvest and after storage in controlled atmosphere with extremely low O_2 (CA-ELO) (0.5 kPa O_2). The treatments evaluated were summer pruning in December, January, and February, in addition to a control treatment (without summer pruning). The experiment was carried out in the 2018/2019 and 2019/2020 growing seasons. The fruit were evaluated at harvest and after eight months of CA-ELO (0.5 kPa O_2 + <0.5 kPa CO_2/1.5 ± 0.2°C/92 ± 2% RH) storage, at chamber opening, and after 7 days of shelf-life at 23 ± 3°C and 60 ± 5% RH. The quality of the fruit was evaluated through of soluble solids, flesh firmness, titratable acidity, fruit color, and physiological disorder incidence (sunburn and flesh browning), in addition to enzymatic activity and concentration of functional compounds. There was no significant effect of the summer pruning time on fruit flesh firmness, soluble solids, titratable acidity, and rot incidence after storage under CA-ELO plus 7 days of shelf life. Summer pruning in February resulted in fruit with higher peel red color development, which in general contains a higher concentration of functional compounds at harvest (total phenolic compounds and total antioxidant activity), and lower flesh browning incidence in 'Fuji' apples stored under CA-ELO. The nitrogen (N) concentration and nitrogen/calcium ratio (N/Ca) in the second growing season were lower in fruit from plants pruned in February, compared to no summer pruning or earlier pruning. The superoxide dismutase and peroxidase enzyme activity were lower in fruit from treatments with summer pruning in January and February, whereas the polyphenol oxidase enzyme activity was lower when summer pruning was conducted in February.

Keywords: *Malus domestica* Borkh.; postharvest; functional compounds; physiological disorders.

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Introduction

Fruit quality is determined during its development, when it is still attached to the plant, being influenced by the environmental conditions of cultivation (Liu, Zhang, & Zhao, 2013), especially fruit exposure to sunlight (Jackson, 1970). Pruning is one of the practices commonly performed on fruit plants. There are several types of pruning, with different purposes, but, in general, it influences the plant's growth and for absorption of water and nutrients (Filho, Medina, & Silva, 2011). Pruning is a practice used to balance between vegetative and productive growth of plants (Zhang, Koc, Wang, & Jiang 2018), which can change the crop load and fruit quality, in addition to improving the quality of light in the plant canopy (Musacchi & Serra, 2018).

Summer pruning aims to control the canopy size of plants, improve light availability, and reduce diseases (Ashraf & Ashraf, 2014). Summer pruning in apple trees improves fruit quality through the physiological modification of plants and the alteration of the canopy environment, mainly due to higher sunlight penetration (Cooley & Autio, 2011). By removing vigorous and upright water sprouts and reducing plant vigor, summer pruning promotes balance (Maughan, Black, & Roper, 2011) and influences the relationship between vegetative and reproductive growth (Cañón, González, Alcalde, & Schwarze, 2014). Fruit quality in terms of color, size, soluble solids content, and fruit flavor can be improved with this practice, because of the greater amount of solar radiation that reaches the interior of the plant canopy (Trevisan, Gonçalves, Chavarria, Antunes, & Herter, 2006).

Summer pruning can affect different fruit attributes, depending on the species evaluated. Summer pruning of sweet cherry trees affects the distribution of sugar in the plant; flower buds of trees pruned in summer have higher sugar content in the most illuminated parts of the canopy (Vosnjak, Mrzlic, & Usenik, 2022). The authors suggest that this practice may be a measure adopted to control the concentration of sugars in the flower buds, especially because a strong and well-nourished flower bud can result in a good production prospect for the next growing season. In blueberry, summer pruning influenced the level of disease and the occurrence of insects in the orchard in addition to affecting the flowering and fruit harvest dates and yield (Lee et al., 2015).

Managing the vegetative growth of apple trees by summer pruning is an extremely important practice to maintain a good yield and optimize fruit quality (Cline, Embree, Hebb, & Nichols, 2008). 'Reinette du Canada' apple trees pruned in the summer produced fruit with lower soluble solids (SS) and titratable acidity (TA) concentration at harvest and during refrigerated storage, in addition, to pruning influenced the mineral concentration of fruit (Guerra & Casquero, 2010). Although summer pruning may contribute to the production of redder fruit, it can compromise the production of photoassimilates and change the growth shoot/root ratio if performed incorrectly (Dotto et al., 2017). According to these authors, defining the best way and time to carry out this practice is of paramount importance.

Apples can have their metabolism altered during storage due to their exposure to the sun during preharvest. The development of disorders, ripening, and changes in fruit appearance are among these alterations, that is, the stress starts in the orchard and continues during storage (Mc Tavish, Poirier, Torres, Mattheis, & Rudell, 2020). Flesh browning in apples is one of the major problems of storage under a controlled atmosphere with ultralow O_2 (CA-ULO) and a controlled atmosphere with extremely low O_2 (CA-ELO) (Thewes et al., 2021; Ho, Verboven, Verlinden, Schenk, & Nicolaï, 2013). The manifestation of this disorder can also be associated with low Ca concentrations in the fruit (Corrêa et al., 2017) and its relationship with other minerals (Corrêa et al., 2012). Therefore, although summer pruning can improve fruit quality at harvest, studying fruit quality after storage under CA-ELO is essential.

One of the tested hypotheses is that later summer pruning, in January or February, provides higher development of the red color of the fruit. Especially because, in some situations, the summer pruning stimulates the sprouting of buds, shading the interior of the canopy (Schupp & Ferree, 1988; Saure, 1990). Moreover, the time of summer pruning may influence fruit mineral composition as well as oxidative and antioxidant enzymes activity and, consequently, the manifestation of physiological disorders, especially those that appear in 'Fuji' apples after storage under CA-ELO.

Summer pruning is a long-time recommended management practice. However, its effect on fruit quality, as mentioned above, depends on the time of its application, with the need to investigate the most appropriate time to carry out this practice. Additionally, there is no information about the effect of time summer pruning on the quality maintenance of 'Fuji' apples stored under CA-ELO. Considering that a storage condition in CA-ULO and CA-ELO can cause damage to the quality of 'Fuji' apples, especially flesh browning, and summer pruning can alter the fruit quality at harvest, we sought with this work to identify the effect of the summer pruning time on the quality of 'Fuji' apples at harvest and after storage in CA-ELO. Thus, this study aimed to evaluate the effect of the time of summer pruning on the quality of 'Fuji' apples at harvest and after storage under CA-ELO.

Material and methods

The field experiment was carried out in an experimental orchard located in São Joaquim, Santa Catarina State, Brazil (49°55' W and 28°17' S, 1,415 m altitude), with apples of the Fuji variety ('Fuji Standard'), grafted onto the rootstock 'Marubakaido' with 'M.9' interstock . The experiment was carried out in an orchard planted in 1999, with spacing of 4 m between rows and 1.5 m between plants (1,666 plants ha^{-1}) and shape training in a central leader under conventional management. The average size of the plants was 3.7 m in height, 3.1 m wide, and 1.7 m thick, with a trunk diameter equivalent, on average, to 17.5 cm. The experimental design was randomized in blocks, with four replications in the 2018/2019 growing season and five replications in the 2019/2020 growing season, each replication consisting of three plants. The data referring to climate conditions, temperature range (difference between the low and high daily temperature), precipitation, average temperature, and hours of insolation from February to April in the evaluated growing season are shown in Figure 1. For the determination of the insolation, a sensor of the duration of the incidence of direct solar radiation (CSD3; Campbell Scientific Ltd.; UK) was used, coupled to a datalogger (CR1000 of Campbell Scientific Ltd.; UK). The sensor determines the time during which direct solar radiation exceeds the 120 W m^{-2} level. Results were expressed in hours.

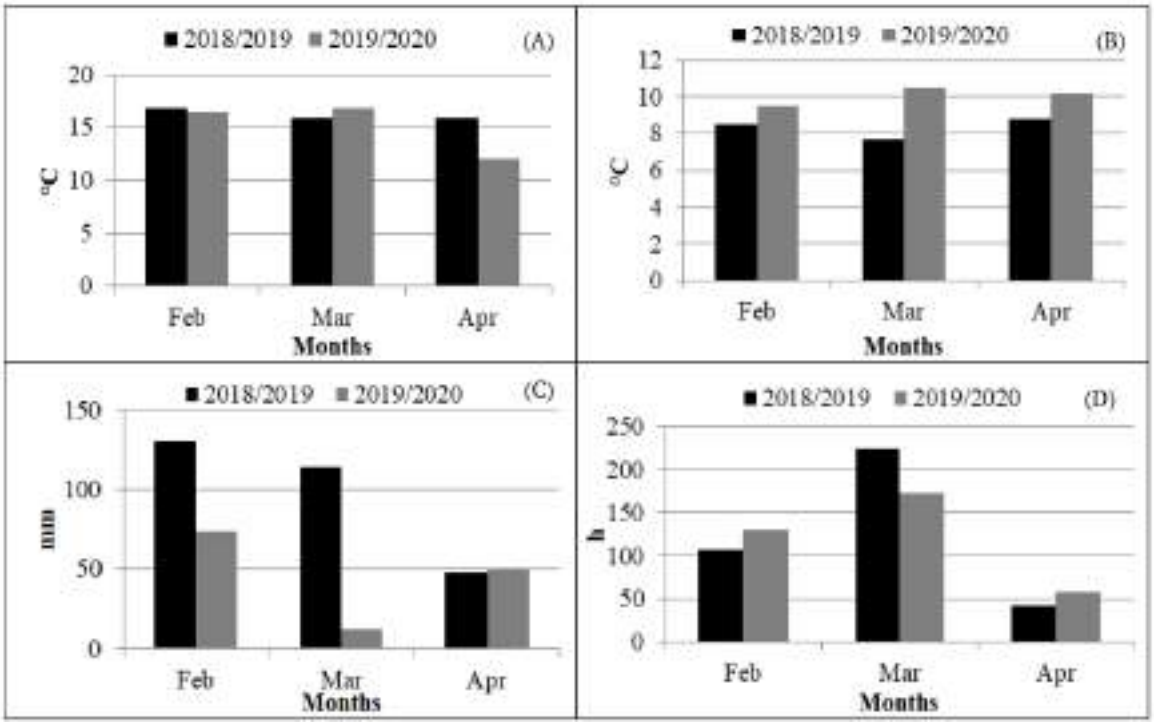

Figure 1. Weather data on average temperature (A), temperature range (B), precipitation (C) and hours of insolation (D) of the São Joaquim weather station for the 2018/2019 and 2019/2020 growing season (Company of Agriculture Research and Rural Extension of Santa Catarina- EPAGRI, 2020).

Treatments consisted of different times of summer pruning (December, January, and February; southern hemisphere) and no summer pruning (control). Summer pruning was carried out at the beginning of the second week of the respective month by removing undesirable shoots and those that were poorly positioned and shaded the fruit. Heading back cuts were made throughout the entire plant, to remove any vigorous and upright water sprouts that were in each plant. In the winter pruning, vigorous branches and those with a diameter greater than 1/3 of the diameter of the main branch were removed. The dates of full bloom and harvest were October 18, 2018 and April 11, 2019 for the 2018/2019 growing season as well as October 14, 2019 and April 8, 2020 for the 2019/2020 growing season, respectively. The crop load adjustment was performed by a combination of chemical thinning followed by manual thinning (Meland, 1997). For chemical thinning, 200 mL 100 L^{-1} of Maxcel® were applied when fruit were 5 to 8 mm in diameter. The hand thinning was performed when fruit reached 12 to 20 mm in diameter, considering as a criterion to keep two fruit per limb and one fruit per spur buds.

All fruit from the central plant of each replication were harvested at maturity and taken to the laboratory. At harvest, the fruit that were up to 2 m high on the plant and those that were above that height were harvested separately. However, only fruit that were up to 2 m tall from the plant were used. All these fruit were classified according to the percentage of red color coverage in the fruit epidermis into four classes: 1) 0-25% red color, 2) 26-50% red color, 3) 51-75% red color, and 4) 76–100% red color (Layne, Jiang, & Rushing, 2001). After that, samples containing 30 and another containing 40 fruit were assembled, according to the percentage of fruit in each of the classes. The sample of 30 fruit was evaluated at harvest and a sample of 40 fruit was evaluated at the exit of the chamber after eight months of storage.

The storage condition consisted of CA-ELO (0.5 kPa O_2 + <0.5 kPa of CO_2 at 1.5 ± 0.2 C/92 ± 2% RH). After harvesting, the fruit were placed in a hermetically closed mini-chamber, in which the atmosphere condition was controlled. The installation and correction of gas concentrations were based on the methodology described by Fernandes et al. (2021), with modifications. To avoid the accumulation of CO_2, hydrated lime was used inside the microchambers. The controlled atmosphere condition was established by injecting gaseous nitrogen from high-pressure cylinders to reduce O_2 down to 1.5 kPa, and over the next 15 days of storage, the O_2 gradually decreased to 1.0 kPa, and after 15 days of storage, the O_2 gradually decreased to 0.5 kPa, remaining under this condition for another seven months, with the daily verification and correction of

the gas concentrations. The monitoring and correction of the partial pressures of gases in the mini-chambers were carried out using an electronic gas analyzer for O_2 and CO_2 (Schelle®, Germany). The O_2 was replaced by injecting atmospheric air whenever its partial pressure was below the established level, resulting from consumption by fruit respiration. Sachets with hydrated lime (50 g kg^{-1} of fruit) were added inside the mini-chamber to keep CO_2 < 0.5 kPa.

The percentage of fruit with more than 50% of the surface covered with red color was evaluated visually by counting the number of fruit that had a red-pigmented surface equivalent to >50% of the total surface of the fruit, relative to the total number of fruit.

Fruit with sunburn incidence, which is characterized by the presence of a bronze-yellow color on the surface, were counted and the results were expressed as a percentage of fruit with sunburn relative to the total number of evaluated fruit.

Peel color was evaluated in terms of hue angle values ($h°$) using a CR 400 colorimeter (Konica Minolta, Japan). The readings were carried out in the redder (red color) and less red (background color) regions of the fruit.

Flesh firmness (expressed in N) was quantified at two opposite ends of the fruit in the equatorial portion using an electronic penetrometer with an 11 mm diameter tip (Güss Manufacturing Ltd, South Africa) after removing a thin epidermis layer.

The titratable acidity (TA) and soluble solids (SS) were determined using two wedges taken from opposite sides of the fruit and crushed in an electric mixing machine to extract the juice. The TA values (% malic acid) were obtained by titration of 0.1 N NaOH, using a TitroLine® Easy automatic titrator (Schott Instruments, Germany), in a solution containing 5 mL of fruit juice homogenized with 45 mL of distilled water until pH 8.1. The SS concentration (%) was determined on a PR201α digital refractometer (Atago®, Japan), using an aliquot of juice from the fruit. Subsequently, the SS/TA ratio was calculated.

The concentrations of mineral elements (skin+flesh) (expressed in mg kg^{-1} FW), which make it the same for all parameters, were quantified using the methodology described by De Martin et al. (2017). The N was determined using 0.2 g of the fresh sample digested with 2.0 mL of concentrated sulfuric acid (Merck, Germany) and 3.0 mL of hydrogen peroxide and kept at 350°C. The sample volume was filled to 50 mL with distilled water at the end of the digestion. Sample distillation and titration were performed by the semi-micro Kjeldahl method, according to the methodology described by Tedesco, Gianello, Bissani, Bohnen, and Volkweiss (1995).

The rot incidence (%) was evaluated after the fruit left the chamber plus seven days of shelf life. The incidence of flesh browning (expressed in %) was visually evaluated, observing the fruit that showed some change in pulp color, especially brown color. from a cross-section, the fruit that showed some flesh browning and/or small cavities in the internal regions of the flesh were counted.

The peel and flesh were separated for the total phenolic compounds (TPC) and total antioxidant activity (TAA) analysis and superoxide dismutase (SOD), peroxidase (POD), and polyphenol oxidase (PPO) enzyme activity. The 1 cm slice was cut from the equatorial region of each fruit, being then peeled, composing the peel sample. Two wedges were taken from the opposite sides of this slice, which composed the flesh sample. The obtainment of extracts and TAA analysis were performed according to the methodology proposed by Stanger, Steffens, Soethe, Moreira, and Amarante (2017).

The extracts for the TPC analysis were obtained according to the methodology of Rufino et al. (2007). For the TPC analysis through spectrophotometry, the methodology was adapted from that described by Roesler et al. (2007). First, 2.5 mL of Folin-Ciocalteau (Sigma-Aldrich, Brazil), and 0.5 mL of the sample extract were added to test tubes. After 3 min., 2.0 mL of 10% sodium carbonate (Cinética, Brazil) was added. The tubes were shaken and incubated for 1 hour in the dark. After this period, the samples were centrifuged and the reading was performed on a microplate reader (EnSpire, PerkinElmer, USA), at a wavelength of 765 nm. The standard curve was made with gallic acid (Biotec, Brazil) at concentrations of 0, 10, 30, 50, 70, 90, and 100 µL L^{-1}. Results were expressed in mg of gallic acid equivalent per 100 g of sample.

The enzymatic extract used for the analysis of SOD, POD, and PPO activity was obtained from the homogenization for 1 minute of 500 mg of the fruit sample in an Ultra Turrax with 5 mL of the extraction medium, composed of 0.100 M potassium phosphate buffer (pH 7.0, Vetec, Brazil), containing 1 mM insoluble polyvinylpyrrolidone (PVP, Vetec, Brazil), and 1 mM ethylenediaminetetraacetic acid (EDTA, Synth, Brazil). After homogenization, 50 µL of 10% Triton (Vetec, Brazil) was added to the mixture. The samples were left to stand for 15 minutes in the refrigerator and then centrifuged at 4°C for 15 minutes at 1,241.6 × g (CR22N, Hitachi, Japan). The supernatant was stored in Eppendorf tubes at −50°C for further analyses.

The SOD enzyme activity was determined according to the method described by Giannopolitis and Reis (1977), with modifications. The reactions were carried out in three separate tubes. In the first tube, determined as blank, aliquots of 50 µL of crude extract from the flesh sample and 25 µL of crude extract from the peel were added to 2.95 and 2.975 mL of the reaction medium, respectively, composed of 50 mM potassium phosphate buffer (pH 7.8, Synth, Brazil), 13 mM methionine (Vetec, Brazil), 75 µM nitro blue tetrazolium chloride (NBT, Sigma-Aldrich, Brazil), 100 nM EDTA (Vetec, Brazil), and 2 mM riboflavin (Vetec, Brazil), and remained in reaction while covered by aluminum foil for 10 minutes. The second tube, the control, received only the working solution, remaining for the same reaction time, but under lighting. The third tube received the working solution and samples, remaining for 10 minutes in the reaction under lighting. The sample readings were performed using a microplate (EnSpire, PerkinElmer, USA) reader at λ = 560 nm. The results were expressed in µmol $min.^{-1}$ mg^{-1} protein.

The quantification of the POD enzyme activity was determined according to the methodology described by Hammerschmidt, Nuckles, and Kuć (1982), with modifications. An aliquot of 100 µL of the crude extract from peel and 300 µL of the flesh was added to 2.9 and 2.7 mL of the reaction buffer, respectively, composed of 5 mL of 50 mM potassium phosphate buffer (pH 6.0, Vetec, Brazil), 12.5 µL of pure guaiacol (Vetec, Brazil), and 16.3 µL of pure H_2O_2 (Vetec, Brazil). A microplate reader (EnSpire, PerkinElmer, USA) was used to measure the absorbance of the samples for 13 minutes, every 30 seconds, at 25°C and λ = 470 nm. The POD enzyme activity was determined according to the slope of the straight line in the interval from 0 to 13 minutes. The results were expressed in µmol min^{-1} mg^{-1} protein.

The quantification of the PPO enzyme activity was determined according to the method described by Kar and Mishra (1976), with modifications. The reactions were carried out in three tubes. Aliquots of 0.3 mL of 0.2 M potassium phosphate buffer solution (pH 6.7, Vetec, Brazil) and 1.85 mL of 0.1 M catechol solution (Vetec, Brazil), dissolved in the buffer solution, were added to the first tube. The second tube, called the sample blank, received 0.3 mL of the crude extract sample and 1.85 mL of deionized water. Finally, 0.3 mL of sample and 1.85 mL of 0.1 M catechol (Vetec, Brazil) were added to the third tube. Readings were performed in a microplate reader (EnSpire, PerkinElmer, USA) at λ = 395 nm. The results were expressed in µmol $min.^{-1}$ mg^{-1} protein.

Protein quantification was based on the absorption of the Coomassie Brilliant Blue G-250 reagent proposed by Bradford (Bradford, 1976). For the reaction, 290 µL of Bradford reagent (Sigma-Aldrich) were mixed with 10 µL of the enzymatic extract. Then, readings were performed on a microplate reader (EnSpire, PerkinElmer, USA) at 595 nm.

The enzyme activity (SOD, POD, and PPO) and calcium, nitrogen, magnesium, and potassium concentrations were evaluated only in the 2019/2020 growing season. The results of the mineral concentration allowed for calculating the N/Ca, K/Ca, and (K+Mg)/Ca ratios.

The data were subjected to analysis of variance, and the means were compared using the LSD test at a 5% probability when significant ($p < 0.05$), indicating the coefficient of variation of the data sets. When necessary, the data were transformed using the formula arc sen $((P+1.5)/100)^{1/2}$. Statistical analyses were performed in the program R v. 4.1.2 (R Core Team, 2021).

Results and discussion

The percentage of apples with more than 50% red color was significantly higher when summer pruning was carried out in February in both growing seasons (Table 1). The control and summer pruning treatments carried out in December and January did not differ from each other. The summer pruning carried out in February and January provided fruit with a more intense red color in the 2018/2019 growing season, compared to that with no summer pruning or earlier pruning. However, this result was not observed in the 2019/2020 growing season. Studies (Ashraf & Ashraf, 2014; Uselis et al., 2020) have proven the effect of summer pruning in improving fruit red color. Summer pruning of 'Rubin' apples had a positive effect on fruit red color (Uselis et al., 2020). However, as observed in the present study, performing summer pruning at the correct time is essential to obtain good results in terms of red fruit color, considering that early pruning did not have a positive effect on this important variable for quality.

Table 1. Percentage of fruit with more than 50% red color, epidermis color (*h°;* peel red color and peel background color) and sunburn incidence in 'Fuji' apples at harvest as a function of summer pruning time in the 2018/2019 and 2019/2020 cycles productives.

Pruning	Fruit with more than 50% red color (%)	Epidermis color (*h°*)		Sunburn (%)
		Red color	Background color	
Without summer pruning	37.5 b	58.6 b	107.1 a	52.2 a
December	39.0 b	61.5 a	108.1 a	41.1 b
January	41.5 ab	48.6 c	105.6 b	36.5 b
February	49.4 a	48.3 c	106.9 a	37.4 b
CV (%)	5.8	3.2	0.7	5.2
Without summer pruning	54.5 b	49.9 a	107.3 a	5.7 b
December	66.9 b	43.6 a	106.6 a	16.0 a
January	60.0 b	45.6 a	107.2 a	6.3 b
February	83.3 a	43.3 a	107.4 a	17.7 a
CV (%)	17.8	11.3	1.3	19.3

*Means followed by the same letter in the columns, for each growing season, do not differ from each other by the LSD test ($p < 0.05$). CV: Coefficient of variation.

Climate conditions are fundamental for the red coloring of apples, which may have favored the development of the red color of fruit in the 2019/2020 growing season when compared to the previous growing season. The Figure 1 showed the average temperature, temperature range, precipitation, and insolation during the period of fruit ripening. Harvest was carried out in mid-April for both growing seasons (the data for April are shown up to harvest). The average temperature in the months before harvest and insolation during this period was variable. However, there was a higher temperature range during the fruit ripening stage in the 2019/2020 growing season, which may have favored the red color development. Fenili et al. (2019) also found that one of the climate factors determining satisfactory red color in 'Daiane' and 'Venice' apples was the higher temperature range observed during fruit ripening.

The exposure of fruit to light is important for their final color at harvest (Jing, Feng, Zhao,Wu, & Chen, 2020). The red peel color of the apple is due to anthocyanin accumulation, which is very important for fruit quality, as redder apples have a higher market value (Honda & Moriya, 2018). Light regulates the expression of genes involved in the biosynthesis of anthocyanins, promoting the synthesis of this pigment (Feng, Li, Ma, & Cheng, 2014a). Although the light incidence was not evaluated, fruit from plants pruned in February possibly received higher light interception in the period in which there was higher red color development, as pruning was carried out approximately two months before harvest and very vigorous branches and those that shaded the fruit were removed.

In some situations, summer pruning may not favor fruit coloring, as this practice can encourage buds to sprout, hindering the entry of light into the crown (Schupp & Ferree, 1988; Saure, 1990). This effect may explain the results of the fruit red color from plants pruned in December and January, but mainly in December when no positive effect of summer pruning was observed. The Figure 2 showed that plants from the summer pruning treatment carried out in December presented crown architecture similar to the control during the dormancy period, but different from that subjected to pruning in February, evidencing the resumption of vegetative growth of plants after the summer pruning in these treatments.

Figure 2. Architecture of plants subjected or not to summer pruning at different times during the winter rest period.

Flesh firmness, SS, TA, and the SS/TA ratio did not differ between the control treatment (without summer pruning) and summer pruning, regardless of the season (data not shown). In the 2018/2019 growing season, apples from plants subjected to summer pruning in January showed a more yellowish background color than fruit from other treatments (Table 1). However, no difference among treatments was observed in the following growing season.

The final color of apple fruit is developed during the ripening process, with the synthesis of secondary compounds and degradation of chlorophyll in this process (Musacchi & Serra, 2018). Nevertheless, other ripening attributes, such as flesh firmness, SS and TA do not support this hypothesis of anticipating ripening with summer pruning, regardless of the time of completion.

Sunburn incidence was significantly higher in fruit from plants not pruned in the 2018/2019 growing season and fruit from summer pruning treatments in December and February in the 2019/2020 growing season (Table 1). Sunburn is caused by high solar radiation intensity that falls on the fruit, and its symptoms can vary from white, orange-yellow, or orange spots to dark brown spots, with a burnt appearance (Racsko & Schrader, 2012). Considering that the improvement in the fruit red color was a result of the higher incidence of sunlight, it was expected that fruit from plants subjected to later summer pruning would present a higher sunburn incidence, but it only occurred in the second growing season. However, sunburn is not only common when there is greater solar radiation, but other factors also contribute to this problem. The high solar radiation, associated with high air temperatures, low relative humidity, and high altitudes, in addition to when the cold or mild weather is abruptly followed by hot and sunny weather, can favor the development of sunburn (Lal & Sahu, 2017; Piskolczi, Varga, & Racskó, 2004). These factors may have influenced the results obtained, both the difference among crops and treatments.

In the 2018/2019 growing season, the TPC concentration in the apple peel and flesh was significantly higher in fruit from plants submitted to summer pruning in February and fruit from unpruned plants (Table 2). In the 2019/2020 season, the TPC concentration in the peel did not differ between the control and summer pruning treatments in December and February, being lower in the summer pruning carried out in January. The TPC concentration in the fruit flesh in the 2019/2020 growing season was significantly higher in fruit treated with summer pruning in February. The hypothesis is that the plants that would receive the summer pruning could have a higher content of phenolic compounds, justified by the greater light intensity that reaches the fruit of these plants, especially those pruned later in the summer. Good light conditions are of paramount importance to produce apples with compounds that promote human health (Drogoudi & Pantelidis, 2011). The synthesis of phenolic compounds is intensified when the fruit is exposed to light during growth (Di Stefano, Scandurra, Pagliaro, Di Martino, & Melilli, 2020). The TPC concentration in the apple peel is a direct response to light availability (Feng, Li, Ma, & Cheng, 2014b). Summer pruning in February, in general, provides greater accumulation of phenolic compounds in apples, possibly due to the improvement in the internal insolation of the plant and, consequently, greater exposure of the fruit to light.

Table 2. Total phenolic compounds (TPC; mg EAG 100 g^{-1} FM) and total antioxidant activity (TAA) by the DPPH and ABTS methods (µMol Trolox 100 g^{-1} FM) in the peel and flesh of 'Fuji' apples at harvest as a function of summer pruning time.

Treatment	Peel			Flesh		
	TPC	DPPH	ABTS	TPC	DPPH	ABTS
			2018/2019			
Without summer pruning	168 ab	1343 b	1518 a	20.2 a	155.3 a	123.4 b
December	160 b	1290 b	1362 a	17.6 bc	133.9 b	110.8 b
January	118 c	1277 b	1166 b	15.9 c	156.6 a	115.5 b
February	178 a	1773 a	1435 a	19.0 ab	167.3 a	157.6 a
CV (%)	7.4	8.4	8.0	6.7	7.9	14.9
			2019/2020			
Without summer pruning	462 a	2748 a	2874 b	30.1 bc	129.7 ab	275.1 b
December	500 a	2105 ab	4040 a	34.2 ab	96.8 c	299.6 b
January	444 b	1131 c	2578 b	29.6 c	104.1 bc	304.5 ab
February	495 a	1969 b	3854 a	36.7 a	135.7 a	333.1 a
CV (%)	4.5	25.8	9.3	9.7	16.3	7.3

Means followed by the same letter in the columns, for each growing season, do not differ from each other by the LSD test ($p < 0.05$). CV: Coefficient of variation.

The total antioxidant activity (TAA) determined in the peel by the DPPH method was significantly higher in fruit from plants pruned in February in the 2018/2019 growing season. In the flesh, fruit from control treatment, summer pruning in January, and summer pruning in February presented significantly

higher TAA by the DPPH method than those from summer pruning conducted in December. In contrast, the ABTS method provided significantly higher TAA values in the peel for the control, summer pruning in December, and summer pruning in February treatments than the summer pruning carried out in January in the 2018/2019 season. The highest TAA in the flesh was provided by the summer pruning in February. In the 2019/2020 growing season, pruning in December and February provided significantly higher TAA in the fruit peel, while the highest values in the flesh were observed in fruit from plants submitted to summer pruning in February, not differing from summer pruning in January (Table 2). As for the content of phenolic compounds, in general the fruit of plants pruned in February contained higher TAA, possibly due to the higher light intensity that reached the fruit. Especially when compared to the other summer pruning seasons, visually the pruning in the month of February provided a lower intensity of vegetative growth stimulated by the pruning (data not shown), therefore, it possibly allowed more light to enter the canopy of the plants, favoring the accumulation of antioxidant compounds.

Phenolic compounds are considered natural antioxidants present in large amounts in fruit (Haminiuk, Maciel, Plata-Oviedo, & Peralta, 2012) and their synthesis can be influenced by the light conditions available during apple development (Sun, Xin, Gao, Wang, & Li, 2014). Cervantes et al. (2019) observed that the more the strawberry fruit were exposed to light, the higher the TAA.

The Ca, K, and Mg contents as well as the K/Ca and (K+Mg)/Ca ratios in the fruit did not show differences among treatments. The N concentration and N/Ca ratio in fruit from plants pruned in February were lower than in the other treatments (Table 3). A high N/Ca ratio can be harmful to fruit quality, as it increases the susceptibility to the occurrence of disorders related to Ca deficiency (Stüpp, Rosa, Amarante, Mafra, & Steffens, 2013). In the plant, N can be remobilized from leaves to fruit through the phloem (Miqueloto, Amarante, Steffens, Santos, & Mitcham, 2014; De Martin et al., 2017). The removal of shoots in the pruning carried out in February may have led to lower N transport to the fruit, which possibly caused the lower N concentration and N/Ca ratio in the fruit.

Table 3. Mineral concentration (mg kg^{-1} FW) and mineral ratios in 'Fuji' apples as a function of summer pruning time.

Treatment	N	Mg	K	Ca	N/Ca	K/Ca	(K+Mg)/Ca
				2019/2020			
Without summer pruning	311 a	46.8 a	1688 a	37.3 a	8.3 ab	45.4 a	46.6 a
December	303 a	41.2 a	1738 a	37.7 a	8.0 b	46.3 a	47.4 a
January	342 a	45.3 a	1822 a	36.3 a	9.5 a	50.2 a	51.5 a
February	199 b	41.6 a	1752 a	37.0 a	5.4 c	47.6 a	48.8 a
CV (%)	13.2	11.1	5.9	6.5	12.7	8.9	8.7

Means followed by the same letter in the columns do not differ from each other by the LSD test ($p < 0.05$). CV: Coefficient of variation.

After storage plus 7 days shelf life, fruit peel background color, and rot incidence did not differ among treatments (Table 4). Studies show that the loss of fruits by rotting during storage is directly linked to the health of the fruits and also to the storage conditions, for storage for long periods. When the fruits are stored in ULO, there are lower losses due to rot, thus becoming the best alternative to reduce losses related to pathogenic microorganisms during storage (Balla & Holb, 2008).

Table 4. Rot incidence and fruit peel background color in 'Fuji' apples as a function of summer pruning time, after storage under CA-ELO plus 7 days shelf life.

Treatment	Rot incidence (%)	Peel background color
	2018/2019	
Without summer pruning	32 a	105,4 a
December	39 a	106,5 a
January	30 a	106,4 a
February	32 a	106,6 a
CV (%)	14,7	0,8
	2019/2020	
Without summer pruning	15,5 a	107,1 a
December	18,5 a	106,6 a
January	17,1 a	107,2 a
February	19,9 a	107,3 a
CV (%)	19,1	1,2

Means followed by the same letter in the columns do not differ from each other by the LSD test ($p < 0.05$). CV: Coefficient of variation.

Flesh firmness, TA, and SS/TA ratio were not influenced by the pruning period in both growing seasons (Table 5). Possibly, because these attributes did not respond significantly to the summer pruning times at harvest, there was also no significant response after storage in CA. The SS content was lower in the fruit of pruned plants in December, compared to the fruit of unpruned plants, in the 2018/2019 season. The percentage of fruit with flesh browning was lower, in both seasons, in plants pruned in February (Table 5).

Table 5. Flesh firmness, soluble solids (SS), titratable acidity (TA), SS/TA ratio, and percentage of fruit with flesh browning in 'Fuji' apples as a function of summer pruning time, after storage under CA-ELO plus 7 days shelf life.

Treatment	Firmness (N)	SS (%)	TA (% malic acid)	SS/TA	Flesh browning (%)
			2018/2019		
Without summer pruning	68.3 a	12.6 a	0.26 a	47.9 a	21.0 a
December	68.8 a	11.8 b	0.23 a	50.5 a	16.6 a
January	69.0 a	12.2 ab	0.24 a	53.1 a	19.9 a
February	69.8 a	12.5 a	0.23 a	55.0 a	3.8 b
CV (%)	3.6	2.3	9.9	9.7	16.3
			2019/2020		
Without summer pruning	76.5 a	14.8 a	0.24 a	62.5 a	12.1 a
December	74.8 a	15.0 a	0.25 a	59.2 a	10.9 a
January	73.2 a	14.4 a	0.25 a	56.4 a	15.9 a
February	76.1 a	15.1 a	0.25 a	61.7 a	1.7 b
CV (%)	2.5	7.0	8.8	11.1	18.9

Means followed by the same letter in the columns, for each growing season, do not differ from each other by the LSD test ($p < 0.05$). CV: Coefficient of variation.

Flesh firmness and SS concentration are important attributes for fruit quality (Lu, 2004), and together with TA and epidermis color (red and background color), which define the sensory characteristics of the fruit, influence the consumer's decision to buy an apple. Therefore, the fact that these attributes such as flesh firmness, SS, TA and epidermis color were not affected by the time of summer pruning is an indication that there were no significant losses from this management practice in stored fruit.

Summer pruning in February reduced the incidence of flesh browning in both growing seasons (Table 5). Flesh browning is a physiological disorder that impairs fruit appearance and flavor, as it can lead to a strange flavor (Murata, Tsurutani, Tomita, Homma, & Kaneko, 1995). One of the factors that may explain the result is the lower N/Ca ratio found in fruit from plants pruned in February (Table 3). De Martin et al. (2017) concluded that flesh browning in 'Rocha' pears is associated with lower Ca concentration, higher K concentration, and higher N/Ca, Mg/Ca, and K/Ca ratios. A lower N/Ca ratio can lead to a lower occurrence of flesh browning because Ca plays a fundamental role in maintaining the structure of the cell wall of fruit (Gao, Xiong, Li, Chen, & Zhu, 2019) and preserving the integrity of cell membranes (Miqueloto et al., 2014).

Table 6. Enzyme activity (μmol $min.^{-1}$ mg^{-1} protein) of polyphenoloxidase (PPO), superoxide dismutase (SOD), and peroxidase (POD) in the flesh of 'Fuji' apples after storage under CA-ELO plus 7 days shelf life, as a function of summer pruning time. Data from the 2019/2020 growing season.

Treatment	PPO	SOD	POD
Without summer pruning	69.8 a	61.1 a	5.9 a
December	72.3 a	57.9 ab	5.8 a
January	71.4 a	38.7 bc	4.0 b
February	39.9 b	26.6 c	2.9 b
CV (%)	27.0	38.7	21.5

Means followed by the same letter in the columns do not differ from each other by the LSD test ($p < 0.05$). CV: Coefficient of variation.

The lowest polyphenol oxidase PPO activity was observed in the flesh of the fruit from plants subjected to summer pruning in February. The superoxide dismutase (SOD) and peroxidase (POD) enzyme activities were lower in fruit from treatments with summer pruning carried out in January and February (Table 6). The browning process in fruit and vegetables is due to the oxidation of phenolic compounds, resulting from the loss of membrane integrity, which ends up exposing the substrate to enzymes, especially POD and PPO, leading to browning (Li et al., 2018). Indeed, PPO plays an important role in the flesh browning process in apples, as it oxidizes polyphenols to quinones, which are then polymerized to form brown-colored pigments (Murata et al., 1995). Similar to PPO, POD is also related to the browning of fruit and vegetables. The loss of cell membrane integrity and cell compartmentalization releases POD into chloroplasts and other cell organelles (Li et al., 2018). Fruit from plants pruned in February possibly

had less oxidative stress and there may not have been the induction of antioxidant enzymes in the flesh, such as SOD, justifying the lower enzyme activity in these fruit.

Conclusion

Late summer pruning yields redder 'Fuji' apples with higher functional property and less flesh browning after storage under a controlled atmosphere. Summer pruning of 'Fuji' apple trees in February provides fruit with significantly higher peel red color development, significantly higher amounts of functional compounds at harvest, and less incidence of flesh browning in fruit stored under a controlled atmosphere with extremely low oxygen (CA-ELO), possibly related to the mineral composition and enzymatic activity of the fruit. The summer pruning time had no significant effect on other physicochemical attributes of ripening and after storage.

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Metabolic Profiling of 'Elstar' and 'Nicoter' Apples: Impact of Storage Time, Dynamic Controlled Atmosphere and 1-MCP Treatment

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Abstract: The aim of this work was to evaluate the effect of CA and DCA on sugars, tricarboxylic acid cycle (TCA), anaerobic metabolism and some volatile compounds of 'Elstar' and 'Nicoter' apples. This study also aimed to evaluate the effect of ethylene action blocking by 1-MCP (0.650 ppm). The storage conditions tested for both cultivars were (1) CA; (2) DCA-CF; (3) DCA-RQ 1.3; (4) DCA-RQ 1.5; (5) DCA-CD 1.1; and (6) DCA-CD 1.3. The lowest oxygen limit (LOL) was higher for the 'Nicoter' apples, and the three DCA methods were able to detect this difference between the cultivars. Sorbitol had a trend of accumulation when the fruit was stored under DCA-RQ and DCA-CD, especially in higher RQ and CD, showing a negative Pearson correlation with the oxygen partial pressure over the storage period. The 1-MCP treatment induced sorbitol accumulation even when the fruit was stored under CA. The TCA intermediaries, such as citrate, 2-oxoglutarate, succinate, fumarate and oxaloacetate, were the most affected by the atmosphere conditions and the 1-MCP treatment for both cultivars. Malic acid was more affected by the storage time than the atmosphere conditions. Succinate and fumarate had an accumulation trend when the fruit was stored under DCA-RQ.

Keywords: *Malus domestica* Borkh; anaerobic metabolism; sugar metabolism; acid metabolism

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1. Introduction

During the postharvest life of apple fruit, the respiratory process is important to maintain the fruit's energy pool and cellular activity [1,2]. However, if it is too high, fruit quality will be reduced due to reserve starvation, such as sugars and organic acids [1]. To decrease fruit metabolism, the storage temperature is reduced, and the gaseous atmosphere is changed, resulting in a so-called controlled atmosphere storage (CA) [3,4]. Under CA storage, the oxygen partial pressure is reduced to near 1.0 kPa and carbon dioxide is increased to partial pressures near 2.0 kPa, depending on the cultivar [3,4]. The change in temperature and atmosphere composition affects the electron transport chain, tri-carboxylic acid cycle (TCA), sugars and anaerobic metabolism, suppressing overall fruit metabolism and extending the postharvest life of fruit.

Oxygen partial pressure reduction is effective to decrease sugar and acid consumption until a certain level, which is the anaerobic compensation point (ACP), a point with minimal CO_2 release [5,6]. If the O_2 partial pressure is reduced below ACP, anaerobic metabolism starts, increasing glycolysis speed and, consequently, sugar consumption [7,8] and the production of anaerobic metabolism compounds, such as acetaldehyde and ethanol [9–12],

which can cause damage to the fruit. Thus, the use of methods to detect the lowest oxygen limit (LOL) during storage under oxygen partial pressures below 1.0 kPa is important in order to avoid low O_2 damage and changes in sugar, TCA and anaerobic metabolism.

The LOL can be monitored in real time during storage based on several fruit physiological responses. There are methods that monitor the production and accumulation of anaerobic metabolism compounds [13,14], chlorophyll fluorescence (DCA-CF) [15] and respiratory quotient (DCA-RQ) [10,16] and, more recently, some of these methods have been based on the CO_2 release of fruit (DCA-CD) [17,18]. However, there are no reports in the literature evaluating the effect of these technologies, especially DCA-RQ and DCA-CD, on the dynamics of TCA acids, sugars and the switch between aerobic and anaerobic metabolism after long-term storage. In the literature, it is shown that the accumulation of TCA and derived acids, such as glutamate and succinate, are induced by the use of high CO_2 partial pressure (3.0 kPa) [19], but little is known about the effect of extremely low oxygen (<0.4 kPa) under DCA. Additionally, with the new DCA method based only on CO_2 release (DCA-CD), it is possible to modulate the CO_2 release of fruit to a minimal level using a low anaerobic metabolism factor [18] and, consequently, maintain the oxygen partial pressure as close as possible to the ACP. With this method, it could be possible to reduce the aerobic metabolism to the lowest level possible, inducing anaerobic metabolism of fruit and, consequently, reducing TCA and sugar consumption to a minimum.

Another technology used worldwide to further reduce fruit metabolism is blocking of ethylene action by 1-methylcyclopropene (1-MCP) [20–22]. According to Bekele et al. [23], the 1-MCP application affects sugars and organic acids metabolism, delaying the consumption of some TCA intermediaries when 'Jonagold' apples were stored under regular atmosphere and CA. However, under extremely low oxygen partial pressures, as in DCA, the effect of 1-MCP on sugar and TCA metabolism is still not clear. Early studies showed that 1-MCP application suppresses the anaerobic metabolism of 'Galaxy', 'Pink Lady' and 'Fuji' apples stored under DCA-RQ 1.5 (oxygen below 0.3 kPa) [24], but this work did not specify to what pathway the fruit metabolism is switched when treated with 1-MCP. Thewes et al. [8] verified that sorbitol also accumulated when the fruit was stored under extremely low oxygen partial pressures (DCA-RQ), and the authors hypothesized that sorbitol accumulation could be an additional means of nicotinamide adenine dinucleotide (NADH) oxidation and protect the cell membrane from leakage. Thus, the evaluation of a combined effect of DCA plus 1-MCP treatment would be helpful to understand how fruit metabolism runs under extremely low oxygen partial pressures and the effect of ethylene action under these conditions.

The aim of this work was to evaluate the effect of CA, DCA-CF, DCA-RQ and DCA-CD on sugars, TCA, anaerobic metabolism and some volatile compounds of 'Elstar' and 'Nicoter' apples after harvest, at 6 and 9 months of storage plus 7 d of shelf life at 20 °C. This study also aimed to evaluate the effect of ethylene action blocking by 1-MCP in order to understand the effect of ethylene on these variables when the fruit was stored under CA and DCA.

2. Materials and Methods

2.1. Fruit Harvest and Storage Conditions

Two experiments were performed at the Lake of Constance Research Centre for Fruit Cultivation (KOB) Germany, with apples of the cultivars Elstar and Nicoter. The fruit was harvested at optimal maturity for long-term storage. Immediately after the harvest, the fruit was submitted to a selection process, and 20 samples of each fruit were selected. Four samples were used in each treatment.

After the sampling process, fruit was cooled to 5 °C, maintained for one week and thereafter cooled down to the storage temperature, 3 °C for 'Nicoter' and 1 °C for 'Elstar'. These two storage temperatures were based on the recommendations for both cultivars. The fruit was stored in 250 L chambers. These chambers were gas-tight to allow the application of CA and DCA conditions. The storage conditions tested for both cultivars

were (1) CA; (2) dynamic controlled atmosphere based on chlorophyll fluorescence (DCA-CF); (3) dynamic controlled atmosphere based on respiratory quotient 1.3 (DCA-RQ 1.3); (4) DCA-RQ 1.5; (5) dynamic controlled atmosphere based on carbon dioxide (DCA-CD 1.1); and (6) DCA-CD 1.3. All the storage conditions were without and with 1-MCP treatment (650 ppb). The CO_2 partial pressures were 2.0 and 1.0 kPa for CA stored 'Elstar' and 'Nicoter' apples, respectively. For DCA, the CO_2 partial pressures were 1.2 and 1.0 kPa for 'Elstar' and 'Nicoter' apples, respectively. Each chamber was connected to an automatic CA control system (Isollcel®, Bolzano, Italy) to continuously control the atmosphere inside the chambers. The oxygen partial pressure variation for both cultivars and storage conditions are shown in Figure 1.

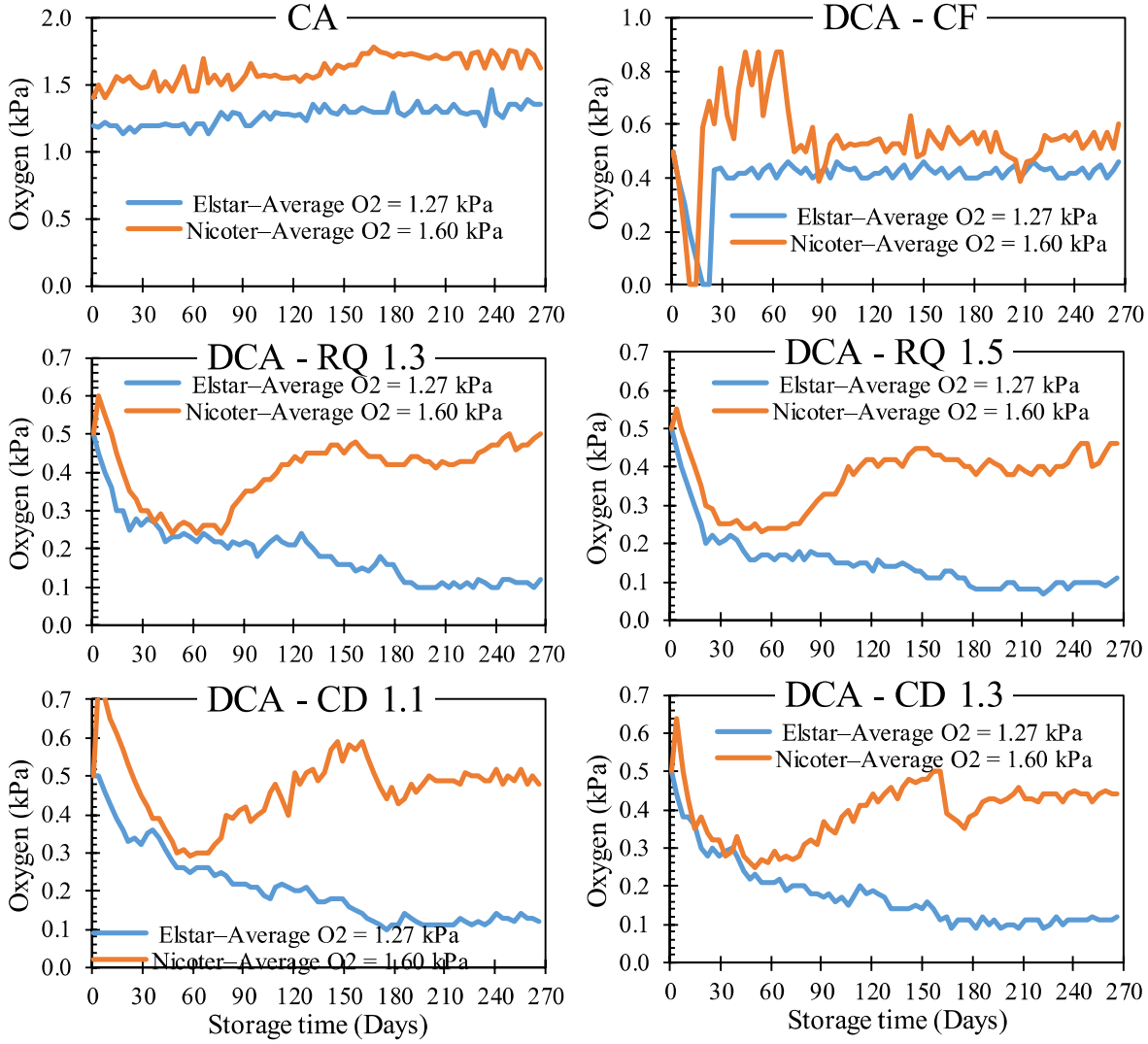

Figure 1. Oxygen setpoint variation for fruit stored under controlled atmosphere (CA) and dynamic controlled atmosphere based on chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD) over 9 months at a temperature of 1 °C for 'Elstar' and 3 °C for 'Nicoter'.

2.2. 1-MCP Treatment

To treat the fruits with 1-MCP, they were put into a chamber together with a solution containing 1-MCP (SmartFresh™, 0.14% of active ingredient). The dose used was 650 ppb. The fruit was exposed to 1-MCP treatment for 24 h at a temperature 5 °C and thereafter stored in the atmosphere condition described above.

2.3. Storage Temperature and Relative Humidity

Over the nine months of storage, the temperature was monitored daily with the aid of mercury thermometers. It was maintained at 3 ± 0.1 °C for 'Nicoter' and 1 ± 0.1 °C for 'Elstar' apples. The relative humidity was set at 94 ± 2% by adding a container with calcium chloride (7.5 g kg^{-1} of fruit) in each chamber.

2.4. Monitoring Fruit Metabolism

The fruits' bio-response to low oxygen partial pressure was monitored based on chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and CO_2 release (DCA-CD). For DCA-CF, two batches of 6 fruit were monitored with a CF sensor in real time throughout the storage period [25]. To perform the DCA-RQ method, the experimental chambers were closed for 13 to 14 h, and the O_2 and CO_2 concentrations were measured before and after this period. The ratio between CO_2 release and O_2 uptake was calculated to give the RQ [16]. Two RQ treatments, 1.3 and 1.5, were tested in this experiment, and the RQ was calculated two times per week. When the RQ was below the setpoint, the pO_2 was decreased, and if the RQ was above the setpoint, the pO_2 was increased. To monitor the optimal oxygen partial pressure based on CO_2 production only, the method described by Thewes et al. [18] was used.

2.5. Moments of Metabolite Evaluation

The ethylene production, respiration rate, sugars, TCA acids, anaerobic metabolism and some volatiles were evaluated after harvest, at 6 and 9 months of storage plus 7 d of shelf life at 20 °C, as described below.

2.5.1. Ethylene Production and Respiration Rate

The ethylene production and respiration rate were determined for eight fruits per replicate. Each replication was enclosed in an airtight glass container (4.25 L) for 2 h. Afterwards, 1 mL of headspace gas was injected into a gas chromatograph (Carlo Erba, Fractovap Series 2150, Electrometer, Mod. 180, Milano, Italy) equipped with a flame ionization detector and a stainless-steel column of 0.9 m × 1/8 in packed with activated aluminum oxide (60 mesh). The oven temperature was set to 100 °C, and the injector temperature was set to 175 °C. Nitrogen was used as a carrier gas. The ethylene production was calculated based on a standard. The respiration rate was measured with an infrared gas analyzer (Hartmann and Braun GmbH, Frankfurt, Germany) with a flow rate of 17 L h^{-1} using the same experimental setup as already described for ethylene. These analyses were performed according to Thewes et al. [8].

2.5.2. Sample Preparation for Metabolite Evaluation

All the metabolites were evaluated in juice obtained from 20 apples per replicate as reported in Wang et al. [26]. Before juice extraction, the fruit was cooled to 0 °C. Immediately after pulp cooling, horizontal slices of the equatorial region of the fruit were taken, the seeds were removed, and the slices were centrifuged under low temperature to avoid chemical and enzymatic oxidation of the samples (the maximum juice temperature during sample preparation was 5 °C). For evaluation of the sugars and TCA intermediaries, 500 µL of juice were put into 2 mL tubs and mixed with 1500 µL of cold bi-distilled water. This solution was filtered with a 45 µm nylon filter, frozen in liquid nitrogen and stored under −30 °C until the analyses. To evaluate the anaerobic metabolism compounds and volatiles, 10 mL of the juice samples were taken and immediately put into 20 mL vials and frozen to −30 °C until analysis.

2.5.3. Individual Sugar Measurement

The sugar content was determined with high-performance liquid chromatography (HPLC, Bischoff, Leonberg, Germany), using an adapted method from Wang et al. [26]. The sugars were analyzed isocratically on a 305 × 7.8 mm HC-75 Ca^{2+} form cation exchange

column (Hamilton, Reno, NV, USA). The mobile phase was bi-distilled water at a constant flow of 0.4 mL min^{-1}. The temperature of the column was isothermal at 80 °C. A reflectance index detector (Bischoff, Leonberg, Germany) was used to determine the concentration of each type of sugar. The time for each sample run was 32 min. The sorbitol and individual sugars (sucrose, glucose and fructose) were identified and quantified by the comparison of retention times and the area of standards for each compound.

2.5.4. Organic Acid Determination

The organic acid was evaluated with high-performance liquid chromatography (HPLC, Bischoff, Leonberg, Germany), with a method adapted from Wang et al. [26]. The acids were determined on a Rezex RDA-organic acid H^+ column (Phenomenex, Torrance, CA, USA), 7.8 mm × 300 mm. The mobile phase was H_2SO_4 (50 mmol L^{-1}) at a constant flow of 0.5 mL min^{-1}. The temperature of the column isothermal was 70 °C. A UV detector at 210 nm (Bischoff, Leonberg, Germany) was used to determine the acids' concentrations. The acids were identified and quantified by comparison of the retention time and area of standards. The time for each sample run was 18 min.

2.5.5. Volatile Metabolite Evaluation

A vial containing 10 mL of juice was taken and mixed with 3 g NaCl and 10 µL of 3-octanol standard solution (81.8 µg mL^{-1}) inside a 20 mL vial that was hermetically sealed with a PTFE-coated silicone lid. From this solution, the metabolites were extracted from the headspace via solid phase microextraction (HS-SPME). A divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco, 50/30 µm × 20 mm) was preconditioned following the manufacturer's protocol. Before being exposed to the fiber, the vial was submerged into a water bath at 35 °C for 5 min. After 5 min, the fiber was exposed to the headspace of the sample for 60 min under constant stirring at the same temperature.

The metabolites were quantified and identified on a Shimadzu QP2010 Plus gas chromatography coupled to mass spectrometry (GC/MS; Shimadzu Corporation, Kyoto, Japan). The fiber was thermally desorbed into the injection port at a temperature of 250 °C for 10 min, in a splitless mode. The compounds were separated in a polar-fused silica Zebron capillary column: ZB-WAX, 30 m × 0.25 mm i.d., 0.25 µm film thickness (Phenomenex, Aschaffenburg, Germany). Helium was used as a carrier gas at a constant flow rate of 1.2 mL min^{-1}. The initial column temperature was set at 35 °C and held for 3 min. Then, a temperature gradient of 2 °C min^{-1} was started until 80 °C was reached, followed by a 5 °C min^{-1} increase until 230 °C was reached, and the isothermal conditions were maintained for 5 min. The detector was operated in the electron impact ionization mode with an ionization energy of +70 eV and a scan mass range from 35 to 350 *m/z*, at a temperature of 230 °C. A series of homologous saturated n-alkanes (C7–C30) was analyzed under the same conditions to calculate a linear retention index (LRI). The concentrations of all the compounds were determined by internal standardization, according to the method proposed by Both et al. [27]. The analytes were identified based on comparisons with standards and mass spectra available in the National Institute of Standards and Technology (NIST) library and by comparing the calculated LRI with those available in the scientific literature.

2.6. Statistical Analysis

A variance analysis (ANOVA) at 5% of error probability was carried out. Data that showed significant difference by ANOVA were subjected to the LSD test at 5% error probability. The experiment was conducted in a completely randomized scheme with a factorial arrangement. Additionally, a Pearson correlation network analysis was carried out in order to show the linear relationships between the variables evaluated. This analysis was performed with CytoScape® using the app MetScape® [28].

3. Results and Discussion

3.1. Oxygen Setpoint Variation over the Storage Period

Over the 9 months of storage, the fruit's response to low oxygen stress was monitored based on three methodologies, DCA-CF, DCA-RQ and DCA-CD, and the results are shown in Figure 1. All three DCA methods resulted in fruit with lower oxygen partial pressure as compared to CA stored fruit (Figure 1). A noteworthy fact is that the two cultivars had a completely different response to low oxygen partial pressure, with higher LOL for the 'Nicoter' apples. The higher oxygen setpoint of the 'Nicoter' apples is probably because this cultivar does not adapt to extremely low oxygen partial pressure [8] and has low skin permeability [29]. The oxygen setpoint behavior was similar when the fruit was stored under DCA-RQ and DCA-CD for both cultivars (Figure 1). These results are extremely important, because they show that the new DCA method (DCA-CD) had the capability to accurately detect the LOL over the storage period in a similar way as compared to DCA-RQ. Additionally, our results demonstrate that the LOL can be accurately monitored by measuring only the CO_2 release rate of fruit by DCA-CD [18]. In practical terms, this is very important because the CO_2 release rate can be measured in rooms without any additional device.

3.2. Ethylene Production and Respiration Rate of Fruit

The ethylene production increased from harvest to 9 months of storage, when the fruit was stored under CA without 1-MCP for both cultivars (Figure 2). However, when the fruit was stored under DCA or treated with 1-MCP, the ethylene production increased less for 'Elstar' and decreased for the 'Nicoter' apples. The lower ethylene production when the fruit was stored under DCA is probably a result of low oxygen partial pressure during storage (Figure 1). This happens because oxygen is necessary to produce ethylene from ACC [11,12,30,31]. On the other hand, the effect of DCA on ethylene production can be a result of ethanol accumulation [32–36]. A noteworthy fact is that the 1-MCP application had no effect on ethylene production when the fruit was stored under DCA-RQ 1.5 and DCA-CD 1.3 for the 'Elstar' apples and all the DCA conditions for the 'Nicoter' apples. For the 'Elstar' apples stored under CA, DCA-CF, DCA-RQ 1.3 and DCA-CD 1.1, the 1-MCP application decreased the ethylene production, especially after 9 months of storage (Figure 2).

It is important to evaluate if a storage condition is effective to decrease fruit metabolism to determine the respiration rate (CO_2 release) during shelf life [2]. The storage under DCA was more effective in fruit metabolism reduction from harvest to 6 months of storage as compared to CA, but the respiration rate increased from 6 to 9 months of storage, evidencing an increase in fruit metabolism, regardless of 1-MCP treatment (Figure 2). The lower respiration of the fruits stored under DCA, with the exception of DCA-RQ 1.5 for the 'Nicoter' apples, is due to lower oxygen partial pressure storage (Figure 1). Several early studies reported a reduced respiration rate by storage under DCA [11,16,30]. Nevertheless, the three DCA methods differently affect the switch between aerobic and anaerobic metabolism, as discussed below, and the storage of the 'Nicoter' apples under DCA-RQ 1.5 increased the respiration rate due to high anaerobic metabolism after 6 months of storage (Figure 4), corroborating the results of the literature [8,37]. A noteworthy fact is that the 1-MCP treatment had no effect on the respiration rate during shelf life, regardless of the storage condition, showing that its applications did not reduce the respiration further when the 'Nicoter' apples were stored under low oxygen partial pressures (Figure 1).

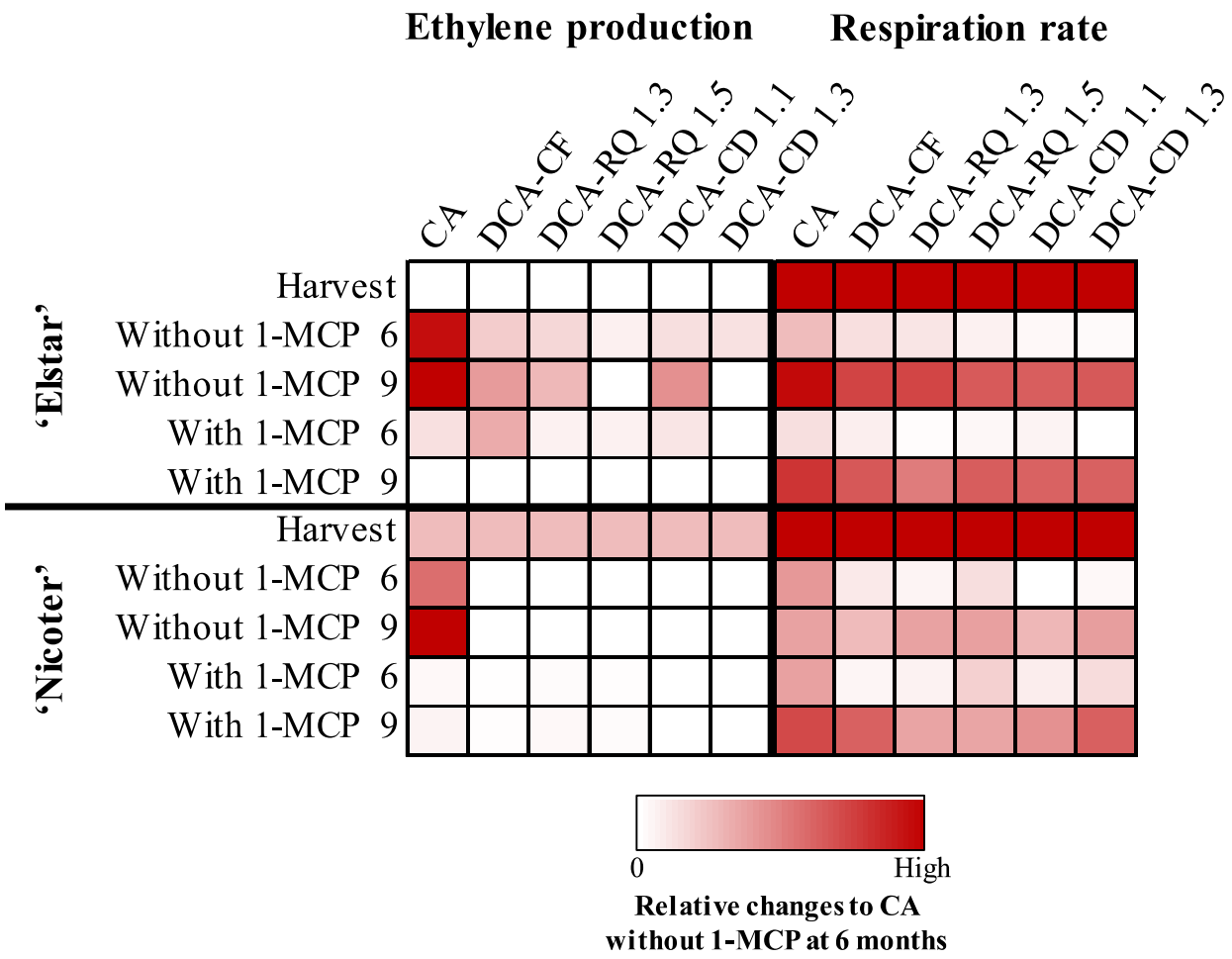

Figure 2. Heat map showing the relative changes for ethylene production and respiration rates of 'Elstar' and 'Nicoter' apples after harvest, at 6 and 9 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment (650 ppb), plus 7 d of shelf life at 20 °C.

3.3. Effects of CA, DCA and 1-MCP on Metabolite Changes over the Storage Period

The CA and DCA storage conditions changed the metabolism (CO_2 release) of apples over the storage period (Figure 2), but little is known about the dynamics of sugars, acids and anaerobic metabolism when the fruit was stored under DCA, especially DCA-RQ and DCA-CD. Thus, we evaluated the effect of CA and DCA, without and with 1-MCP, on the dynamics of sugars, TCA and anaerobic metabolites (Figures 3 and 4). The sucrose concentration increased from harvest to 6 months of storage, regardless of the atmosphere condition and 1-MCP treatment, remaining constant thereafter for the 'Elstar' apples. However, for the 'Nicoter' apples, the sucrose concentration increased from harvest to 6 months of storage and decreased thereafter at the evaluation made after 9 months of storage (Figure 4). In the literature, there are studies reporting that the sucrose content decreases [26] or increases during fruit ripening [38] in a normal atmosphere. We showed that in CA and DCA storage, the sucrose content increased from harvest to 6 months of storage, either with or without ethylene-blocking action with 1-MCP. According to Doerflinger et al. [38], this sucrose increase is due to starch degradation, which releases glucose, fructose and sucrose.

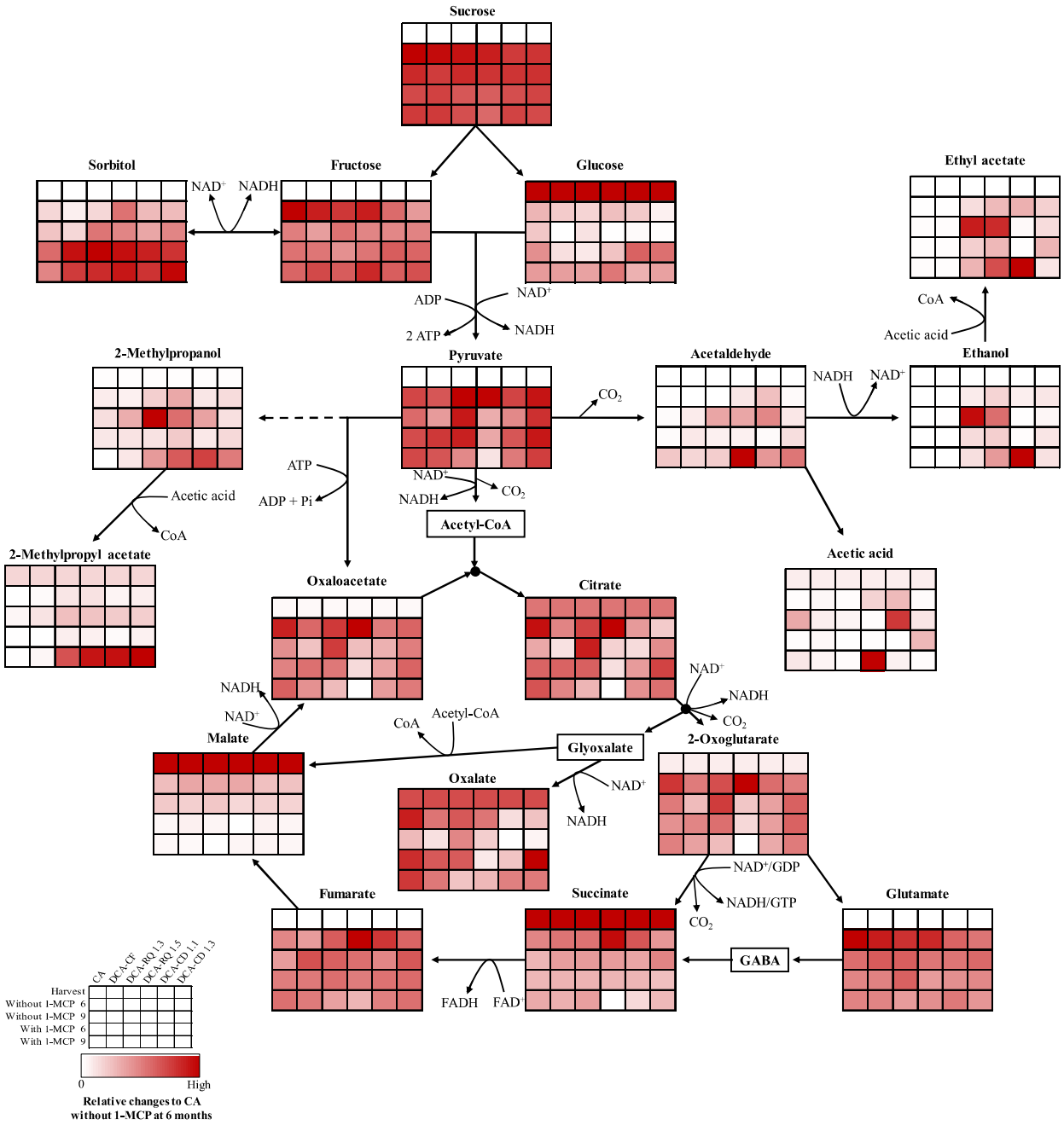

Figure 3. Heat map showing the relative changes of sugars, organic acids and some volatile compounds of 'Elstar' apples after harvest, at 6 and 9 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment (650 ppb), plus 7 d of shelf life at 20 °C.

Sucrose is a disaccharide composed of fructose and glucose. The glucose concentrations were higher at harvest for both cultivars (Figures 3 and 4). These results corroborate the results of [8], which found a reduction in glucose from harvest until the end of storage. From 6 to 9 months of storage, the glucose concentration remained almost constant, with some influence of the storage condition. For the 'Elstar' apples stored under DCA-CD, after 6 and 9 months, and DCA-RQ 1.5 after 9 months, the 1-MCP treatment resulted in higher glucose content when compared to fruit without 1-MCP (Figure 3). When the fruit was treated with 1-MCP, the storage under DCA-CD over 6 months and DCA-RQ 1.5

over 9 months resulted in a higher glucose content as compared to the other treatments (Figure 3). In 'Jonagold' apples, the 1-MCP treatment resulted in accumulation of glucose over the storage period in a normal atmosphere (20.9 kPa O_2 + 0.04 kPa CO_2) [23]. However, 'Nicoter' stored under DCA-CF, DCA-RQ 1.3 and DCA-CD had higher glucose content after 9 months of storage without 1-MCP, differing from CA and DCA-RQ 1.5 (Figure 4). The 1-MCP treatments had no effect on glucose content for the 'Nicoter' apples (Figure 4). Thewes et al. [8] reported that apples stored under DCA consumed glucose first and fructose thereafter, corroborating the present results.

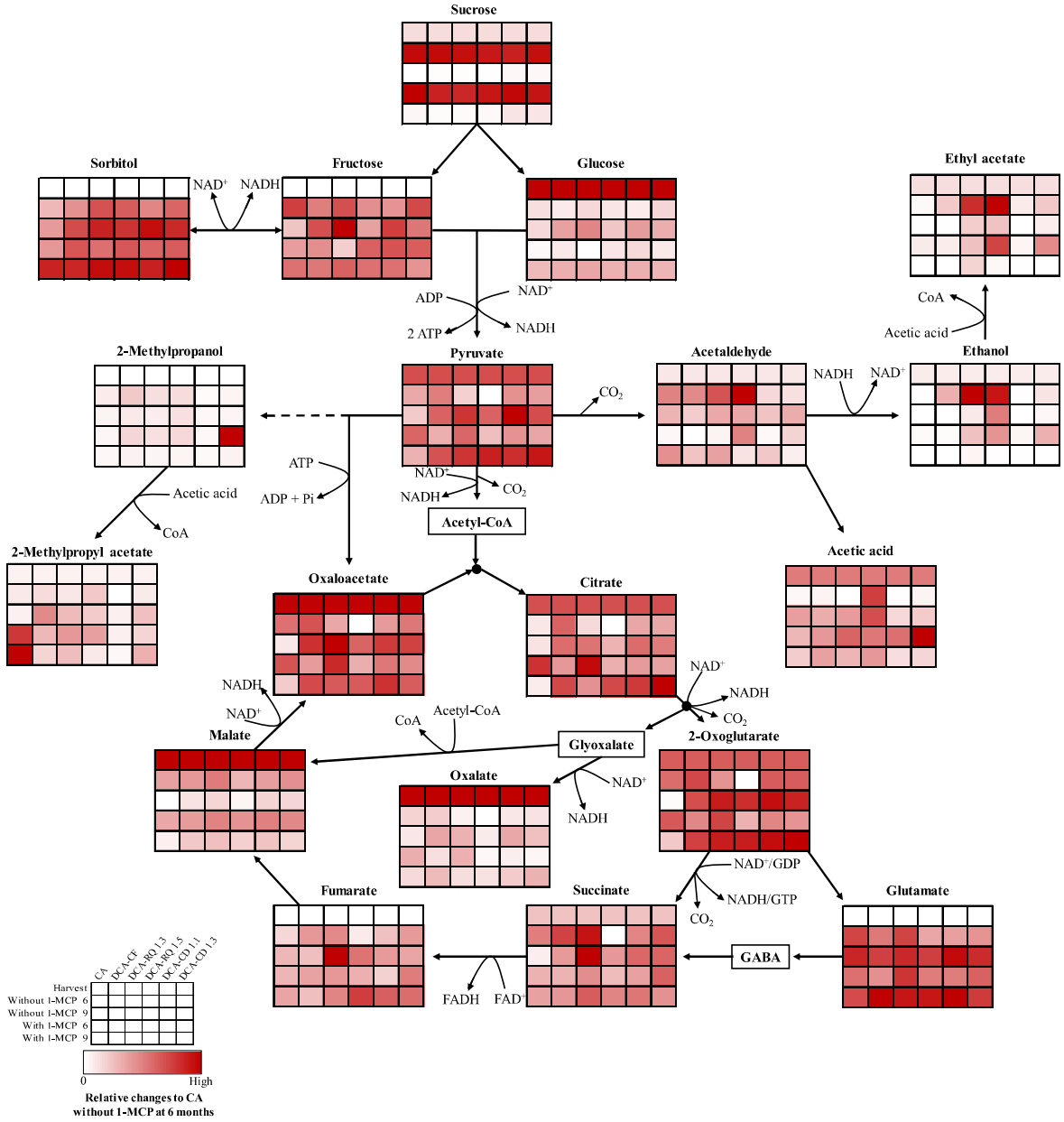

Figure 4. Heat map showing the relative change (compared to CA without 1-MCP) of sugars, organic acids and some volatile compounds of 'Nicoter' apples after harvest, at 6 and 9 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment (650 ppb), plus 7 d of shelf life at 20 °C.

At harvest, the fructose content was low, and its concentration was higher at the evaluation after 6 months of storage (Figures 3 and 4). These results are consistent with

early studies that reported an increase of fructose over the storage period [8,20,26,38]. For the 'Elstar' apples without 1-MCP, the fructose concentration was higher when fruit was stored over 6 months under CA, DCA-CF and DCA-RQ, differing from those stored under DCA-CD (Figure 3). On the other hand, when the fruit was treated with 1-MCP, the storage under DCA-RQ 1.5 resulted in the highest fructose concentration after 9 months (Figure 3). For the 'Nicoter' apples stored without 1-MCP, CA, DCA-RQ 1.3 and DCA-CD 1.3 resulted in a higher fructose concentration after 6 months (Figure 4). On the other hand, after 9 months of storage without 1-MCP, the fruit under DCA-CF, DCA-RQ 1.5 and DCA-CD 1.1 had a higher fructose concentration as compared to the other atmosphere conditions. When fruit was treated with 1-MCP, the storage under DCA-RQ 1.3 over 6 months resulted in the lowest fructose concentration (Figure 4). The 1-MCP treatment combined with DCA-RQ 1.3 probably delayed the accumulation of fructose from sucrose degradation in the 'Nicoter' apples. Fructose is the most abundant [26,39] and sweetest sugar in apples [40], so fruit with higher fructose will be sweeter, but the apples' sweetness is also a result of the interaction with other sugars, especially sorbitol and volatile compounds [39].

Sorbitol is a sugar alcohol present in apple fruit, and its concentration decreases over the storage period in normal atmosphere conditions [26,38]. However, in the present study, its concentration increased over the storage period, especially when the fruits were stored under DCA-RQ and DCA-CD (Figures 3 and 4), showing a trend of its accumulation with the oxygen decreasing, as shown by the Pearson correlation (Figures 6–8). These results agree with Thewes et al. [8], who reported an increase in sorbitol in fruit stored under DCA-RQ. Additionally, sorbitol can protect the fruit cells from stress damage, as demonstrated in yeast [41–43]; in our study, the stress condition was probably the ethanol accumulation (Figures 3 and 4). A noteworthy fact is that the 1-MCP treatment increased the sorbitol accumulation in almost all the storage conditions (Figures 3 and 4), with the exception of the 'Nicoter' apples stored under CA over 6 months. The involvement of sorbitol in preventing chilling damage on 'Granny Smith' apples and Arabidopsis thaliana is described in the literature, with its concentration increased by the 1-MCP treatment [44]. The higher sorbitol accumulation of fruit stored under DCA-RQ and DCA-CD or 1-MCP treatment can contribute positively to fruit aroma, because it confers a sweet, fruity aroma to the fruit [39].

The metabolism of glucose and/or fructose in glycolysis will produce pyruvate, ATP and NADH [19,41]. Pyruvate is a key metabolite in the fruit respiration process, and in normal atmosphere conditions (20.9 kPa O_2 + 0.04 kPa CO_2), the pyruvate is preferential metabolized via TCA, but if the oxygen partial pressure is below the ACP, pyruvate is directed to lactic acid and ethanolic fermentation [45,46]. It can also be converted to amino acids [19,23], which are volatile compound precursors. The 'Elstar' apples stored under DCA-RQ and DCA-CD 1.3 had the highest pyruvate accumulation after 6 months of storage (Figure 3). When the 'Elstar' fruits were stored under DCA-RQ 1.5, the oxygen decreased to extremely low partial pressures (Figure 1), resulting in an accumulation of TCA intermediaries, such as citrate, 2-oxoglutarate, succinate, fumarate and oxaloacetate (Figure 3), showing that the low oxygen level suppressed aerobic respiration, resulting in higher pyruvate accumulation after 6 months without 1-MCP. Pyruvate accumulation results in cytosol acidification, which activates the pyruvate decarboxylase (PDC) enzyme [45], which probably converted almost all the pyruvate to acetaldehyde in the 'Nicoter' apples (Figure 4), especially at the evaluation that took place after 6 months of storage. The higher flow of pyruvate to acetaldehyde after 6 months of storage explains the reduction in TCA intermediaries under DCA-RQ 1.5 (Figure 4). If we look to the metabolites' accumulation when fruit was stored under DCA-RQ 1.3, it is noteworthy that the pyruvate concentration was high after 6 and 9 months of storage, but the TCA intermediates accumulated more after 9 months of storage for the 'Elstar' apples (Figure 3). These results demonstrate that after 6 months of storage, the oxygen partial pressure (Figure 1) did not suppress the electron transport chain sufficiently enough to result in accumulation of anaerobic metabolism compounds when the 'Elstar' fruits were stored under DCA-RQ 1.3, but after

9 months of storage, the oxygen setpoint was lower (Figure 1), and the TCA intermediaries accumulated, starting anaerobic metabolism (Figure 3). In the literature, it is reported that significant anaerobic metabolism generally starts in storage under RQ higher than 1.3 [11,30,47] but it depends on the temperature and the time that the fruit remains under low oxygen [9].

An interesting result is that the 'Elstar' apples stored under DCA-CD 1.1, which resulted in oxygen partial pressures lower than DCA-CF and higher than DCA-RQ 1.3 (Figure 1), had higher acetaldehyde concentrations as compared to fruit stored under DCA-RQ 1.3 after 6 months storage without 1-MCP (Figure 3). Acetaldehyde is very toxic for the cells [34], so it is actively converted to other compounds, such as ethanol and acetic acid. The fruits stored under DCA-CD 1.1 without 1-MCP preferentially converted acetaldehyde to acetic acid, and the fruits stored under DCA-RQ preferentially converted to ethanol (Figure 3). These results suggest that the oxygen partial pressure lowering to a level close to ACP results in an incremental accumulation of acetaldehyde, which is converted to acetic acid but not to ethanol. This is an important result because acetic acid is a precursor of important esters, such as butyl acetate, 2-methylbutyl acetate and hexyl acetate [48,49], and ethanol is considered an off-flavor of apples [6,50] but it is also an important precursor of esters in 'Fuji' apples [51–53]. However, if we evaluate the anaerobic metabolism compounds' accumulation when the 'Elstar' apples were stored under DCA-CD 1.3, a higher pyruvate accumulation as compared to DCA-CD 1.1 after 6 and 9 months of storage is observed (Figure 3). On the other hand, after 9 months of storage, DCA-CD 1.3 had a higher ethyl acetate accumulation as compared to DCA-CD 1.1, which is a result of the lower oxygen setpoints under DCA-CD 1.3 (Figure 3).

The 'Elstar' fruit stored under CA over 6 months had a high accumulation of organic acids, such as citrate, 2-oxoglutarate, oxaloacetate, oxalate and glutamate (Figure 3). Nevertheless, the accumulation of these acids is not due to O_2 shortage, but to a higher respiration rate (Figure 2), showing that these fruits had a higher metabolic rate. On the other hand, the 'Elstar' apples stored under DCA-CF without 1-MCP over 9 months had low accumulations of TCA intermediaries and anaerobic metabolism compounds (Figure 3). These results show that the storage under DCA-CF reduced aerobic respiration and did not start anaerobic metabolism by the oxygen lowering to ±0.4 kPa (Figure 1). The low metabolism rate, especially the anaerobic metabolism of fruit under DCA-CF, may explain the lower volatile compounds emission [30,53] because the TCA intermediaries, acetic acid and ethanol, are important volatile compound precursors.

When the 'Elstar' apple were treated with 1-MCP, the highest effects on pyruvate and the TCA intermediaries were observed under DCA-RQ 1.5 storage, at the evaluation after 9 months (Figure 3). This storage treatment (DCA-RQ 1.5 + 1-MCP) suppressed pyruvate accumulation, which resulted in higher glucose and fructose concentrations, especially after 9 months of storage (Figure 3). These results demonstrate that the combination of extremely low oxygen (Figure 1) with ethylene-blocking action suppressed the glycolytic pathway, probably because of the low ethylene production (Figure 2) and action. Additionally, the low oxygen over the storage period may result in cytosol acidity (low pH), altering the phosphofructokinase enzyme activity [54]. The lower pyruvate concentration in the fruit stored under DCA-RQ 1.5 + 1-MCP is also a result of its conversion to acetaldehyde, which is converted to acetic acid in a higher proportion as compared to ethanol, contrary to the fruit stored under DCA-RQ 1.5 without 1-MCP (Figure 3). The TCA intermediaries had very low concentrations in the storage under DCA-RQ 1.5 combined with 1-MCP (Figure 3), which is a result of low oxygen (Figure 1) and low pyruvate concentration because the pyruvate plays a central role in fruit respiration, linking glycolysis with TCA and anaerobic metabolism [19].

Comparing the effect of 1-MCP on the anaerobic metabolism, when the 'Elstar' apples were stored under DCA-CD 1.1 and DCA-RQ 1.5, an inverse result is observed. The fruit treated with 1-MCP and stored under DCA-CD 1.1 had increased formation of ethanol and ethyl acetate and low acetic acid accumulation (Figure 3). However, under DCA-RQ 1.5,

the fruit without 1-MCP had higher ethanol and ethyl acetate accumulations, with a low amount of acetic acid. This is probably a result of the modulation of ADH and aldehyde dehydrogenase (ALDH) enzyme activity as a function of the oxygen setpoint and 1-MCP treatment. These results explain in part why the 1-MCP application on 'Galaxy', 'Fuji' and 'Pink Lady' suppressed the ethanol and ethyl acetate accumulations in storage under DCA-RQ 1.5 [24] because it switches the acetaldehyde conversion to acetic acid (Figure 3). Nevertheless, the effect of 1-MCP in suppressing ethanol and ethyl acetate production is dependent on the oxygen setpoint that the fruit retained over the storage period.

In analyzing the conversion of glucose and fructose to pyruvate for the 'Nicoter' apples, a different response as compared to the 'Elstar' apples was observed (Figures 3 and 4). After 6 months of storage, the pyruvate concentration was lower in the fruit under DCA-RQ 1.5 without 1-MCP (Figure 4). The TCA intermediaries also remained in low concentrations when the fruit was stored in this condition, showing that TCA is suppressed by the extremely low oxygen storage (Figure 1). In this case, the pyruvate is metabolized via a fermentative pathway (Figure 4), increasing the accumulation of acetaldehyde, ethanol, ethyl acetate and acetic acid. Analyzing these results, it is clearly demonstrated that the 'Nicoter' apples are much more sensitive to low oxygen over the storage period as compared to the 'Elstar' apples (Figures 1, 3 and 4). This is probably a result of the compact pulp of 'Nicoter' apples, which reduces the gas diffusion in the flesh, increasing the LOL [55], or because of the low skin porosity [29]. On the other hand, after 9 months of storage under DCA-RQ 1.5 without 1-MCP, the oxygen setpoint was increased (Figure 1), reducing the anaerobic metabolism, and the pyruvate flux was directed to the TCA cycle again, increasing the TCA intermediaries as compared to 6 months of storage. These results show that low oxygen stress is reversible, corroborating the results obtained by [15]. Pyruvate is a key molecule in fruit metabolism, linking glycolysis to TCA or anaerobic metabolism [19], and the conversion of pyruvate to acetyl-CoA or acetaldehyde is dependent on the cytosol pH, which modulates the activity of PDC and PDH [45].

Evaluating the 'Nicoter' apples stored under CA without 1-MCP, a reduction in TCA intermediaries from harvest to 9 months of storage was observed (Figure 4). This can be associated with the reduced fruit respiration (CO_2 release) over the storage period when compared to harvest (Figure 2). These results demonstrate that fruit stored under CA without 1-MCP may maintain the climacteric peak and remain close to senescence after 9 months of storage. This was not observed when fruit was stored under DCA without 1-MCP, where an increase in the TCA intermediaries occurred from 6 to 9 months of storage (Figure 4), which was also associated with an increased CO_2 release rate (Figure 2). This shows that the fruit stored under CA probably stay over its respiratory peak, and fruit stored under DCA remained in a state before the peak. These results corroborate those reported in the literature, which suggest that apple fruit has an increment of ethylene production and respiration rate until a peak is reached, and thereafter it decreases [2,56], but this process is delayed by low oxygen storage, such as in DCA [3,57].

Succinate generally accumulates when apples are stored under CA with high CO_2 partial pressures due to the inhibition of succinate dehydrogenase enzyme activity [19,58] or treatment with ethanol [59]. The present study verifies that storing 'Nicoter' apples under DCA-RQ 1.3 without 1-MCP also results in a higher accumulation of succinate as compared to the other storage conditions (Figure 4), a fact that was not observed in the 'Elstar' apples (Figure 3). After 9 months of storage under DCA-RQ 1.3 without 1-MCP, the higher succinate is due to a higher TCA intermediary accumulation in 'Nicoter' apples stored under this condition, such as 2-oxoglutarate, fumarate, citrate and oxaloacetate (Figure 4). According to [58], this is a result of pH reduction, inhibiting the succinate dehydrogenase activity. Storage under DCA-RQ 1.3 probably reduced the cytosolic pH due to anaerobic metabolism, reducing the succinate dehydrogenase activity, especially after 6 months, when only succinate had accumulated. This was also observed when apple fruit of the same cultivars were treated with ethanol vapor [59].

The storage of 'Nicoter' apples under CA + 1-MCP and DCA-RQ 1.3 + 1-MCP over 6 months resulted in higher accumulations of pyruvate and TCA intermediaries, such as citrate, 2-oxoglutarate and oxaloacetate, as compared to the other storage conditions (Figure 4). Additionally, in these two storage conditions (CA and DCA-RQ 1.3), the 1-MCP application had an effect on these metabolite accumulations as compared to fruit stored under the same conditions without 1-MCP. On the other hand, after 9 months of storage with 1-MCP treatment, all the DCA stored fruit had higher concentrations of pyruvate, citrate and 2-oxaloacetate when compared to CA-stored fruit (Figure 4). However, if we compare the fruit without and with 1-MCP at the same atmosphere conditions, only slight differences were observed (Figure 4). These results corroborate those reported in the literature for 'Jonagold' [23] and 'Empire' apples [20].

According to the literature, oxalate has some antioxidant activity [60] and an anti-senescence effect on fruit [61]. In the present study, the oxalate concentration decreased from harvest to 9 months of storage in the 'Elstar' apples, regardless of the atmosphere condition and 1-MCP treatment (Figure 3). However, for the 'Nicoter' apples, there was a drastic reduction in oxalate from harvest to 6 months of storage, and thereafter, it remained almost constant, regardless of the storage condition (Figure 4). The 'Elstar' apples stored under CA over 6 months had higher concentrations of oxalate, and fruit under DCA-CD 1.1 had the lowest concentration (Figure 3). After 9 months of storage without 1-MCP, the DCA-RQ 1.3-stored fruit had a higher oxalate concentration compared to the other storage conditions. In the 1-MCP-treated fruit, the storage under DCA-CD 1.3 resulted in the highest oxalate concentration. This response of oxalate concentration to the storage conditions is a result of the citrate concentrations, which had a similar response to the treatments as compared to the oxalate (Figures 3 and 5). In the literature, it is reported that an oxalate treatment reduced ethylene production in plums [61], but the present study verifies that fruit with lower ethylene production also had lower oxalate accumulation, with some exceptions, such as DCA-CD 1.3 + 1-MCP (Figures 2 and 3).

Several amino acids can be produced from pyruvate, such as valine and alanine [23]. Valine is a precursor of 2-methylpropanol and 2-methylpropyl acetate [62]. The 'Elstar' apples stored under DCA-RQ 1.3 (9 months) and the 'Nicoter' apples stored under DCA-CD 1.3 + 1-MCP over 6 months had higher 2-methylpropanol concentrations as compared to the other atmosphere conditions, but they did not result in higher 2-methylpropyl acetate concentrations (Figures 3 and 4). The 'Elstar' apples stored over 9 months under DCA-RQ and DCA-CD with 1-MCP had higher concentrations of 2-methylpropyl acetate as compared to CA and DCA-CF, probably because of the higher precursor concentration (2-methylpropanol). These results demonstrate that 2-methylpropyl acetate synthesis is not ethylene-dependent because 1-MCP inhibits ethylene action, and the ethylene production in these conditions is very low (Figure 2). In the literature, it is reported that branched-chain volatile compounds, such as 2-methylpropyl acetate, are less affected by 1-MCP than straight-chained compounds [11,63,64]. For the 'Nicoter' apples, the storage under CA + 1-MCP resulted in higher 2-methylpropyl acetate concentrations as compared to fruit stored under DCA, regardless of the storage period and DCA method.

3.4. Linear Relationships Between Metabolites After Storage

To better understand the relationships between the sugars, acids, volatile compounds, CO_2 release and mean oxygen partial pressure during storage, a Pearson correlation ($p < 0.05$) was calculated. The Pearson correlation matrix was used to build a correlation network for each out-turn time evaluation (Figures 5–8). The oxygen setpoint was negatively correlated with sorbitol for both cultivars (Figures 6–8), showing a trend of its accumulation with decreasing oxygen. These results corroborate those in the literature, which reported sorbitol accumulation with decreasing oxygen [8]. The oxygen setpoint was also negatively correlated with ethanol, ethyl acetate and 2-methylpropyl acetate for the 'Elstar' apples after 6 months' storage (Figure 5). This happens because the reduced oxygen results in anaerobic metabolism, resulting in accumulation of fermentative compounds.

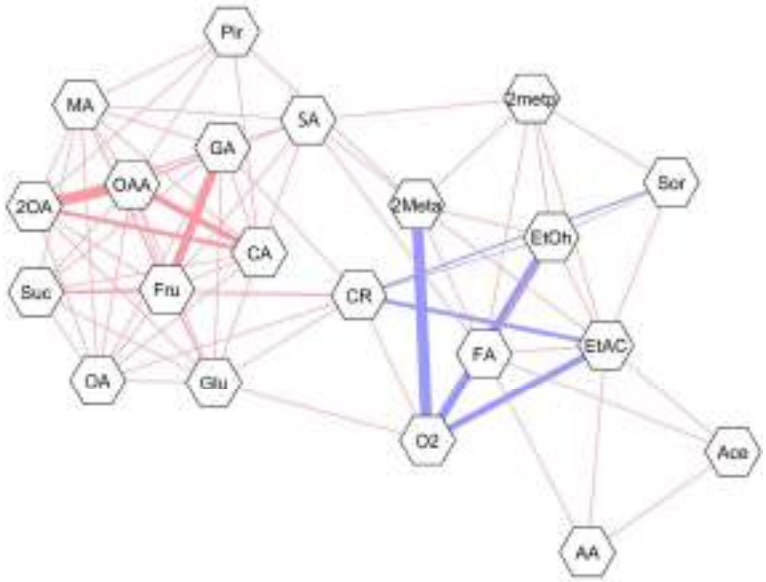

Figure 5. Correlation network for 'Elstar' apples after 6 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment (650 ppb), plus 7 d of shelf life at 20 °C. Blue and red lines show negative and positive correlation, respectively. The sizes of the lines show the correlation magnitude (all correlations shown are higher than 0.5). Suc: sucrose; Glu: glucose; Fru: fructose; Sor: sorbitol; Pir: pyruvate; Ace: acetaldehyde; EtOh: ethanol; EtAC: ethyl acetate; AA: acetic acid; CA: citrate; 2OA: 2-oxoglutarate; GA: glutamate; SA: succinate; FA: fumarate; MA: malate; OAA: oxaloacetate; OA: oxalate; 2metp: 2-methylpropanol; 2meta: 2-methylpropyl acetate; CR: carbon release.

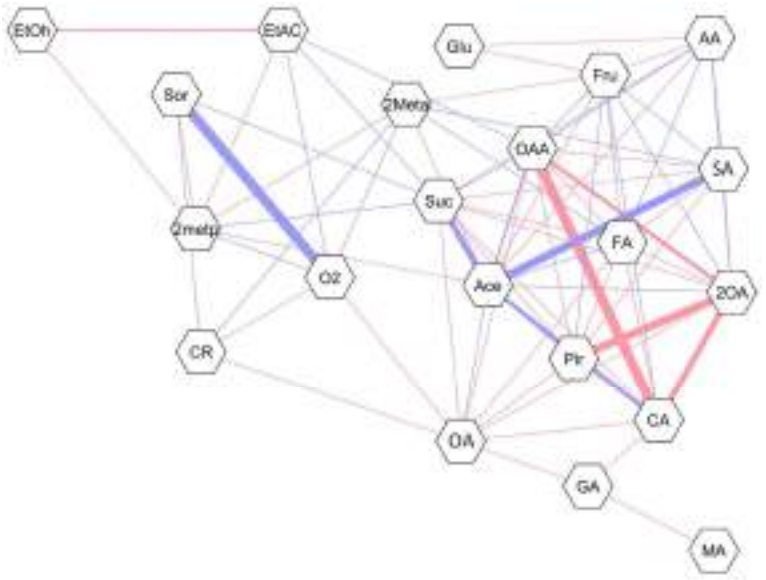

Figure 6. Correlation network for 'Elstar' apples after 9 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment (650 ppb), plus 7 d of shelf life at 20 °C. Blue and red lines show negative and positive correlation, respectively. The sizes of the lines show the correlation magnitude (all correlations shown are higher than 0.5). Suc: sucrose; Glu: glucose; Fru: fructose; Sor: sorbitol; Pir: pyruvate; Ace: acetaldehyde; EtOH: ethanol; EtAC: ethyl acetate; AA: acetic acid; CA: citrate; 2OA: 2-oxoglutarate; GA: glutamate; SA: succinate; FA: fumarate; MA: malate; OAA: oxaloacetate; OA: oxalate; 2metp: 2-methylpropanol; 2meta: 2-methylpropyl acetate; CR: carbon release.

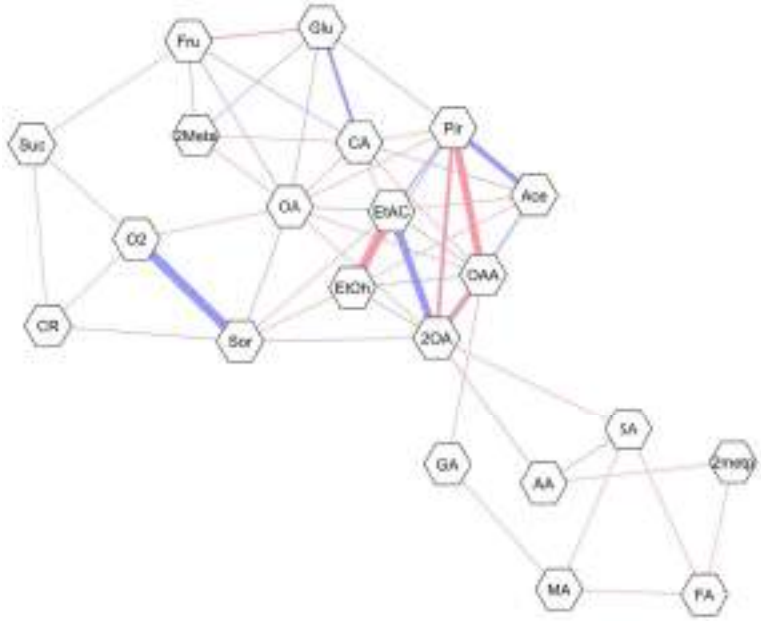

Figure 7. Correlation network for 'Nicoter' apples after 6 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment (650 ppb), plus 7 d of shelf life at 20 °C. Blue and red lines show negative and positive correlation, respectively. The sizes of the lines show the correlation magnitude (all correlations shown are higher than 0.5). Suc: sucrose; Glu: glucose; Fru: fructose; Sor: sorbitol; Pir: pyruvate; Ace: acetaldehyde; EtOH: ethanol; EtAC: ethyl acetate; AA: acetic acid; CA: citrate; 2OA: 2-oxoglutarate; GA: glutamate; SA: succinate; FA: fumarate; MA: malate; OAA: oxaloacetate; OA: oxalate; 2metp: 2-methylpropanol; 2meta: 2-methylpropyl acetate; CR: carbon release.

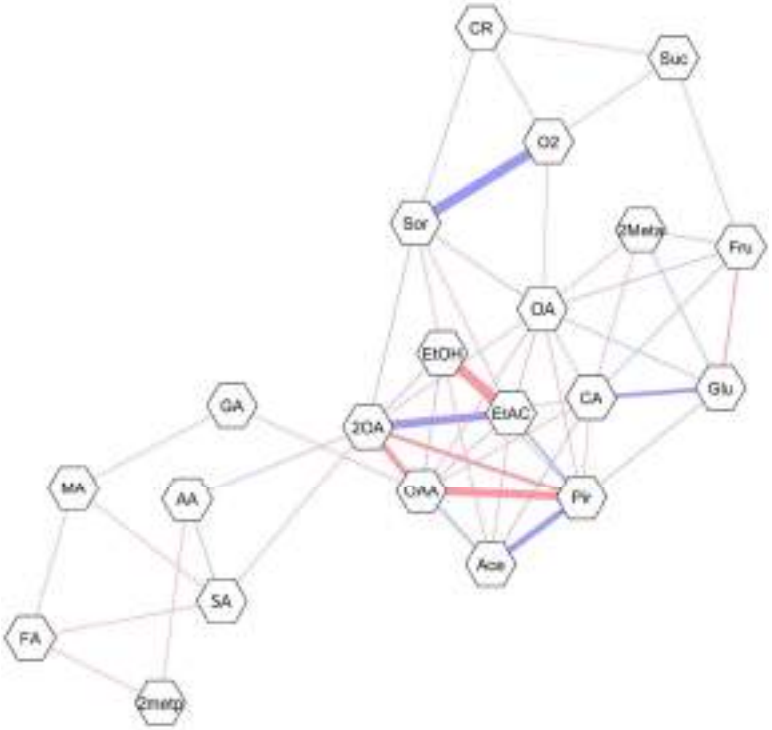

Figure 8. Correlation network for 'Nicoter' apples after 9 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment

(650 ppb), plus 7 d of shelf life at 20 °C. Blue and red lines show negative and positive correlation, respectively. The sizes of the lines show the correlation magnitude (all correlations shown are higher than 0.5). Suc: sucrose; Glu: glucose; Fru: fructose; Sor: sorbitol; Pir: pyruvate; Ace: acetaldehyde; EtOH: ethanol; EtAC: ethyl acetate; AA: acetic acid; CA: citrate; 2OA: 2-oxoglutarate; GA: glutamate; SA: succinate; FA: fumarate; MA: malate; OAA: oxaloacetate; OA: oxalate; 2metp: 2-methylpropanol; 2meta: 2-methylpropyl acetate; CR: carbon release.

The correlation networks also show that the TCA intermediaries of the 'Elstar' apples, such as citrate, 2-oxoglutarate and oxaloacetate, had a positive Pearson correlation among each other (Figures 5 and 6). These results demonstrate that citrate, 2-oxoglutarate and oxaloacetate had a similar response to the storage treatments, regardless of the DCA method and 1-MCP application. On the other hand, for the 'Nicoter' apples, a strong and positive correlation between pyruvate and oxaloacetate after 6 and 9 months of storage was observed (Figures 7 and 8). The pyruvate is probably actively converted to citrate and/or oxaloacetate by the enzymes pyruvate dehydrogenase and pyruvate carboxylase, respectively. The conversion of pyruvate to oxaloacetate uptakes CO_2, which can explain, in part, the apparent low RQ measurements in several studies [12,47,65].

4. Conclusions

In practical terms, this study demonstrates the effects of CA and three DCA methods, either with or without 1-MCP, on the metabolite variation at harvest and after 6 and 9 months of storage at a low temperature, plus 7 d shelf life for 'Elstar' and 'Nicoter' apples. The metabolism of both cultivars differs between each other for the LOL variation over the storage period. The LOL was higher for the 'Nicoter' apples, and the three DCA methods were able to detect this difference between the cultivars, but LOL estimation was lower when the fruit was stored under DCA-RQ and DCA-CD because of the anaerobic metabolism induction. This demonstrates that both DCA methods can be used for commercial apples storage.

The sugar metabolism was differently affected by the DCA methods and 1-MCP treatment, especially sorbitol, which had a trend of accumulation when the fruit was stored under DCA-RQ and DCA-CD, especially in higher RQ and CD, showing a negative correlation with the oxygen partial pressure over the storage period. The 1-MCP treatment induced sorbitol accumulation even when the fruit was stored under CA (higher oxygen partial pressures), showing that sorbitol synthesis by fruit could be a response to stress, such as low oxygen and/or 1-MCP treatment.

The pyruvate flux to TCA was inhibited differently for both cultivars, being inhibited first for the 'Nicoter' apples at higher oxygen partial pressures, demonstrating a higher tolerance to low oxygen for the 'Elstar' apples. TCA inhibition is reversible by increasing the oxygen setpoint, resulting in higher TCA intermediaries in the 'Nicoter' apples after being stored under DCA-RQ 1.5 for 9 months. The 'Elstar' apples stored under DCA-CD 1.1 resulted in higher accumulations of acetaldehyde, which is converted to acetic acid in a higher amount, which is an important acetate ester precursor. The 1-MCP application reduced the accumulation of ethanol and ethyl acetate when the fruit was stored under DCA-RQ 1.5 by directing the conversion of acetaldehyde to acetic acid and not to ethanol for both cultivars studied.

TCA intermediaries, such as citrate, 2-oxoglutarate, succinate and oxaloacetate, were the most affected by the atmosphere conditions and 1-MCP treatment for both cultivars. These acids generally have a positive Pearson correlation among each other. Malic acid was more affected by the storage time than the atmosphere conditions. Succinate and fumarate had an accumulation trend when the fruit was stored under DCA-RQ, i.e., lowest oxygen partial pressures.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10121372/s1, Table S1: Averages of sugars, organic acids and some volatile compounds of 'Nicoter' apples after harvest, at 6 and 9 months of storage under controlled atmosphere (CA) and dynamic controlled atmosphere monitored by chlorophyll fluorescence (DCA-CF), respiratory quotient (DCA-RQ) and carbon dioxide (DCA-CD), either without or with 1-MCP treatment (650 ppb), plus 7 d of shelf life at 20 °C.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Padrão do escurecimento da polpa de novos cultivares de maçã para utilização em alimentos minimamente processados

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Resumo – Este trabalho tem como objetivo avaliar o escurecimento e a mudança total de cor da polpa de diferentes cultivares de maçã através de parâmetros colorimétricos, a fim de identificar aqueles com mínima ocorrência destes fenômenos e potencial para o processamento mínimo. Maçãs dos cultivares Daiane, Elenise, Fuji Mishima, Kinkas, Luiza, Monalisa, Maxi Gala e Venice, provenientes de pomares experimentais de São Joaquim, SC, safra 2018/2019, foram avaliadas em relação à atributos de cor relacionados ao escurecimento da polpa. As maçãs foram cortadas ao meio e dispostas em condições de ambiente. Após o corte, foram realizadas leituras de atributos de cor na polpa dos frutos, utilizando um colorímetro CR 400 (Konica Minolta®), em 14 intervalos de tempo, de 10 a 2.880 minutos. Através dos parâmetros coletados pelo colorímetro (*L**, *a** e *b**), foram avaliados a luminosidade da polpa, o índice de escurecimento e a mudança total de cor. As maçãs 'Fuji Mishima', 'Elenise' e especialmente 'Venice' apresentaram menor escurecimento da polpa em comparação aos demais cultivares avaliados. Esses resultados indicam que possivelmente esses cultivares têm maior potencial para a utilização na indústria de processamento mínimo devido ao menor escurecimento e mudança de cor na polpa dos frutos.

Termos para indexação: *Malus domestica* Borkh; Escurecimento enzimático; Mudança total de cor.

Flesh browning pattern of new apple cultivars for use in minimally processed foods

Abstract – This study aimed to evaluate the browning and the total color change of the apple flesh of different apple cultivars through colorimetric parameters, to identify those with minimal occurrence of these phenomena and potential for minimal processing. Apples from the cultivars Daiane, Elenise, Fuji Mishima, Kinkas, Luiza, Monalisa, Maxi Gala and Venice were evaluated for color attributes related to flesh browning. The apples were cut in half and placed under ambient conditions. After cutting, color attributes were read on the fruit flesh, using a CR 400 colorimeter (Konica Minolta®), at 14-time intervals, from 10 to 2880 minutes. Through the parameters collected by the colorimeter (*L**, *a** and *b**), the flesh luminosity, the browning index and the total color change were evaluated. 'Fuji Mishima', 'Elenise' and especially 'Venice' apples show less flesh browning than the other cultivars evaluated. These results indicate that these cultivars may have greater potential for use in the minimal processing industry due to less browning and color change in the fruit flesh.

Index terms: *Malus domestica* Borkh; Enzimatic browning; Full color change.

Introdução

A maçã (*Malus domestica* Borkh) é o fruto de clima temperado de maior dispersão, comercialização e consumo como fruta fresca no mundo. No Brasil, o agronegócio da macieira tem um histórico importante de organização e planejamento, mas precisa de inovações para o fortalecimento da cadeia e o aumento no consumo do fruto. A produção brasileira foi crescendo conjuntamente à demanda interna, sendo a terceira fruta mais consumida (Cruz *et al*., 2015). Contudo, considerando a população do país e o consumo *per capita* de outros países, o volume consumido ainda é baixo.

A indústria de processamento mínimo da maçã tem grande potencial e pode aumentar o consumo de maçã. Nos EUA, as maçãs minimamente processadas emergiram como lanche popular nos programas de merenda escolar e consumo familiar. Dessa forma, a tendência é que este mercado continue crescendo à medida que mais consumidores exigem um lanche rápido, conveniente, seguro, saudável e nutritivo (Subhashree *et al*., 2017). O aumento na produção e no consumo de frutas minimamente processadas ocorre pelo estilo de vida dos consumidores modernos aliado à maior procura por produtos *in natura* e seus benefícios, reduzindo o consumo de alimentos

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industrializados e o desperdício de alimentos frescos.

O principal problema dos vegetais minimamente processados é o escurecimento enzimático. A maçã é altamente suscetível a este fenômeno devido à sua estrutura e composição fenólica. Em sua composição existem enzimas e substratos específicos para a ação oxidativa (Moreno *et al*., 2016). A intensidade do escurecimento depende da complexa interação entre atividade da enzima polifenoloxidase (PPO), presença do oxigênio molecular (O_2) e concentração dos compostos fenólicos que são os substratos da mesma (Jiang *et al*., 2016). O corte do fruto durante o processamento mínimo possibilita o contato entre a enzima e substrato, de forma que a intensidade e a velocidade do escurecimento da polpa tornam-se dependentes do conteúdo de compostos fenólicos, atividade da enzima PPO e a natural capacidade antioxidante do fruto. Segundo Tappi *et al*., (2019), existe uma variação significativa na atividade da polifenoloxidase em diferentes cultivares. Além disso, a capacidade antioxidante e os compostos fenólicos também apresentam grande variação entre cultivares (Stanger *et al*., 2017).

O Brasil não possui uma indústria estabelecida de maçã minimamente processada e as pesquisas nesta área são muito escassas. Dentre os inúmeros desafios destaca-se a necessidade de encontrar cultivares apropriados em termos de qualidade e tempo de vida de prateleira potencial, uma vez que o cultivar é um dos principais fatores que exercem influência sobre o escurecimento enzimático (Tappi *et al*., 2019). Os cultivares Gala e Fuji, que correspondem a mais de 90% da produção nacional (Kist, 2019), são bastante suscetíveis ao escurecimento enzimático logo após o corte da polpa (Bertrand *et al*., 2015; Tappi *et al*., 2019).

Nos EUA, foram desenvolvidos estudos de modificação genética em cultivares de macieira com intuito de usá-los para o processamento mínimo (Maxmen, 2017). O Brasil não produz maçãs exclusivamente de uso industrial, porém a pesquisa nacional obteve avanços agronômicos na adaptação de macieiras a condições climáticas e com menor suscetibilidade a fitopatógenos (De Martin *et al*., 2018; Denardi *et al*., 2020). O melhoramento também permitiu o surgimento de maçãs com escurecimento enzimático tardio ou ausente, possibilitando à polpa do fruto permanecer inalterada em relação à cor, durante horas, mesmo com estresses mecânicos durante o processamento (Carlesso *et al*., 2022; Ercoli *et al*., 2017; Denardi *et al*., 2020). Assim, frutos com menor taxa ou ausência de escurecimento enzimático podem ser opções em potencial às indústrias de produtos minimamente processados, gerando um apelo sensorial com a cor quase inalterada.

Este trabalho objetivou avaliar o escurecimento e a mudança total de cor da polpa de diferentes cultivares de maçã através de parâmetros colorimétricos, a fim de identificar aqueles com mínima ocorrência destes fenômenos e potencial para o processamento mínimo.

Material e métodos

Maçãs dos cultivares Daiane, Elenise, Fuji Mishima, Kinkas, Luiza, Monalisa, Maxi Gala e Venice foram colhidas de pomares experimentais localizados em São Joaquim, SC, durante a safra 2018/2019. Em seguida, foi efetuada padronização de calibre e eliminação de frutos que apresentavam podridões, lesões ou defeitos.

Após a colheita, os frutos foram imediatamente levados ao laboratório para avaliação dos atributos de cor relacionados ao escurecimento da polpa. As maçãs foram cortadas ao meio, na porção equatorial, e dispostas em bandejas de papelão em condições ambiente (23±2°C de temperatura; 75±5% de umidade relativa - UR). Os frutos foram mantidos em condição de ambiente para induzir o escurecimento da polpa e averiguar o padrão de escurecimento dos cultivares, sem considerar que, após o processamento mínimo, devem ficar sob refrigeração.

Imediatamente após a colheita, foi realizada uma caracterização inicial para determinação dos atributos de maturação dos frutos utilizando, para tanto, três repetições compostas por dez frutos. O índice de iodo-amido foi determinado por meio da comparação do escurecimento da metade peduncular dos frutos tratados com solução de iodo, com uma escala de 1 a 10, onde o índice 1 indica o teor máximo de amido (fruto imaturo) e o índice 10 representa o amido totalmente hidrolisado (fruto completamente maduro). A firmeza de polpa (N) foi determinada na região equatorial dos frutos, em pontos opostos, após a remoção de uma pequena porção da epiderme, com auxílio de um penetrômetro eletrônico (GÜSS Manufacturing Ltd, Cidade do Cabo, África do Sul), equipado com ponteira de 11,0mm de diâmetro. Os valores de acidez titulável (% ácido málico) foram obtidos em amostra de 5mL de suco extraída de fatias dos frutos. Esta amostra foi titulada com solução de NaOH 0,1N, utilizando o indicador ácido-base azul de bromotimol. Os teores de sólidos solúveis (°Brix) foram determinados em um refratômetro digital modelo PR201α (Atago®, Tóquio, Japão), utilizando alíquota do suco obtido para a quantificação da AT. Os dados da caracterização inicial são apresentados na Tabela 1.

Para avaliação dos atributos de cor relacionados ao escurecimento da polpa as maçãs foram cortadas ao meio, na porção equatorial, e dispostas em bandejas de papelão em condição de ambiente (23±2°C de temperatura; 75±5% de umidade relativa - UR). Os cortes foram realizados de forma manual, com lâminas cortantes. Os frutos foram mantidos em condições ambiente para induzir o escurecimento da polpa e averiguar o padrão de escurecimento dos cultivares, sem considerar que, após o processamento mínimo, devem ficar sob refrigeração.

Após o corte, foram realizadas duas leituras de atributos de cor em regiões opostas da polpa dos frutos, utilizando um colorímetro CR 400 (Konica Minolta®, Tóquio, Japão), em 14 intervalos de tempo (0, 10, 20, 30, 60, 90, 120, 180, 240, 360, 600, 1080, 1560 e 2880 minutos). Através dos parâmetros *L**, que determina a luminosidade, *a**, que determina as coordenadas de verde a vermelho e *b**, que determina as coordenadas de azul a amarelo, coletados pelo colorímetro,

Tabela 1. Firmeza de polpa (FP), teor de sólidos solúveis (SS), acidez titulável (AT) e índice de iodo-amido (IA) em maçãs de oito cultivares na colheita, safra 2018/2019
Table 1. Flesh firmness (FP), soluble solids content (SS), titratable acidity (AT) and starch index (IA) of eight apple cultivars at harvest, 2018/2019 season

Cultivar	Data colheita	FP (N)	SS (°Brix)	AT (% ac. málico)	IA (1-10)
Monalisa	05/02	91,5	11,9	0,77	4,9
Luiza	13/02	88,3	11,4	0,43	5,8
Maxi Gala	18/02	74,2	10,7	0,39	7,2
Venice	15/03	81,9	13,0	0,50	6,9
Daiane	18/03	75,0	11,7	0,46	8,8
Fuji Mishima	18/03	75,2	12,5	0,44	7,7
Kinkas	26/04	75,9	12,7	0,49	7,1
Elenise	26/04	71,3	13,9	0,71	7,9

foram avaliados a luminosidade da polpa (*Lightness;* L*), o índice de escurecimento (IE) e a mudança total de cor (E). O IE e E foram obtidos por meio das respectivas equações apresentadas abaixo, conforme Subhashree *et al*. (2017):

$$IE = \frac{100[\left(\frac{a + 1{,}75L}{5{,}635L + a - 3{,}012b}\right) - 0{,}31]}{0{,}17}$$

$$\triangle E = \sqrt{(L* - L*0)^2 + (a* + a*0)^2 + (b* + b*0)^2}$$

A intensidade de escurecimento após o corte das maçãs é representada pelo índice de escurecimento, sendo que, quanto maior o valor do índice, mais intenso é o escurecimento. Os valores de ΔE indicam o quanto a amostra se distanciou do padrão. Quanto mais perto de 0, mais próximo do padrão selecionado. Cada cultivar apresenta tonalidade e luminosidade de polpa específicas; alguns são naturalmente mais claros que outros. Para eliminar a heterogeneidade entre as amostras e avaliar apenas o escurecimento ocorrido após o corte dos frutos, o índice de escurecimento foi normalizado, utilizando o parâmetro inicial como divisor comum, conforme a equação: $IE_{[0;\ 10;\ 20;(...);\ 2880]}/IE_{[0]}$ (Niamnuy *et al*., 2008; Subhashree *et al*., 2017).

Este experimento foi conduzido em delineamento inteiramente casualizado, composto por 5 repetições de 10 frutos, seguindo um esquema fatorial "cultivar" (8) x "intervalo de leitura" (14), após a realização do corte dos frutos. As pressuposições de normalidade dos erros e homogeneidade das variâncias foram avaliadas pelos testes de Shapiro-Wilk e Levene, respectivamente. Os dados foram então submetidos à análise da variância e, quando a interação entre os fatores foi significativa (p<0,05), foi realizado o desdobramento dos efeitos. Para o fator "cultivar" foi realizado o teste de comparação de médias Scott-Knott, enquanto para o fator "intervalo de leitura" foi realizada a análise de regressão.

Resultados e discussão

Os fatores "cultivar" e "intervalo de leitura" apresentaram interação significativa em todas as variáveis analisadas. A redução máxima da luminosidade, o máximo escurecimento e a mudança total de cor na polpa das maçãs variaram em tempo e intensidade, dependendo dos cultivares avaliados. Contudo, substanciais alterações ocorreram, em média, nos primeiros 50 minutos de exposição à temperatura ambiente, após o corte dos frutos (Figuras 1, 2 e 3). As alterações de cor, decorrentes do dano mecânico sofrido pelo tecido das maçãs após o corte, aparecem em alguns minutos e, para Oyarzún *et al*. (2018), com uma manifestação máxima até 1h depois do corte. Portanto, considerando o processo na indústria de produtos minimamente processados, para reduzir essas alterações, frutos recém-cortados devem ser armazenados em temperaturas próximas a 0°C e a manipulação deve ocorrer sob baixas temperaturas. Entre 0°C a 4°C a atividade da PPO tende a diminuir, reduzindo o escurecimento da polpa dos frutos (Tomás-Barberán; Espin, 2001).

Imediatamente após o corte dos frutos, verificou-se que a luminosidade da polpa (valor *L**) no cultivar Maxi Gala é naturalmente mais elevada, seguida pelos cultivares Monalisa, Daiane, Kinkas e Luiza (Tabela 2). Sendo assim, a redução deste atributo tende a ser mais abrupta nesses cultivares após o corte dos frutos e consequentemente ser percebida visualmente com mais facilidade em relação aos demais. O cultivar Fuji Mishima é o que possui a polpa com menor luminosidade, seguido pelos cultivares Elenise e Venice, ou seja, são os cultivares com menor propensão a grandes alterações na luminosidade da polpa após o corte dos frutos.

Foi possível verificar diferenças na luminosidade da polpa entre os cultivares aos 10 minutos após o corte. A maçã 'Maxi Gala' apresentou a maior redução, seguida por 'Monalisa', 'Fuji Mishima' e 'Kinkas', enquanto os demais cultivares tiveram menor redução, sem diferir entre si. Embora haja diferenças intrínsecas de luminosidade e tonalidade da polpa entre os cultivares, alguns deles mantêm os atributos visuais muito próximos do natural, mesmo minutos após o corte da polpa e o início das reações de oxidação.

Após 60 minutos do corte dos frutos, 'Maxi Gala' seguiu como o cultivar com maior redução da luminosidade, em média 47% superior aos demais, seguido pelos cultivares Kinkas, Monalisa, Luiza, Fuji Mishima e Daiane (Tabela 2). O cultivar Venice foi o que apresentou menor redução da luminosidade. Na avaliação realizada 240 minutos após o corte, o cultivar Maxi Gala, seguido dos cultivares Monalisa e Kinkas foram os que apresentaram maior redução da luminosidade. Após 600 minutos, 'Maxi Gala' e 'Monalisa' apresentaram maior redução da luminosidade, enquanto 'Fuji Mishima' não diferiu de 'Venice', como os cultivares com menor redução da luminosidade da polpa. Após 2.880 minutos do corte das maçãs, os

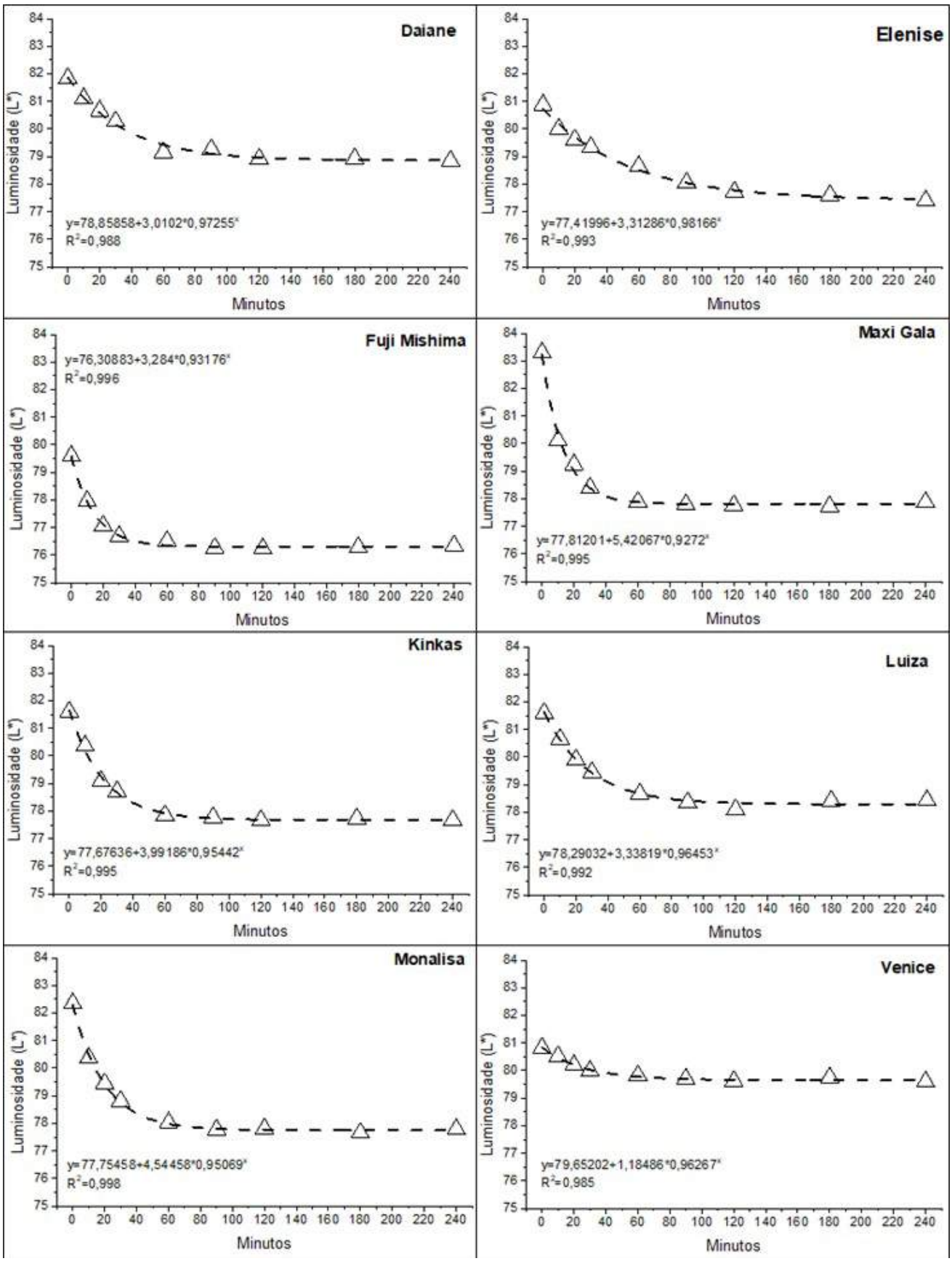

Figura 1. Variação da luminosidade (L*) na polpa de oito cultivares de maçã em função do tempo após o corte dos frutos na região equatorial, durante 240 minutos de exposição em condições ambiente (23±2°C; 75±5% UR), na safra 2018/2019

Figure 1. Variation in luminosity (L) on the flesh of eight apple cultivars as a function of time after cutting the fruits in the equatorial region, during 240 minutes of exposure under ambient conditions (23±2°C; 75±5% RH), 2018/2019 season*

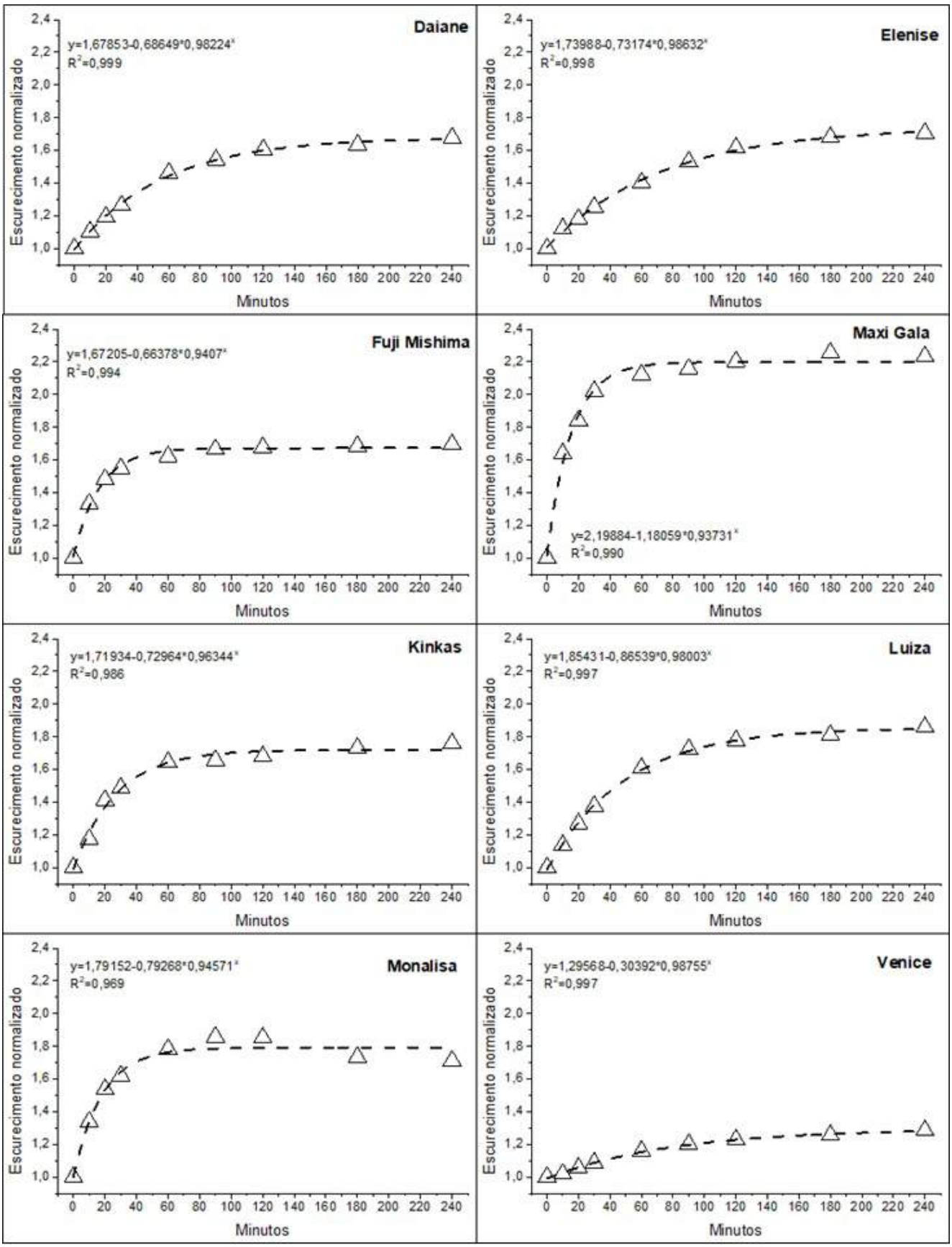

Figura 2. Variação do escurecimento normalizado na polpa de oito cultivares de maçãs em função do tempo após o corte dos frutos na região equatorial, durante 240 minutos de exposição em condições ambiente (23±2°C; UR de 75±5%), na safra 2018/2019

Figure 2. Variation in normalized browning on the flesh of eight apple cultivars as a function of time after cutting the fruits in the equatorial region, during 240 minutes of exposure under ambient conditions (23±2°C; 75±5% of RH), 2018/2019 season

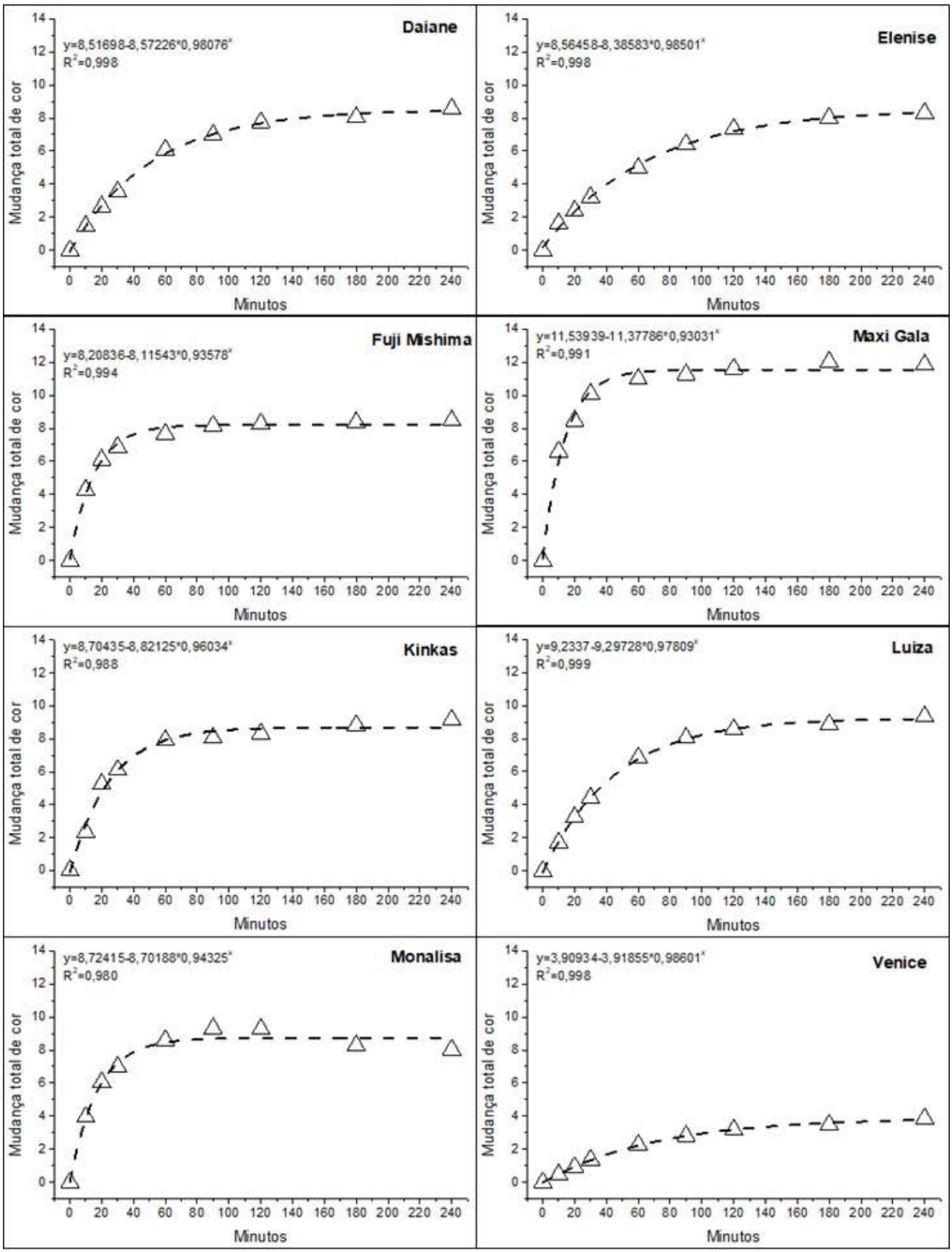

Figura 3. Variação da mudança total de cor na polpa de oito cultivares de maçãs em função do tempo após o corte dos frutos na região equatorial, durante 240 minutos de exposição em condições ambiente (23±2°C; UR de 75±5%), na safra 2018/2019

Figure 3. Variation in the total color change on the flesh of eight apple cultivars as a function of time after cutting the fruits in the equatorial region, during 240 minutes of exposure under ambient conditions (23±2°C; 75±5% of RH), 2018/2019 season

Tabela 2. Luminosidade na polpa imediatamente após o corte (LP) e redução da luminosidade, em unidades de *lightness*, de oito cultivares de maçã em função do tempo após o corte dos frutos, na região equatorial e exposição em condições ambientes (23±2°C; 75±5% de UR), na safra 2018/2019

Table 2. Luminosity on the flesh immediately after cutting (PL) and reduction in luminosity, in lightness units, of eight apple cultivars as a function of time after cutting the fruits, in the equatorial region and exposure in ambient conditions (23±2°C; 75±5% of RH), 2018/2019 season

Cultivar	LP	Redução de luminosidade (L*)				
		10	60	240	600	2880
		min				
Daiane	81,8 c	0,7 d	2,7 c	3,0 d	4,3 b	4,8 c
Elenise	80,9 d	0,9 d	2,2 d	3,5 d	3,9 b	5,3 c
Fuji Mishima	79,6 e	1,6 c	3,1 c	3,2 d	3,3 c	5,0 c
Maxi Gala	83,3 a	3,2 a	5,4 a	5,4 a	5,2 a	7,5 a
Kinkas	81,6 c	1,2 c	3,8 b	3,9 c	4,2 b	7,5 a
Luiza	81,6 c	0,9 d	2,9 c	3,2 d	4,3 b	6,6 b
Monalisa	82,3 b	2,0 b	4,3 b	4,6 b	4,9 a	7,2 a
Venice	80,8 d	0,3 d	1,0 e	1,2 e	2,7 c	1,0 d
CV (%)	0,6	30,4	12,0	12,5	11,6	17,7

Médias seguidas de mesma letra nas colunas não diferem entre si pelo teste de Scott-Knott a 5% de probabilidade.

Means followed by the same letter in the columns do not differ from each other using the Scott-Knott test at 5% probability.

cultivares Maxi Gala, Kinkas e Monalisa apresentaram as maiores reduções de luminosidade na polpa sem diferenças entre si, seguidas pelo cultivar Luiza. O cultivar Venice foi o que apresentou menor redução da luminosidade na polpa durante todo o período de avaliação, até 2.880 minutos após o corte.

Os consumidores julgam a qualidade de frutos minimamente processados com base na sua aparência. O atributo "luminosidade" é considerado um dos principais parâmetros de qualidade visual da polpa de frutos: quanto maior os valores de *L**, maior a aceitabilidade dos consumidores (Rocha; Morais, 2003), porque a polpa não está escura. Segundo Son, Moon e Lee (2011), o armazenamento sob baixa temperatura contribui para retardar a perda de luminosidade em maçãs minimamente processadas.

A compreensão dos detalhes que envolvem o processo do escurecimento enzimático em diferentes cultivares é necessária para que seu controle possa ser realizado. Para Kumar *et al*. (2012), apesar dos muitos esforços realizados com a utilização de tratamentos físicos que permitam a inibição da atividade enzimática da PPO, sem a adição de agentes químicos, estes não são suficientemente eficientes.

Em relação ao escurecimento da polpa, o destaque negativo foi o cultivar Maxi Gala, com valores variando, em média, entre 30 e 40% a mais que os outros cultivares, desde os 10 minutos até 2.880 minutos após o corte dos frutos (Tabela 3). O escurecimento da polpa em maçãs ocorre devido a reações químicas catalisadas principalmente pela enzima PPO. O papel da PPO, na reação de escurecimento enzimático, é oxidar fenóis em quinonas (Cortellino *et al*., 2015). As reações subsequentes levam a polimerização de quinonas em pigmentos marrons.

Aos 10 minutos após o corte, os cultivares Fuji Mishima e Monalisa também apresentaram escurecimento de polpa superior aos demais, porém, inferior ao 'Maxi Gala'. Após 60 minutos do corte, o cultivar Venice já apresentava o menor escurecimento entre todos os cultivares, e manteve-se como o cultivar com menor escurecimento até 2.880 minutos após a realização do corte, com valores médios de 40% a menos de escurecimento. Na avaliação realizada após 60 minutos do corte, 'Daiane' e 'Elenise' também apresentaram um baixo escurecimento, superior apenas ao cultivar Venice, enquanto 'Monalisa' apresentou alto escurecimento, inferior apenas ao cultivar Maxi Gala. Com 240 e 600 minutos do corte, 'Luiza' apresentou escurecimento superior aos demais e inferior ao cultivar Maxi Gala. Após 2.880 minutos do corte, o cultivar Luiza seguiu como o segundo cultivar com maior escurecimento, e o cultivar Fuji Mishima, o segundo com menor escurecimento.

Os cultivares Venice e Maxi Gala foram os destaques positivo e negativo na mudança total de cor, com a menor e maior alteração, respectivamente, durante todo o período avaliado (Tabela 4). A oxidação causada pela atividade enzimática, além de alterar a cor da polpa das maçãs, também pode resultar em perda de qualidade nutricional e proporcionar modificações de sabor (Carvalho *et al*., 2010). Segundo Son, Moon e Lee (2001), a intensidade da cor marrom, resultante da atividade da PPO, depende do perfil de compostos fenólicos no tecido.

A mudança de cor na polpa das maçãs foi mais elevada no cultivar Maxi Gala, em todas as avaliações realizadas, e no cultivar Luiza, aos 2.880 minutos após o corte. 'Venice' apresentou, em todas as avaliações, menor mudança total de cor, sendo que aos 2.880 minutos após o corte dos frutos os resultados foram similares aos do cultivar Maxi Gala aos 10 minutos após o corte. Aos 10 minutos após o corte, os cultivares Fuji Mishima e Monalisa apresentaram mudança total de cor inferior apenas ao cultivar Maxi Gala, e superior aos demais. Em 60 minutos após o corte, 'Kinkas' e 'Fuji Mishima' apresentaram mudança total de cor inferior apenas ao cultivar Maxi Gala, seguidos pelo cultivar Luiza. 'Elenise' apresentou mudança total de cor superior apenas ao cultivar Venice. Em 240 minutos após o corte dos frutos, os cultivares Maxi Gala e Venice apresentaram maior e menor mudança total de cor, respectivamente, enquanto os demais cultivares apresentaram resultados intermediários sem diferirem entre si. Em 600 minutos após o corte, o cultivar Luiza apresentou mudança total de cor inferior apenas ao 'Maxi Gala' e superior aos demais. Após 2.880 minutos do corte dos frutos, 'Luiza' e 'Maxi Gala' apresentaram maior mudança total de cor, sem diferirem entre si, seguidos do cultivar Kinkas, enquanto 'Venice' apresentou, dentre todos, a menor mudança de coloração.

Tabela 3. Escurecimento normalizado na polpa de oito cultivares de macieira em função do tempo após o corte dos frutos na região equatorial e exposição em condições ambiente (23±2°C; UR de 75±5%), na safra 2018/2019
Table 3. Normalized darkening on the flesh of eight apple cultivars as a function of time after cutting the fruits in the equatorial region and exposing them to ambient conditions (23±2°C; 75±5% of RH), 2018/2019 season

Cultivar	Tempo após o corte dos frutos (min)				
	10	60	240	600	2880
Daiane	1,10 c	1,46 d	1,68 c	1,71 c	2,04 c
Elenise	1,12 c	1,40 d	1,71 c	1,79 c	2,05 c
Fuji Mishima	1,33 b	1,62 c	1,69 c	1,70 c	1,85 d
Maxi Gala	1,64 a	2,12 a	2,23 a	2,29 a	2,67 a
Kinkas	1,17 c	1,64 c	1,76 c	1,80 c	2,15 c
Luiza	1,14 c	1,61 c	1,86 b	1,99 b	2,43 b
Monalisa	1,34 b	1,78 b	1,71 c	1,78 c	2,08 c
Venice	1,02 c	1,16 e	1,29 d	1,35 d	1,50 e
CV (%)	6,7	5,7	6,1	6,5	7,3

Nota: Médias seguidas de mesma letra nas colunas não diferem entre si pelo teste de Scott-Knott a 5% de probabilidade.
Means followed by the same letter in the columns do not differ from each other using the Scott-Knott test at 5% probability.

Tabela 4. Mudança total de cor na polpa de oito cultivares de macieira em função do tempo) após o corte dos frutos na região equatorial e exposição em condições ambiente (23±2°C; UR de 75±5%), na safra 2018/2019
Table 4. Total color change on the flesh of eight apple cultivars as a function of time after cutting the fruits in the equatorial region and exposing them to ambient conditions (23±2°C; 75±5% of RH), 2018/2019 season

Cultivar	Tempo após o corte dos frutos (min)				
	10	60	240	600	2880
Daiane	1,5 c	6,1 d	8,6 b	9,0 c	12,4 c
Elenise	1,6 c	5,0 e	8,3 b	9,2 c	11,8 c
Fuji Mishima	4,3 b	7,7 b	8,5 b	8,5 c	10,4 d
Maxi Gala	6,6 a	11,0 a	11,8 a	12,2 a	15,2 a
Kinkas	2,3 c	7,9 b	9,2 b	9,6 c	13,3 b
Luiza	1,7 c	6,9 c	9,3 b	10,6 b	14,5 a
Monalisa	4,0 b	8,6 b	8,0 b	8,7 c	11,9 c
Venice	0,5 d	2,3 f	3,9 c	4,9 d	6,7 e
CV (%)	28,8	10,6	9,8	9,5	8,9

Nota: Médias seguidas de mesma letra nas colunas não diferem entre si pelo teste de Scott-Knott a 5% de probabilidade.
Means followed by the same letter in the columns do not differ from each other using the Scott-Knott test at 5% probability.

Os resultados de luminosidade, escurecimento e mudança total de cor na polpa dos frutos indicam que alguns cultivares apresentam maior potencial para a utilização na indústria de processamento mínimo. Os cultivares Fuji Mishima, Elenise e especialmente Venice apresentaram os melhores resultados. Já o cultivar Maxi Gala, que atualmente é um dos mais produzidos no Brasil (Faoro, 2022), apresentou resultados inferiores, indicando que, dentre os cultivares avaliados, pode não ser uma boa opção para o mercado de processamento mínimo de maçãs, ou que seu emprego para este fim tem maior dependência da aplicação de antioxidantes e da temperatura de manipulação. O cultivar Fuji Mishima também tem um volume de produção elevado no país e pode ser mais bem aproveitado para estes fins. Já os cultivares Elenise e Venice ainda estão em processo de difusão pelo Brasil, porém com boas perspectivas de cultivo devido a vantagens em relação aos clones de cultivares Gala e Fuji, como maior adaptabilidade às condições climáticas do país, resistência a doenças e boa qualidade dos frutos (De Martin *et al*., 2018), além de menor velocidade de escurecimento e de alteração de cor da polpa, que pode tornar os frutos mais aptos para o processamento mínimo, conforme os resultados obtidos.

Para Denardi *et al*. (2020) e Carlesso *et al*. (2022), além do alto potencial para o mercado de frutas frescas, a maçã 'Elenise' pode promover mudanças nas indústrias de maçãs minimamente processadas e de suco de maçã no Brasil, as quais hoje dependem dos cultivares Gala e Fuji, pois apresenta lento escurecimento de polpa devido à baixa reação oxidativa de seus compostos. Os resultados do presente trabalho reforçam a indicação do cultivar Elenise para processamento mínimo, e ainda incluem 'Venice' nesse nicho de mercado em potencial, pois seus frutos também apresentam lento escurecimento de polpa. Estudos futuros devem ser realizados com o objetivo de compreender os principais mecanismos fisiológicos envolvidos no menor grau de escurecimento desses cultivares.

Conclusões

Quando comparadas às demais variedades estudadas, as maçãs do cultivar Venice apresentam menor escurecimento de polpa, tendo potencial para utilização na indústria de minimamente processados, por conta da menor alteração visual dos frutos após os danos mecânicos.

As maçãs dos cultivares Fuji Mishima e Elenise também possuem potencial para o mercado de minimamente processados pelas baixas alterações visuais na polpa dos frutos. Por outro lado, maçãs 'Maxi Gala' e 'Luiza' não são as mais indicadas para o processamento

mínimo, uma vez que apresentam maior índice de escurecimento de polpa e mudança total de cor.

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Treatment of 'Baigent' apple fruit with nitric oxide in controlled atmosphere storage reduces decay and physiological disorders

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ABSTRACT

The study was carried out to evaluate the effects of nitric oxide (NO) treatment at different doses and times during controlled atmosphere (CA) storage on quality of 'Baigent' apples. The evaluated treatments were: control; 10 μL L^{-1} of NO applied only at the beginning of CA storage; 1, 2 or 5 μL L^{-1} of NO applied every 30 d of CA storage; and 5 μL L^{-1} of NO applied at the beginning and at the end of CA storage. Fruit were stored for 7.5 months in 1.2 kPa O_2 + 2.0 kPa CO_2, 1.5 ± 0.2 °C, and relative humidity of 94 ± 2%. NO treatments, except 10 μL L^{-1} NO applied only at the beginning of CA storage, reduced ethylene production. At removal from CA storage, 5 μL L^{-1} NO, applied every 30 d of CA storage or at the beginning and at the end of CA storage, reduced respiratory rates. Control fruit and fruit treated with 2 μL L^{-1} NO every 30 d of CA storage had higher soluble solids content, and control fruit and fruit treated with NO at 1 or 2 μL L^{-1} every 30 d of CA storage had higher titratable acidity. NO treatments, regardless of the dose and application time during CA storage, delayed skin yellowing and reduced hydrogen peroxide content and the incidence of pulp mealiness. The application of 5 μL L^{-1} of NO at the beginning and at the end of CA storage maintained a higher total phenolic compound content and reduced the incidence of flesh browning. Treatment with 5 μL L^{-1} NO at the beginning and at the end of CA storage provided better quality maintenance of 'Baigent' apples.

1. Introduction

Controlled atmosphere (CA) storage maintains apple fruit quality for long periods (up to 7 months for 'Gala' and 10 months for 'Fuji') (Brackmann et al., 2005). However, substantial losses can still occur under CA, especially with prolonged storage, due to the incidence of decay and physiological disorders.

Nitric oxide (NO) is naturally produced in living cells through NO synthase like enzymes and is involved in the regulation and influence of many cellular processes during plant development. Reactive nitrogen species are derived from NO and can act by modifying fatty acids and mainly nucleotides (DNA/RNA) through nitration and are able to regulate gene expression and decrease oxidative stress leading to a longer period of storage of fruit or vegetables (Manjunatha et al., 2010; Pols et al., 2022). NO delays ripening and senescence by the reduction in ethylene synthesis through the inhibition of the enzymes 1-aminocyclopropane 1-carboxylic acid synthase (ACC synthase) and 1-aminocyclopropane 1-carboxylic acid oxidase (ACC oxidase) (Manjunatha et al., 2012; Zuccarelli et al., 2021). In addition, NO inhibits the expression of genes involved in the ethylene biosynthetic pathway (Ferrante and Francini, 2006; Zhu et al., 2006; Liu, et al., 2007; Flores et al., 2008; Zhu et al., 2008; Hayat et al., 2010; Manjunatha et al., 2010; Sis et al., 2012; POLS et al., 2022). NO also induces cell protection from oxidative stress by activating antioxidant enzymes, such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and suppression of lipoxygenase (LOX) (Manjunatha et al., 2010, 2012; Buet et al., 2021; Jiménez-Muñoz et al., 2021), which may prevent the manifestation of physiological disorders.

The pre-storage treatment with NO gas of 'Cripps Pink' apples had a positive effect on the maintaining fruit quality, delayed skin yellowing, and reduced flesh browning (Steffens et al., 2021) and superficial scald (Steffens et al., 2022a). The application of NO gas requires an environment with low O_2, to avoid the reaction of NO with O_2, forming NO_2, and thus guarantee its effectiveness (Liu et al., 2016). Therefore, CA

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conditions used in apple storage constitute a suitable environment for the application and reapplication of NO. In 'Laetitia' plums, the reapplication of NO during CA storage had a more effective effect on fruit quality maintenance than a single application at the beginning of CA storage (Steffens et al., 2022b). As the effect of NO is dependent on concentration and exposure time (Buet et al., 2021), better results can be obtained with lower doses and reapplication during CA storage.

The study was carried out to evaluate the effects of NO treatment at different doses and time during CA storage on quality of 'Baigent' apples.

2. Materials and method

2.1. Plant material, experimental design and treatments

Fruit were harvested (2019/ 2020 crop season) in a commercial orchard located in Vacaria (28° 30′ 39″ S; 50° 55′ 47″ W), State of Rio Grande do Sul, Southern Brazil. Fruit with mechanical damage or damage caused by insects and diseases were discarded. The fruit showed the following ripening attributes at harvest: skin background color with lightness (*L*), chroma (*C*), and hue angle (h°) of 76.0, 39.6, and 100.4, respectively; flesh firmness of 80.4 N, a soluble solids content (SSC) of 12.1% of sugar, and titratable acidity (TA) of 0.378% malic acid.

The evaluated treatments were: control (0 μL L^{-1} of NO); 10 μL L^{-1} of NO applied only at the beginning of CA storage; 1, 2 or 5 μL L^{-1} of NO applied every 30 d of CA storage; and 5 μL L^{-1} of NO applied at the beginning and at the end of CA storage. The experiment followed a completely randomized design, with five repetitions and each repetition/experimental unit consisting of 25 fruit.

Fruit of all treatments were stored in 60 L experimental micro chambers, under CA (1.2 kPa O_2 + 2.0 kPa CO_2, 1.5 ± 0.2 °C, and relative humidity of 94 ± 2%) for 7.5 months (Brackmann et al., 2017). CA condition was established by injecting gaseous nitrogen from high-pressure cylinders to reduce O_2 down to 5 kPa, and over the next 10 d of storage, the O_2 gradually decreased to 1.2 kPa through O_2 consumption by fruit respiration. The partial pressures of CO_2 were obtained by injecting this gas inside the minichambers up to the desired concentrations (Gularte et al., 2022).

CO_2 and O_2 levels were checked daily, with the aid of an electronic gas analyzer (Schelle, Germany). The correction of atmospheres was performed manually, using nitrogen gas injection from high pressure cylinders to reduce CO_2 and O_2. When necessary, CO_2 supplementation was also carried out with gas from high pressure cylinders, while the increase in O_2 concentrations was carried out by the injection of atmospheric air (Gularte et al., 2022).

The NO used for application in the microchambers came from a high-pressure cylinder (standard mixture containing 1.000 μL L^{-1} of NO + balance N_2, WhiteMartins®). The gas was injected up to pre-established concentrations in the treatments. The applications were performed soon after the correction of gases (monitoring), and the nope remained around 24 h inside the microchambers. After 24 hours, the monitoring was performed again. The first application of NO occurred on 10 d of storage, when the O_2 partial pressure reached 1.2 kPa. NO was reapplied every 30 d from the first application or at the end of storage, depending on treatment. The last NO application (for treatments with reapplication every 30 d) occurred 5 d before the end of storage.

2.2. Evaluation of fruit quality attributes

Fruit were evaluated for respiratory and ethylene production rates, skin background color and incidence of decay at the chamber opening. After 7 d of shelf life, fruit were evaluated for respiratory and ethylene production rates, skin background color, flesh firmness, SSC, TA, total phenolic content (TPC), hydrogen peroxide content and incidence of flesh browning, mealiness and decay.

To determine respiratory rates and ethylene production, approximately 1 kg of fruit was placed in airtight glass flasks (with a volume of 6.0 L) for 120 min (Steffens et al., 2022b). The values of respiratory rates and ethylene production were expressed in ηmol CO_2 kg^{-1} s^{-1} and ηmol C_2H_4 kg^{-1} s^{-1}, respectively.

The skin background color (*L*, *C* and h°) on the greener region at the equator of the fruit was evaluated by using a Minolta colorimeter (model CR 400). Flesh firmness (N), TA (% of malic acid), and SSC (% of sugars) were evaluated according to the methodology described by Thewes et al. (2015). The incidence of decay (%), and TPC were evaluated according to the methodology described by Stanger et al. (2018), the results of TPC were calculated based on the standard curve of gallic acid and expressed as g gallic acid equivalent (GAE) in kg^{-1} fresh weight. Hydrogen peroxide content was assessed according to the method described by Loreto and Velikova (2001) and were expressed in g H_2O_2 kg^{-1} fresh weight.

The incidence of flesh browning and mealiness were evaluated through a cross section in the equatorial region of the fruit. For flesh browning, those that visually showed any type of browning were counted as browning fruit. The mealiness was determined through manual compression of the fruit, being counted as floury fruit those that presented a dry and spongy appearance.

2.3. Statistical analysis

Data were subjected to analysis of variance (ANOVA), using the statistical program SISVAR 5.6 (Ferreira, 2019) and means of treatments compare by the Scott-Knott test ($p < 0.05$). Percentage data (incidence of decay, flesh browning and mealiness) were transformed through the arcsine formula ($[(x+0.5)/100]^{1/2}$) before ANOVA.

3. RESULTS AND DISCUSSION

The application of 5 μL L^{-1} of NO, both every 30 d of CA storage and at the beginning and at the end of CA storage, reduced the ethylene production assessed after 1 day of shelf life. However, 5 μL L^{-1} of NO applied at the beginning and at the end of CA storage showed the highest ethylene production rate on the third day of shelf life. At 5 d of shelf life, the highest rate of ethylene production (with a climacteric peak) was observed for the control, and the lowest rates for treatments with 5 μL L^{-1} of NO, applied every 30 d of CA storage or at the beginning and at the end of CA storage, and with 2 μL L^{-1} of NO applied every 30 d of CA storage. At 7 d of shelf life, 10 μL L^{-1} of NO applied at the beginning of CA storage showed the highest ethylene production rate, followed by the control, while the other treatments showed the lowest rates. In general, NO application maintained the smaller peak of ethylene production throughout the shelf life. Furthermore, the application of NO every 30 d of storage, at doses of 2 and 5 μL L^{-1}, suppressed the climacteric peak of ethylene production (Fig. 1 A). In 'Cripps Pink' apples, the application of NO at the beginning of storage also reduced the rate of ethylene production (Steffens et al., 2021).

Inhibition of ethylene biosynthesis by NO occurs due to decreased expression of ACC oxidase and also occurs the binding of NO to ACC oxidase, forming a binary complex that is later chelated by ACC to form a stable ternary complex "ACC-ACC oxidase- NO", due to the inhibition of ACC oxidase, an accumulation of ACC occurs, which due to the effect of NO will result in a stoichiometric reduction of ACC to 1-(malonylamino) cyclopropane-1-carboxylic acid (MACC) thus decreasing ethylene biosynthesis (Manjunatha et al., 2012; Pols et al., 2022). In 'Laetitia' plums, reapplication of NO at a lower dose (2 μL L^{-1}) during CA storage also reduced ethylene production, (Steffens et al., 2022b). Therefore, the effect of NO on ethylene production depends on the dose and frequency of application. Furthermore, the effects of NO depends on the fruit species (Manjunatha et al., 2010), and possibly the time of exposure to the gas (Buet et al., 2021).

The respiratory rate at 1 day of shelf life was lower in fruit treated with 5 μL L^{-1} of NO, every 30 d of storage and also at the beginning and at the end of CA storage (Fig. 1B). However, for the rest of the shelf life

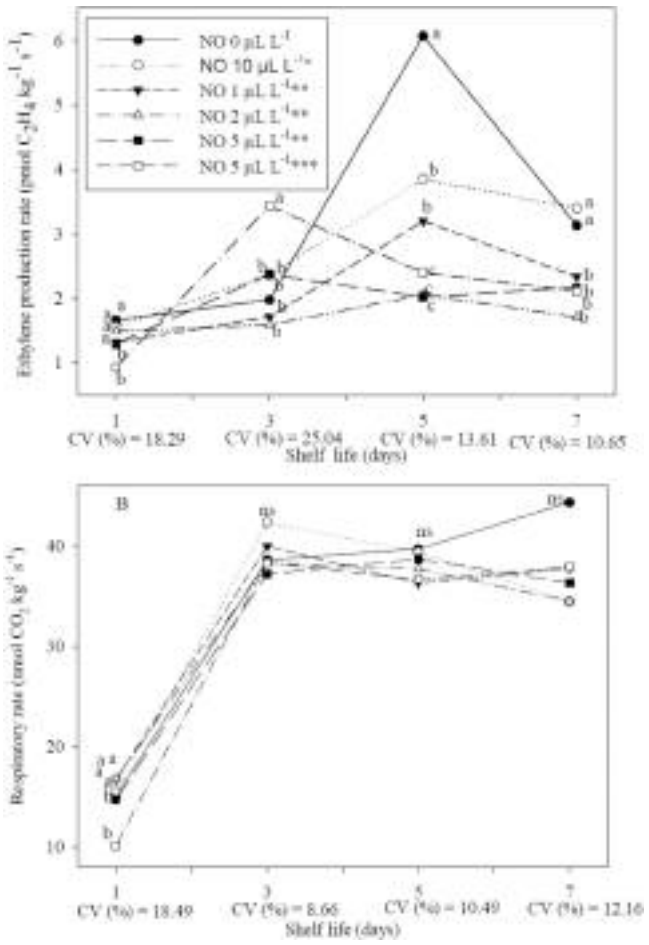

Fig. 1. Ethylene production rate (pmol C_2H_4 kg^{-1} s^{-1}) (**A**) and respiratory rates (ηmol CO_2 kg^{-1} s^{-1}) (**B**) of 'Baigent' apples, after eight months of controlled atmosphere (CA) storage (1.2 O_2 + 2.0 CO_2; 1.5±0.2 °C and RH of 94±2%), followed by one to seven days of shelf life (23±2 °C and RH of 65±5%). Fruit were treated with nitric oxide (NO) at different doses and moments during CA storage. Means followed by different letters on each date differ by Scott-Knott test ($p < 0.05$). ns: not significant. *NO: applied at the beginning of CA storage (when the storage atmosphere was established); **NO: applied at the establishment of the CA storage and every 30 days until the end of CA storage; *** NO: applied at the beginning and at the end of CA storage.

period, there was no difference between treatments (Fig. 1B). In 'Galaxy' (Brackmann et al., 2017) and 'Cripps Pink' (Steffens et al., 2021; 2022a) apples and 'Laetitia' plums (Steffens et al., 2022b), the treatment with NO reduced the respiratory rate of the fruit. In 'Cripps Pink' apples, this effect was dose and exposure time-dependent (Steffens et al., 2022a). According to Kang et al. (2015), in a study carried out on peach, the application of NO resulted in the negative regulation of several enzymes that participate in respiration, such as phosphoglycerate kinase, phosphoglycerate mutase, triose phosphate isomerase, as well as of cytochrome *c* oxidase. In 'Laetitia' plums, the effect of NO persisted during the shelf life period (Steffens et al., 2022b). In 'Packham's Triumph' pears, the application of 10 µL L^{-1} of NO reduced the respiratory rate during 7 d of shelf life, but this was not observed for fruit treated with the dose of 20 µL L^{-1} (Hendges et al., 2016). In 'Cripps Pink' apples, NO treatment, with 10 µL L^{-1} for 8 h and with 20 µL L^{-1} for 2 h, reduced the respiratory rate after 7 d of shelf life. However, the application of 10 µL L^{-1} of NO for 2 or 4 h did not affect the respiratory rate (Steffens et al., 2021). Steffens et al. (2022a) reported in 'Cripps Pink' apples stored under CA that the pre-storage treatment with NO at 10 and 20 µL L^{-1} for 2 h reduced the respiratory rate assessed after 7 d of shelf life. Published results show that fruit treatment with NO might reduce respiration, but the magnitude of this effect depends on the dose, exposure time, application frequency, storage condition, fruit maturation/ripening stage, and fruit specie/cultivar.

There was no difference between treatments for the background skin color of the fruit assessed at chamber opening. However, after 7 d of shelf life, fruit treated with NO, regardless of the dose and moment of application during CA storage, showed a higher h^o value (greener skin background color) than the control (Table 1), possibly reflecting NO effect reducing ethylene production (Fig. 1 A). NO also reduces the activity of chlorophyllase enzyme, responsible for the degradation of chlorophyll, delaying the loss of skin green color (Guo et al., 2013). In 'Cripps Pink' apples stored under CA, the pre-storage treatment with NO, regardless of dose and exposure time, also delayed the loss of green background color of the skin (Steffens et al., 2021). In 'Packham's Triumph' pears, the treatment with NO also delayed the degreening of the skin (Hendges et al. 2016). However, NO applied at the beginning of the cold storage did not affect the background color of 'Cripps Pink' apple.

There was no difference between treatments for flesh firmness (Table 2). Similar results have been reported for flesh firmness in 'Galaxy' (Brackmann et al., 2017) and 'Cripps Pink' (Steffens et al., 2022a) apples, as well as in 'Laetitia' plums (Steffens et al., 2022b), treated with NO and stored in CA. However, in cold-stored 'Feicheng' peaches (Sun et al., 2011), 'Amber Jewel' plums (Singh et al. 2009), and 'Packham's Triumph' pears (Hendges et al. 2016), the treatment with NO increased flesh firmness of the fruit. Therefore, NO effects on flesh firmness might be affected by treatment procedure (dose, exposure time, application frequency) and fruit specie/cultivar (Steffens et al., 2021).

NO treatment reduced the SSC compared to the control, except NO at 2 µL L^{-1} applied every 30 d of CA storage (Table 2). However, SSC values were above 12% in all treatments, attending consumer preference indexes for apples regarding this attribute (Harker et al. 2002; 2008). Steffens et al. (2021, 2022a) also reported reduced SSC content in 'Cripps Pink' apples treated with NO.

Control fruit and fruit treated with NO at 1 and 2 µL L^{-1}, every 30 d of CA storage, had higher TA. Fruit treated with NO, at 10 µL L^{-1} only at beginning of CA storage, and at 5 µL L^{-1}, every 5 d of CA storage and at the beginning and at the end of CA storage, had lower TA values (Table 2). Steffens et al. (2022a) did not observe a reduction in the TA of 'Cripps Pink' apples treated with NO at 10 and 20 µL L^{-1}, for 2 h, at the beginning of CA storage. However, Steffens et al. (2021) observed reduction of TA in 'Cripps Pink' apples treated with NO at 10 µL L^{-1}, by increasing the treatment period from 2-4 h to 8 h. Therefore, the effect of NO on TA of the fruit is dependent on dose and application time during storage.

Fruit treated with 5 µL L^{-1} of NO at the beginning and at the end of

Table 1
Skin background color (L^*, C^* and h^o) of 'Baigent' apples after eight months of controlled atmosphere (CA) storage (1.2 O_2 + 2.0 CO_2; 1.5±0.2 °C and RH of 94 ±2%), followed by seven days of shelf life (23±2 °C and RH of 65±5%). Fruit were treated with nitric oxide (NO) at different doses and moments during CA storage.

Treatments (NO)	Removal from CA storage			Seven days of shelf life		
	L	*C*	*h°*	*L*	*C*	*h°*
0 µL L^{-1}	73.6 ns	40.8 ns	96.0 ns	68.3 ns	38.7 ns	80.5 b
10 µL L^{-1}*	73.9	39.3	97.0	71.9	40.2	90.4 a
1 µL L^{-1}**	73.8	38.7	95.7	72.2	40.7	92.3 a
2 µL L^{-1}**	72.0	38.8	97.8	71.5	40.5	91.7 a
5 µL L^{-1}**	72.2	40.2	99.5	71.8	40.5	90.8 a
5 µL L^{-1}***	73.1	39.6	98.4	71.7	40.8	91.4 a
CV (%)	2.7	2.7	2.8	2,9	3.6	4.6

Means followed by different letters within columns differ by Scott-Knott test ($p < 0.05$). ns: not significant. *NO: applied at the beginning of CA storage (when the storage atmosphere was established); **NO: applied at the establishment of the CA storage and every 30 days until the end of CA storage; *** NO: applied at the beginning and at the end of CA storage.

Table 2
Flesh firmness (FF; N), soluble solids content (SSC; %), titratable acidity (TA; % of malic acid), total phenolic content (TPC; g GAE kg^{-1} fresh weight) and hydrogen peroxide content (H_2O_2; g H_2O_2 kg^{-1} fresh weight) of 'Baigent' apples after eight months of controlled atmosphere (CA) storage (1.2 O_2 + 2.0 CO_2; 1.5 ±0.2 °C and RH of 94±2%), followed by seven days of shelf life (23±2°C and RH of 65±5%). Fruit were treated with nitric oxide (NO) at different doses and moments during CA storage.

Treatments (NO)	FF	SSC	TA	TPC	H_2O_2
0 μL L^{-1}	69.2^{ns}	14.0 a	0.298 a	0.21 c	0.015 a
10 μL L^{-1}*	68.8	12.7 b	0.261 b	0.18 c	0.012 b
1 μL L^{-1}**	71.0	13.2 b	0.278 a	0.28 b	0.012 b
2 μL L^{-1}**	70.6	13.4 a	0.290 a	0.28 b	0.009 b
5 μL L^{-1}**	68.8	13.1 b	0.269 b	0.26 b	0.010 b
5 μL L^{-1}***	67.5	12.8 b	0.257 b	0.32 a	0.009 b
CV (%)	2.7	3.3	5.6	9.4	16.5

Means followed by different letters within columns differ by Scott-Knott test ($p < 0.05$). ns: not significant. *NO: applied at the beginning of CA storage (when the storage atmosphere was established); **NO: applied at the establishment of the CA storage and every 30 days until the end of CA storage; *** NO: applied at the beginning and at the end of CA storage.

CA storage had the highest TPC value. Control fruit and fruit treated with 10 μL L^{-1} of NO only at beginning of CA storage showed the lowest TPC values, while fruit treated with 1, 2, or 5 μL L^{-1} of NO every 30 d of CA storage showed intermediate results (Table 2). Pretreatment with NO for 2 hours prior to CA storage of 'Cripps Pink' apples also increased the TPC (Steffens et al., 2022a). In mushrooms, the treatment with NO increased TPC and the activity of the phenylalanine ammonia-lyase and chalcone synthase enzymes, showing activation of secondary metabolism (Dong et al., 2012).

Control fruit had a higher hydrogen peroxide content than fruit treated with NO, regardless of dose and application time during CA storage (Table 2). Reactive oxygen species (ROS) can be induced by several abiotic factors, in addition to senescence. The control fruit showed a more accentuated peak of ethylene production, when compared to the other treatments with NO (Fig. 1A), more yellow skin (Table 1), and higher mealiness incidence (Table 3), which are indicators of a more advanced ripening and possibly the beginning of fruit senescence, therefore reflecting a higher hydrogen peroxide content. Ren et al. (2020) also reported a lower hydrogen peroxide content in response to NO treatment and attributed the result to a higher antioxidant activity.

NO application, except for 5 μL L^{-1} of NO applied every 30 d of CA storage, reduced decay incidence compared to the control at the chamber opening. After 7 d of shelf life, all fruit treated with NO had reduced incidence of decay compared to the control (Table 3). Steffens et al. (2021; 2022a) did not report reduction of decay in 'Cripps Pink' apples treated with NO at the beginning of storage. NO treatment reduced decay caused by *Colletotrichum gloeosporioides* in 'Tainong' mangoes, (Ren et al., 2020), and suppressed spore germination and mycelium growth of *Penicillium expansum* in tomatoes (Lai et al., 2011). NO impairs membranes integrity of pathogen cells due to oxidative damage, and suppresses spore germination and mycelial growth (Ren et al., 2020). In addition, NO might increase TPC in the fruit (Table 3) that constrains pathogen growth (Sivankalyani, et al., 2016).

Table 3
Incidences (%) of decay, mealiness and flesh browning of 'Baigent' apples after eight months of controlled atmosphere (CA) storage (1.2 O_2 + 2.0 CO_2; 1.5±0.2 °C and RH of 94±2%). Fruit were treated with nitric oxide (NO) at different doses and moments during CA storage. Decay was assessed after removal from CA storage and seven days of shelf life (23±2 °C and RH of 65±5%), while mealiness and flesh browning were assessed only after seven days of shelf life.

Treatments (NO)	Decay		Mealiness	Flesh browning
	Removal from CA storage	Seven days of shelf life		
0 μL L^{-1}	6.0 a	16.8 a	6.5 a	6.5 a
10 μL L^{-1}*	0.0 b	4.1 b	0.0 b	4.2 a
1 μL L^{-1}**	2.0 b	8.0 b	1.0 b	4.4 a
2 μL L^{-1}**	1.0 b	3.0 b	0.0 b	7.3 a
5 μL L^{-1}**	5.0 a	5.0 b	0.0 b	4.2 a
5 μL L^{-1}***	1.0 b	3.9 b	0.0 b	0.0 b
CV (%)	46.5	51.5	24.5	24.4

Means followed by different letters within columns differ by Scott-Knott test ($p < 0.05$). *NO: applied at the beginning of CA storage (when the storage atmosphere was established); **NO: applied at the establishment of the CA storage and every 30 days until the end of CA storage; *** NO: applied at the beginning and at the end of CA storage.

Control fruit had the highest incidence of mealiness. NO, regardless of dose and application time during CA storage, prevented the incidence of the disorder. Fruit treatment with 5 μL L^{-1} of NO at the beginning and at the end of CA storage reduced flesh browning incidence (Table 3). This treatment also increased TPC values (Table 2). The reduction of phenolic compounds results from their oxidation by the polyphenol oxidase enzyme. In longan fruit, the application of sodium nitroprusside, a NO donor, reduced the activity of polyphenol oxidase and peroxidase enzymes, maintained a higher TPC, and delayed flesh browning (Duan et al., 2007). Flesh browning in apples manifests during the storage and is most evident during the shelf life. In this sense, the application of NO gas at the end of CA storage contributes to the protection of fruit against physiological disorders by reducing the activity of enzymes causing oxidation of phenolic compounds.

The management of NO gas during CA storage of 'Baigent' apples preserved fruit quality, by reducing decay and physiological disorders and delaying the yellowing of the fruit skin. Regarding flesh firmness, TA, and SSC. For 'Gala' strains (including 'Baigent'), the loss of fruit flesh firmness is one of the main problems during storage. However, in the present study, fruit from all treatments had flesh firmness ≥ 62 N, considered adequate for maximum consumer acceptance (Harker et al., 2008). According to Harker et al. (2002), fruit with flesh firmness ≤ 53 N are more prone to mealiness. Therefore, the high flesh firmness of the fruit explains the low mealiness incidence (0–6.5%) observed in our study. Flesh firmness is an important indicator to define the conservation potential of apples, consumer acceptance and susceptibility to the manifestation of physiological disorders and decay (Betinelli et al., 2017). Therefore, fruit with higher fresh firmness at harvest have a greater storage potential.

In this study, we did not observe any effect on NO on apple flesh firmness, corroborating the results obtained by Steffens et al. (2021, 2022a) and Brackmann et al. (2017). In this sense, future studies should be carried out to investigate the effect of fruit treatment with NO for long term CA storage (≥ 10 months), as well as for other CA conditions (1-methylcyclopropene [CA+1-MCP], ultra low oxygen CA [CA-ULO], extremely low oxygen CA [CA-ELO] and dynamic CA [DCA]). Fruit response to NO treatment should also evaluate the effects of cultivar, fruit maturity stage and orchard edaphoclimatic condition (production area).

4. Conclusions

'Baigent' apples treated with NO, regardless of the dose or time of application in CA storage, hadlower ethylene production peaks, incidences of mealiness and decay, as well as less yellowing skin color.

The application of 5 μL L^{-1} of NO at the beginning and at the end of CA storage provided a higher content of total phenolic compounds and reduced the incidence of flesh browning.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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ORIGINAL ARTICLE

Blue mold control and volatile compounds in "Fuji" apples treated with chitosan emulsion combined with essential oils

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Abstract

The aim of this work was to evaluate the effects of chitosan emulsion (CE) in combination with essential oils (Eos) on blue mold control, as well as on aroma and quality attributes of "Fuji" apples after storage. Treatments evaluated were control, CE (1%) and CE (1%) combined with citronella, clove and copaiba EOs (all EOs at 500 $\mu l\,L^{-1}$). Fruits treated with EC and EC + OEs showed lower total phenolic compounds in the peel in all treatments. Fruits treated with EC combined with citronella OEs (242.4 OD mg^{-1} of protein min^{-1}) and clove (205.8 OD mg^{-1} of protein min^{-1}) showed lower peroxidase activity in fruit peel tissue. Therefore, CE and CE combined with citronella, clove and copaiba essential oils reduced the development of blue mold in "Fuji" apples. In addition, the treatments with lower severity of blue mold showed lower peroxidase activity in the peel and pulp tissues of the fruit, indicating induction of resistance by essential oils.

Novelty impact statement:

- Chitosan biofilm reduces *rot by P. expansum* in "Fuji" apples, and when in addition to chitosan, essential oils of citronella, clove and copaiba are used, there was an increase in the reduction of disease severity.
- The aroma of the fruit did not present a characteristic aroma of the essential oils used with the method of application used.
- There were no changes in fruit quality attributes with the use of chitosan and chitosan biofilm with the addition of essential oils.

1 | INTRODUCTION

Apple (*Malus domestica* Borkh.) is among the most produced temperate fruit trees in southern Brazil. The majority of produced apple fruit is stored and sold throughout the year. In 2018/2019, Brazil produced 1.02 billion tons of apple fruit, according to the Hortifruti Brazil 2020 yearbook, with Santa Catarina accounting for approximately 50% of total production (De Martin et al., 2019). "Fuji" apples are more susceptible to the development of rots during storage in comparison to other cultivars (Sanhueza et al., 2006).

Blue mold (*Penicillium expansum* Link.) is the main postharvest disease in apples. *P. expansum* affects fruit during the postharvest and might produce mycotoxins, such as patulin (Frisvad & Samson, 2004; Pitt & Hocking, 2009). Mycotoxins represent a threat to public health, as they can cause chronic and acute symptoms in humans and animals, including vomiting, reproductive losses, growth reduction and immunosuppression (Errampalli, 2014).

Postharvest decay is a recurring problem to the apple industry that affects the quality and the marketing value of fruit, with growing concern regarding environment and human health, leading

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to a search for alternative means of decay control (Li et al., 2017). Pathogens resistance to some fungicides applied at postharvest on apples, such as pyrimethanil and fludioxonil (Li & Xiao, 2008), reinforces this concern. In addition, society is increasingly concerned about food safety and environmental contamination with synthetic fungicides, demanding sustainable agricultural production (Ogoshi et al., 2019).

Essential oils (EOs) can be used for decay control and have low toxicity, many of them being recognized as safe by the Food and Drug Administration (Bakkali et al., 2008). In addition, due to their different mechanisms of action, EOs reduce the risk of pathogens developing resistance (Yap et al., 2014). However, the application of EOs might cause undesirable aromas in fruit.

EOs can be emulsified with edible coatings for treatment of fruit (Ali et al., 2016; Azarakhsh et al., 2014; Maqbool et al., 2011). An alternative product used for pathogen control is chitosan, a polycationic ß–1.4 polymer linked to D-glucosamine, defined as chitin diacetate, a natural polysaccharide extracted from the shell or exoskeleton of crustaceans (Botelho et al., 2010). Chitosan can delay mycelial growth, decrease conidia germination and cause morphological changes in the germ tube of some pathogens (Liu et al., 2007). Additionally, chitosan is an excellent matrix for the encapsulation of EOs, allowing the controlled release of volatile compounds (Maes et al., 2019). The encapsulation of EOs in chitosan emulsions (CEs) might reduce postharvest losses caused by blue mold in apples, without negatively affecting aroma and other fruit quality attributes.

This work was carried out to evaluate the effects of CE combined with citronella, clove and copaiba EOs on blue mold control, as well as on aroma and quality attributes of "Fuji" apples after storage.

2 | MATERIALS AND METHODS

"Fuji" apples were harvested in a commercial orchard, in 2018/2019 and 2019/2020, in São Joaquim, SC (49°56′ W; 28°17′ S; altitude of 1350 m) and stored under controlled atmosphere (CA; 1.0 kPa O_2 + 0.5 kPa CO_2) for 7 months. After being removed from CA storage, fruit was transported to the laboratory for sample homogenization, application of treatments and evaluations. The ripening stage of fruits at the time of experiment set up was characterized in terms of flesh firmness, titratable acidity (TA) and soluble solids (SS) in three samples of 15 fruits. Flesh firmness (N) was assessed at the equatorial region of fruit, on two opposite sides, after removing a small portion of the peel, with an electronic penetrometer (GÜSS Manufacturing Ltd, South Africa) equipped with a 7.9 mm diameter probe. TA (% malic acid) was analyzed in a 10 ml sample of juice, obtained by processing fruits in a centrifuge. This sample was diluted in 90 ml of distilled water and titrated with 0.1 N NaOH solution to pH 8.1 using an automatic titrator (SCHOTT Instruments, TitroLine® Easy, Germany). SS (°Brix) was assessed with a refractometer (Atago®, model PR201α, Japan) using an aliquot of the juice obtained to analyze TA.

Treatments evaluated were control (distilled water + Tween® 20; 1%, volume by volume), CE and CE combined with citronella, clove and copaiba EOs. The chitosan concentration in the emulsion was 1% (volume by volume), and all EOs were used at the concentration of 500 µl L^{-1}.

EOs used in this work were the same as those used in the work by Vieira et al. (2018). Clove and citronella EOs presented respectively 5 and 23 identified volatile compounds. In the clove EO, eugenol accounted for 86.6% of the volatile composition. In the citronella EO, citronellal, geraniol and citronellol accounted for 36.2%, 18.9% and 12.8%, respectively, of the volatile composition. Copaiba EO used in this work was acquired from company Ferquima Indústria e Comércio de Óleos Essenciais, in which β-caryophyllene accounted for 51% of the volatile composition: Clove, citronella and cinnamon EOs were the same studied by Vieira et al. (2018).

CE (1%; volume by volume) was prepared by dissolution of chitosan (throughout constant stirring) in 1% acetic acid (volume by volume) until the production of a viscous solution, followed by the addition of 1% glycerin and 1% Tween 20, and then of 500 µL of EOs (citronella, clove and copaiba). The final mix was sonicated in a sonicator (BANDELIN, Sonoplus HD 2200, with a MS 72 tip, Germany) in four series of two minutes, until complete homogenization.

Fruit intended for inoculation was perforated in the equatorial region, on two opposite sides, with an electronic TAXT-Plus® texture analyzer (Stable Micro Systems Ltd, Surrey, United Kingdom) equipped with 2 mm diameter probe. The probe perforated a 4 mm depth in the fruit. Fruit was then inoculated with 10 µl of a suspension of *P. expansum* spores (10^6 spores ml^{-1}). The spore suspension was prepared with sterilized distilled water and 0.05% Tween® 20. The number of spores was counted using Neubauer chamber (hemacytometer) (Youssef et al., 2010).

Fruit was sprayed with each treatment until complete fruit coverage 24 h after inoculation with *P. expansum*. After the application of treatments, fruits were kept in ambient condition (23 ± 3°C and 63 ± 5% of RH) for 7 days, simulating the marketing period. The experiment was followed by a completely randomized design, with four replicates and an experimental unit consisting of 15 inoculated fruits and 15 fruits without inoculation.

Inoculated fruit was daily evaluated for lesion diameter caused by *P. expansum*. The area under the disease progress curve (AUDPC) was calculated according to the formula: AUDPC = Σ [(Si + Si + 1)/2. (Ti + 1-Ti)], where Si = disease severity, mean diameter of the ith observation; Si + 1 = disease severity, mean lesion diameter at the i + 1 assessment; Ti = time in days of the ith observation; Ti + 1 = days of evaluation i + 1. Si was calculated as the sum of the mean diameters of lesions divided by the total number of lesions or fruits.

After 7 days under ambient conditions, in order to verify a possible defense mechanism to the infection process, skin and flesh tissues were collected for analysis of total phenolic compounds (TPC) and peroxidase (POD) activity, in 2018/2019 and 2019/2020. Skin and flesh samples were also collected for analysis of volatile compounds in 2018/2019.

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Extracts for TPC analysis were obtained from 5 g of the sample (skin and flesh tissues), homogenized with 10 ml of methanol 50%. The solution remained at rest for 60 min at 8°C temperature and then centrifuged (Hitachi, CR22N, Japan) under refrigeration (4°C) for 20 min at 10,000 rpm. The supernatant was filtered in a 25-ml volumetric flask. The residue of the first extraction was added 10 ml of 70% acetone, homogenized and allowed to rest for another 60 min at room temperature and then centrifuged again for 20 minutes at 10,000 g. The supernatant was transferred to the volumetric flask (which contains the first supernatant), and the volume was completed to 25 ml with distilled water. Extracts were reserved for TPC analysis.

The TPC content was determined using the Folin-Ciocalteu reagent, as described by Roesler et al. (2007). Gallic acid solution was prepared by diluting 10 mg of gallic acid in 100 ml of methanol P.A. Sodium carbonate solution was prepared by diluting 10 g of the reagent in distilled water in a 100-ml flask. Folin-Ciocalteu was diluted at 1:3 ratio in distilled water. Gallic acid standard curve was obtained by preparing gallic acid solution at concentrations of 0, 10, 30, 50, 70, 90 and 100 $\mu l\,L^{-1}$. For analysis, 2.5 ml of Folin-Ciocalteu, 0.5 ml of sample and 2.0 ml of sodium carbonate solution (10%) were added to each tube, in that order. For the blank, the same reagents previously mentioned and 0.5 ml of methanol were used, replacing the sample. Tubes were vortexed (Gehaka, AV-2, São Paulo) and sealed with parafilm and then incubated for one hour in the darkness. Samples were centrifuged (Hitachi, CR22N, Japan), and reading was performed in microplate reader at the wavelength (λ) of 765 nm. Results were expressed in mg of gallic acid equivalents per 100 g fresh weight (mg GAE $100\,g^{-1}$ fresh weight).

All steps for enzymatic essay of POD activity were carried out at 4°C. POD enzyme was extracted according to method described by Hammerschmid (Hammerschmidt et al., 1982; Youssef et al., 2020) with modifications. Fresh samples of 0.5 g (macerated in liquid nitrogen) were added to 5 ml of 0.100 M potassium phosphate buffer solution (pH 7.0) (Vetec, Duque de Caxias, Brazil), containing 1 mM of insoluble polyvinylpyrrolidone (PVPP) (Vetec, Duque de Caxias, Brazil) and 1 mM ethylenediaminetetraacetic acid (EDTA) (Synth, Diadema, Brazil) for each 100 ml of buffer solution. These samples were then homogenized in an ultra-turrax (Heidolph, Silent Cruscher M, Germany) for 1 minute, with the addition of triton (0.1%; volume by volume) (Vetec, Duque de Caxias, Brazil).

For POD essay, the extract was left for 15 min at 8°C and then centrifuged at 12,000 rpm for 10 min at 4°C (Hitachi, CR22N, Japan), and 0.30 ml of the supernatant was collected. Immediately after the addition of 2.7 ml of reaction mixture, containing potassium phosphate buffer (Vetec, Rio de Janeiro, Brazil): guaiacol: H_2O_2 (5 ml: 0.0125 ml: 0.0163 ml) (Vetec, Rio de Janeiro, Brazil), enzymatic activity was quantified as the increase in absorbance per minute at $\lambda = 470$ nm. Result was expressed in OD mg^{-1} protein $minute^{-1}$.

The volatile compounds were isolated through the technique of solid-phase microextraction (SPME). Analyses were performed in a GC–MS system (PerkinElmer, Germany), consisting of a gas chromatograph (model Clarus 680 GC) coupled to a mass spectrometer (model Clarus SQ 85). Fruit sample (1 g of flesh and skin tissues), 1 g of NaCl and 5 ml of deionized water were put in a vial (capacity of 40 ml), sealed with screw cap and PTFE-coated silicone septum (Agilent Technologies, Germany). The sample was then submitted to pre-extraction incubation (equilibrium), under agitation, for 10 min at 30 ± 1°C, and then, the SPME fiber (CAR/PDMS) mounted in a holder was exposed to the gas phase of the sample for 25 min. Subsequently, the fiber was exposed for 10 min for desorption at 250°C, in the chromatograph injector. The gas chromatograph was equipped with a HP5 column, having helium as the carrier gas at flow rate of 1 ml min^{-1}. The initial temperature was 30°C, for 5 min, with a heating ramp of 2°C min^{-1} up to 120°C, remaining at this temperature for 3 min, with a total running time of 34 min. Compounds were identified based on the chromatograph library, which indicates the probability of similarity of each compound with data contained in the software. Data were submitted to analysis of variance, and treatment means were compared by Tukey's test ($p < .05$). AUDPC data were submitted to Pearson correlation test with TPC and POD activity. Statistical analysis was carried out with SAS software (SAS Institute, 2002).

3 | RESULTS AND DISCUSSION

At the time of experiment set up, fruit had in, 2018/2019, SS of 14.0 °Brix; TA of 0.242% malic acid and flesh firmness of 68.1 N. In 2019/2020, fruits had SS of 15.2 °Brix, TA of 0.230% of malic acid and flesh firmness of 74.2 N.

In both seasons, fruit treated with CE and CE in combination with EOs had lower AUDPC in comparison to the control. In 2018/2019, the AUDPC reduced in comparison to the control by 7.8%, 8.6%, 8.6% and 7.6% for CE, CE + citronella EO, CE + clove EO and CE + copaiba EO, respectively (Table 1). Canaver and Di Piero (2011) also reported reduced blue mold severity and incidence in apples treated by immersion in chitosan suspensions. Chitosan can reduce the incidence of postharvest diseases by directly interfering with mycelium growth and activating various defense responses in the plant tissue (Agrawal et al., 2002; Guerra-Sanchez et al., 2009; Moura et al., 2019). Plants infected by pathogens might synthesize low molecular weight peptides and proteins (called pathogen-related proteins) with antimicrobial action. These proteins are hydrolytic enzymes (chitinase and β-1,3-glucanase) which are part of the general defense mechanism of plants (Hadwiger, 2013) and can be positively induced through the application of chitosan (Hoat et al., 2013).

In 2019/2020, the lower AUDPC was achieved with CE combined with citronella EO, while CE combined with clove and copaiba EOs provided intermediate results, compared to the control (Table 1). For this season, the AUDPC reduced in comparison to the control by 6.2%, 14.3%, 8.7% and 9.3% for CE, CE + citronella EO, CE + clove EO and CE + copaiba EO, respectively. The antifungal activity of EOs is related to their hydrophobicity, which allows them to interact with lipids in the membranes, changing permeability and causing disturbances in the cells (Costa et al., 2011). According to

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TABLE 1 Area under the disease progress curve (AUDPC), total phenolic compounds (TPC) and peroxidase activity (POD) in the flesh and skin tissues of "Fuji" apples

Treatment	AUDPC (cm)	TPC (mg EAG.100 g^{-1} FW)		POD (OD mg^{-1} of protein min^{-1})	
		Flesh	Skin	Flesh	Skin
	2018/2019				
Control	10.5 a	32.7 ab	620.8 a	39.5 ns	340.9 a
Chitosan	9.7 b	33.6 a	387.0 b	38.9	296.9 ab
Chitosan + Citronella	9.6 b	32.68 ab	377.1 b	37.4	272.4 b
Chitosan Clove	9.6 b	26.5 b	463.3 b	33.9	205.8 c
Chitosan + Copaiba	9.7 b	31.9 ab	452.7 b	35.1	312.6 ab
CV (%)	1.93	8.53	13.8	23.3	7.27
	2019/2020				
Control	16.1 a	60.8 ab	1224.3 a	26.3 a	228.4 b
Chitosan	15.1 b	52.1 c	878.7 b	17.5 b	343.4 a
Chitosan + Citronella	13.8 c	59.7 bc	1007.3 b	13.7 b	99.3 c
Chitosan Clove	14.7 bc	67.9 a	978.5 b	14.3 b	121.1 c
Chitosan + Copaiba	14.6 bc	65.4 ab	950.7 b	15.0 b	162.1 bc
CV (%)	2.58	5.15	6.62	19.1	21.8

Note: Means followed by the same letter in the columns are not different by Tukey's test ($p < .05$). ns: not significant ($p \geq .05$). Fruit was removed from controlled atmosphere storage, inoculated with *Penicillium expansum* link, and submitted to postharvest treatments with chitosan emulsion (1%) and chitosan emulsion (1%) combined with different essential oils (EOs; 500 µl L^{-1}). Fruit was left for seven days under ambient conditions (23 ± 5°C/63 ± 2% RH). Data of two seasons (2018/19 and 2019/2020).

Pontes et al. (2019), the strong antimicrobial action of citronella EO is attributed to citronellal and geraniol. The antimicrobial action of clove EO is attributed to eugenol and caryophyllene (Zhang et al., 2017), while in copaiba EO are β-caryophyllene, kaurenoic acid and γ-murolene (Zimmermam-Franco et al., 2013).

Concentration and the form of application of EOs also influence the effectiveness of blue mold control in apples. Vieira et al. (2018) did not report control of blue mold in "Fuji" apples treated with citronella vapor (500 µl L^{-1}), after 30 d of storage. However, our work shows that fruit treated with CE + citronella EO, using the same EO dosage reported by Vieira et al. (2018), reduced blue mold in 2019/2020, compared to fruit treated with CE and the control. Essential oils are extremely volatile at room temperature, so to increase their efficiency, an alternative is to incorporate them into edible coatings. Therefore, the lower volatilization rate of the major EOs compounds when incorporated into emulsions provides longer lasting effect and better control of blue mold.

Control fruit had the highest TPC in the skin tissue and higher incidence of blue mold, indicating that the increase in TPC alone is not enough to prevent the development of this type of rot in "Fuji" apples. The increase in the phenolic content is a common defense response of the plant tissue against the infection process caused by microorganisms (Wallis & Galarneau, 2020). For infections caused by fungi, the increase in the concentration of phenolic compounds occurs through increases in the concentration of flavonoids (Wallis & Galarneau, 2020). Therefore, other mechanisms of defense against pathogen infection might be involved, besides the increase of phenolic compounds.

In 2018/2019, POD activity in the flesh tissue of the fruit was not different between treatments. In 2019/2020, fruit treated with CE and CE + EOs had lower POD activity in the flesh tissue than the control. In both seasons, POD activity in the skin tissue was lower in fruit treated with CE + citronella EO and CE + clove EO than in the control (Table 1). Felipini and Di Piero (2009) also reported in apples treated with chitosan no increase of POD activity, but reduced severity of the disease caused by *C. acutatum*, through inhibition of conidia germination and mycelial growth of the fungus. However, in 2019/2020, fruit treated with CE had higher POD activity in the skin tissue than the control (Table 1). In addition, there was positive correlation between POD activity in the skin tissue and AUDPC in 2018/2019 ($r^2 = 0.59$) and 2019/2020 ($r^2 = 0.60$), and between POD activity in the flesh tissue and AUDPC in 2019/2020 ($r^2 = 0.92$). The positive correlation between POD activity and AUDPC indicates defense response induction to the infection process, causing H_2O_2 accumulation. H_2O_2 is involved in several signal transduction pathways, activating cell defense responses (Sharma et al., 2019), in addition to its toxic effects to pathogens (Zhang et al., 2021).

Treatments with CE or CE + EOs did not affect flesh firmness, SS and TA attributes of the fruit in both seasons (data not shown). Vieira et al. (2018) have reported similar results in "Fuji" apples treated by volatilization with citronella, clove, rosemary and cinnamon EOs.

Fruit treated with EOs did not exhibit the presence of volatile compounds characteristic of EOs aroma (Table 2). However, "Fuji" apples treated by volatilization with citronella, cinnamon, clove, rosemary and tea tree EOs exhibit aroma characteristic of these EOs (Vieira, 2016). The encapsulation of EOs in emulsions allows

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TABLE 2 Profile of volatile compounds (relative area; %) in "Fuji" apples

Class	Compound	Treatment				
		Control	Chitosan	Chitosan + citronella	Chitosan + clove	Chitosan + copaiba
Acid	2-Methylbutanoic acid	0.62 ± 0.19	nd	nd	0.50 ± 0.27	nd
Alcohol	1-Butanol	0.75 ± 1.49	3.73 ± 2.34	3.97 ± 2.00	5.26 ± 2.64	3.99 ± 0.52
	2-Ethylhexanol	12.44 ± 2.15	2.49 ± 1.69	nd	5.95 ± 0.97	4.69 ± 0.57
	2-Ethylhexanol	8.51 ± 2.42	12.15 ± 4.26	13.97 ± 4.37	14.03 ± 3.29	16.07 ± 2.11
	2-Hexenol	nd	0.19 ± 0.38	nd	nd	nd
	Hexane	10.63 ± 1.19	11.40 ± 2.35	9.66 ± 0.72	16.57 ± 5.61	12.18 ± 2.08
Aldehyde	Hexanal	29.17 ± 6.54	30.50 ± 6.82	30.26 ± 4.13	26.19 ± 1.07	26.41 ± 5.19
Ester	Ethyl acetate	3.09 ± 5.11	nd	nd	nd	nd
	Butyl 2-methylbutyrate	0.56 ± 0.11	1.31 ± 0.63	0.84 ± 0.47	1.20 ± 0.81	1.76 ± 0.28
	Butyl butyrate	0.17 ± 0.20	nd	nd	nd	nd
	Ethyl butyrate	2.81 ± 3.71	1.57 ± 1.86	nd	nd	1.68 ± 2.23
	Propyl butyrate	1.29 ± 0.17	0.27 ± 0.25	0.23 ± 0.28	3.96 ± 7.93	1.02 ± 0.24
	2-methylhexyl butyrate	3.21 ± 1.56	5.68 ± 3.24	2.59 ± 1.11	4.81 ± 3.77	5.69 ± 1.50
	Hexyl hexanoate	nd	0.64 ± 0.41	0.24 ± 0.24	nd	nd
	Ethyl hexanoate	0.27 ± 0.41	1.21 ± 0.84	0.37 ± 0.40	1.22 ± 0.76	1.08 ± 0.29
	2-Methylbutyl acetate	0.38 ± 0.26	1.03 ± 0.30	0.77 ± 0.28	0.59 ± 0.27	1.27 ± 0.20
	2-Methylbutyl 2-methylbutyrate	0.62 ± 0.72	1.42 ± 1.19	1.58 ± 0.60	nd	4.78 ± 0.17
	Ethyl 2-methylbutyrate	2.33 ± 2.12	3.35 ± 2.94	1.89 ± 0.78	1.08 ± 0.86	3.26 ± 1.42
	2-Methylbutyl hexanoate	0.17 ± 0.20	0.21 ± 0.25	0.19 ± 0.19	0.09 ± 0.11	nd
	Ethyl propionate	0.25 ± 0.61	nd	nd	nd	nd
Sesquiterpene	α-Farnesene	5.21 ± 1.74	6.67 ± 2.99	8.50 ± 4.63	4.88 ± 4.51	9.66 ± 3.02

Note: Fruit was removed from controlled atmosphere storage and submitted to postharvest treatments with chitosan emulsion (1%) and chitosan emulsion (1%) combined with different essential oils (at 500 μl L^{-1}). Fruit was assessed after seven days under ambient conditions (23 ± 5°C/63 ± 2% RH), in 2018/2019. Values represent the mean ± standard error. Nd = not detected.

Source: PubChem Compound Summary. https://pubchem.ncbi.nlm.nih.gov/compound/. Access: January 2021.

the controlled release of volatile compounds present in EOs (Maes et al., 2019) and does not change the volatile profile of foods (Froiio et al., 2019).

The volatile profile analysis shows the predominance of aldehydes, followed by alcohols, esters and the sesquiterpene α-farnesene. Hexanal, 1-hexanol and 2-methyl-1-butanol were the main volatiles in "Fuji" apples, but they were not different between treatments. 2-Ethyl hexanol was also relevant, and it was higher for the control. Esters represent the main class of volatiles of apple aroma, and their content was considerably low (Table 2), possible as a result of fruit being stored under CA (1.0 kPa O_2 + 0.5 kPa CO_2) for seven months, that reduces the production of esters and increases the production of aldehydes (Both et al., 2014). The esters found in greater proportion were hexyl 2-methyl butyrate, ethyl acetate, ethyl butyrate and ethyl 2-methyl butyrate, however with no difference between treatments (Table 2). In general, the application of CE combined with citronella, clove and copaiba EOs did not change the profile of volatile compounds responsible for the aroma of "Fuji" apples.

The effects of clove and citronella EOs incorporated into CE on blue mold control were below those obtained by Vieira et al. (2018), who applied the same EOs by volatilization. According to Zhou et al. (2018), the antimicrobial activity of emulsions with EOs was less effective than the use of pure EOs. Other preparations of CE combined with EOs may provide better results, and further studies are encouraged. The treatments assessed did not change the sensory quality attributes of "Fuji" apples, which indicates that higher EOs concentrations can be tested in order to obtain better results in the control of the blue mold, without impacting negatively the sensory characteristics of fruits.

4 | CONCLUSIONS

In general, CE and CE combined with citronella, clove and copaiba essential oils reduced the development of blue mold in "Fuji" apples, with better results achieved with CE combined with citronella essential oil.

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The treatment of "Fuji" apples with CE combined with citronella, clove and copaiba essential oils did not affect physico-chemical attributes and aroma of the fruit.

Treatments with lower severity of blue mold had lower POD activity in skin and flesh tissues of the fruit, indicating resistance induction by essential oils.

AUTHOR CONTRIBUTIONS

Angélica Schmitz Heinzen: Conceptualization; data curation; writing – original draft; writing – review and editing. **Cristiano André Steffens:** Resources; supervision; writing – review and editing. **Rogério Laus:** Resources. **Cristina Soethe:** Data curation; writing – review and editing. **Tiago Miqueloto:** Writing – review and editing. **Adriana Lugaresi:** Writing – review and editing. **Karina Soardi:** Writing – review and editing. **Cassandro Vidal Talamini do Amarante:** Resources; software; writing – review and editing.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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Ecophysiology and Vegetative and Productive Behavior of 'Chardonnay' Vines under Protected Cultivation Systems in Serra Catarinense of Brazil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CVTA, CAS and KCS designed the study and wrote the protocol. Authors CLF, AL and ASH performed the statistical analysis and wrote the first draft of the manuscript. Author KCS managed the analyses of the study and the translation of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study aimed to evaluate the effect of protected cultivation, with anti-hail screen or plastic cover, on phenological behavior, water potential, gas exchange, vegetative growth and vine production under protected cultivation systems in an altitude region of Santa Catarina.
Place and Duration of Study: The experiment was carried out in São Joaquim, SC, in the 2018/19, 2019/20 and 2020/21 harvests, with the cultivar Chardonnay on Paulsen 1103 rootstock.
Methodology: The treatments consist of an uncovered environment (control) and crops protected with white screen anti-hail (4 mm x 7 mm) and clear plastic raffia cover (160 µm).
Results: The anti-hail net did not interfere in the phenological stages, photosynthesis and gas exchange of the plants and reduced the RFA, vegetative growth and production, in relation to the plastic cover, and similar development conditions to the control treatment (without cover). The plastic cover accelerated the maturation process, reduced photosynthetically active radiation (RFA),

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promoted vegetative growth, delayed leaf senescence and increased productivity about to plants without cover. The highest red to far red ratio was observed in an uncovered system. The light saturation point was higher in plants in the cultivation system protected with plastic cover. Plants under plastic cover showed an anticipation of fruit maturation about to the screen and without cover.

Keywords: Altitude wines; microclimate; wine tourism; hail net; plastic cover.

1. INTRODUCTION

Although Brazil does not have a tradition in wine production, as with many European countries, there is a considerable area dedicated to the cultivation of grapes and wine production, such as the temperate production zones of Campanha in the Rio Grande do Sul, the Serra Gaúcha and the Serra Catarinense [1].

The wines produced in the Serra Catarinense region (above 800 m altitude) are highlighted in the national scenario [2]. The factors that make the cultivation of high-altitude wine grapes interesting, culturally and economically, are associated with the differentiated flavor and aroma, which, combined with wine tourism, make the culture increasingly attractive to the consumer and profitable to the producer [3].

However, places with an altitude of more than 700 m are more prone to hail and late frosts [4], in addition to high rainfall at the time of sprouting and harvest [5], high relative humidity, and excessive leaf wetness, conditions that require the application of chemical products to control diseases, a factor that affects the quality of wines [6]. Another limiting factor is the anticipation of harvest in many vintages, not allowing the grapes to reach the appropriate ripening period, which affects the productivity and oenological attributes of the wines produced [6, 7].

Considering the aspects of climate and grape production in regions of the Serra Catarinense, many producers are adopting cultivation in a protected environment with the use of anti-hail screen [8] or plastic cover [9], aimed at protecting plants from hail and late frosts [10]. However, the impacts of protected cultivation on plant physiology and fruit quality in altitude regions for grape cultivation are still scarce in the literature. The implementation of the plastic cover and anti-hail mesh in the Serra Catarinense region requires studies, mainly on factors related to physiological and microclimatic responses, vegetative and productive behavior [11].

The use of plastic covers increases the temperature inside the canopy and reduces humidity, evapotranspiration, and incident solar radiation [12, 13, 14]. In regions of Rio Grande do Sul, which have a tradition of protected cultivation and the production of quality wines, the works are more advanced regarding the interference of the use of plastic covering. However, in the Serra Catarinense region, little is known about the effects of using this technology.

The objective of this work was to evaluate the effects of microclimatic conditions, phenology, gas exchange, light interception, water potential in plants, vegetative and productive behavior of vines managed in an uncovered management system, undercover with an anti-hail screen and transparent plastic tarpaulin in altitude region in the Serra Catarinense.

2. MATERIAL AND METHODS

The experiment was carried out in 2018/19, 2019/20, and 2020/21 cycles with the cultivar 'Chardonnay', at the Monte Agudo Winery, located in São Joaquim, SC, at 1,264 m altitude, with geographic coordinates 28º14'54' 'S and 49º47'52''W. The vineyard has been implanted for 11 years (2007 to 2018), on Paulsen 1103 rootstock. The conduction system is “Y”, with a spacing of 2.90 m between rows and 1.30 m between plants, obtaining a density of 2,870 plants per hectare. Pruning is mixed with four canes per plant and 4 to 6 buds per cane.

The climate classification of the region of São Joaquim, SC, is Cfb, according to Köppen [15], with moderate temperature and well-distributed rain, mild summer, the occurrence of frosts in winter and autumn, with average temperatures below 20° C, except in summer, when temperatures exceed 28ºC. In winter, the average temperature is below 14°C, with minimum temperatures below 8°C.

The air under the plastic cover had lower values of relative humidity in both seasons. This

condition can be attributed to the plastic barrier to leaf wetness.

The treatments evaluated were the control, without the use of plastic cover, with anti-hail screen and plastic cover.

The material used in the cover was plastic braided transparent polyethylene canvas (160 µm thick), waterproofed in the first year (2018) of use in the vineyard. After the grapes were harvested, the material (plastic tarpaulin) was removed, to increase durability, and replaced immediately after pruning in the next agricultural cycle (allowing durability of up to 20 years). The plastic cover was arranged along the planting line, following a north-south orientation, seated on metallic wires and these over steel arches (tunnels with a height of 1.30 m from the plant to the plastic and 3 m wide), fixed on the structure of the "Y" system.

The white anti-hail screen, with a mesh opening of 4 mm x 7 mm, fixed in the same arrangement, seated on the same metallic structure used for the plastic, on metallic wires, and these on steel arches (tunnels with a height of 1, 30 from the plant to the plastic and 3 m wide), fixed on the structure of the "Y" system (only placed for experimental purposes).

Phenology evaluations were carried out from the beginning of sprouting to the end of the cycle, plant water potential, gas exchange, the intensity of different wavelengths, photosynthetically active radiation, photosynthetic pigments, vegetative and productive attributes.

After performing the winter pruning in the productive cycles, after 14 days, evaluations were carried out to estimate the sprouts of the plants for the phenology calculations. Data were collected from 1 plant of each repetition, at 14-day intervals, throughout the vegetative/productive cycle of the 2018/19 and 2019/20 harvests. Phenology was expressed as a percentage of branches per point of the phenological scale developed by Lorenz et al. [16], on the evaluation dates. In the same plant, the number of canes and total leaves per plant was evaluated.

For the microclimatic characterization, the maximum, minimum, average temperatures, and relative humidity were obtained using a data logger (NOVUS, LOGBOX-RHT-LCD) at the height of the bunches. The equipment was in the field from August to September in the 2019/20 and 2020/21 harvests.

In the evaluations of microclimatic variables in the two seasons (2019/20 and 2020/21), changes caused by the use of plastic covering on the crop rows were observed. Plants under plastic cover had higher maximum, average and minimum air temperatures compared to those uncovered and under the screen (Table 1). The average temperature difference between the plants under the uncovered system and the anti-hail screen was 0.2 ºC, and 0.5 ºC between the uncovered system and the plastic cover in the year 2019/20. In the year 2020/21, these differences were 0.1 ºC in the uncovered system and anti-hail screen, and 0.6 ºC between the uncovered and the plastic cover, respectively. Studies carried out by Pedro Júnior et al. [13] cite higher temperature in the bunch of 'Syrah' vines produced under the plastic cover, compared to the uncovered environment. It can be seen, in general, that the plastic cover delays the loss of heat in comparison to the uncovered system and the anti-hail screen. Therefore, temperature directly affects plant development and the hormonal balance of fruit trees [17], as well as the vegetative development and quality of the grapes produced [18].

The radiation spectrum was obtained with a spectrophotometer (APOGGEE INSTRUMENTS – model SS – 110, Japan), with detection of wavelengths from 340 to 810 nm, at midday. From the data obtained, the total radiation available in the control treatment and the radiation available for plants under the anti-hail screen and plastic cover were quantified. The total radiation (without interference from the physical barrier) was quantified as 100% of the available light, and the other values were calculated as a function of the total radiation, ~~and~~ then, the percentage of light retained by the physical barrier was determined. The spectroradiometer measured the amount of light in the ultraviolet (300-390 nm), blue (450-490 nm), green (490-580 nm), red (620-700 nm), far-red (700-750 nm) ranges. The red/far-red (V/Vd) ratio was calculated considering these last two wavelength ranges. Photosynthetically Active Radiation (RFA) was determined with a cepometer, Licor model leaf area index (LAI-2200C – Licor, USA).

Table 1. Microclimatic variables of maximum, minimum, average temperatures and relative humidity (%) in an uncovered cultivation environment, covered with anti-hail screen and with plastic cover, in the 'Chardonnay' cultivar, from September to October, in the 2019 harvests /20 and 2020/21, São Joaquim, SC

Microclimatic Variables	Uncovered	Screen	Plastic cover
		Harvest 2019/20	
Max. canopy (°C)	34,5	34,2	34,7
T. min. canopy (°C)	7,1	7,1	7,5
T. average canopy (ºC)	20,6	20,4	21,1
UR. canopy (%)	79,1	81,4	77,9
		Harvest 2020/21	
Max. canopy (°C)	32,2	32,1	32,4
T. min. canopy (°C)	8,1	8,1	8,7
T. average canopy (ºC)	20,0	20,1	20,6
UR. canopy (%)	81,2	80,6	79,6

The determination of leaf water potential (Ψfoliar) was performed with a pressure chamber (PMS Instrument Co, model 1000, USA). The evaluations were carried out on leaves opposite the grape bunches, from 7 am to 3 pm, in December of the 2019/20 and 2020/21 harvests, with intervals of two hours between the evaluations.

The number of shoots and leaves was estimated per plant. The variables were analyzed according to the vegetative stage of the plants. Four representative shoots were marked on the plants, with the aid of graduated rulers, and measurements were performed on one plant by repetition. The shoots and leaves were not removed from the plants to avoid causing damage and injuries. The leaf attributes were quantified through the evaluation of area, dry mass and specific area, using ten leaves in each repetition. The precise moment when all these measurements were made was at the beginning of maturation Leaf area (cm^2) was quantified using a leaf area integrator (Li-Cor, model LI-3100, USA). The dry mass (g) was quantified on an analytical balance, after drying in an oven at 65ºC, with forced air circulation, for 72 hours. The specific leaf area (cm^2 g^{-1}) was calculated by dividing the area (cm^2) by the dry mass (g) of the leaves. The fresh weight of leaves on a plant (kg $plant^{-1}$) was estimated by the number of leaves on the plant and multiplied by the fresh mass (g).

The levels of chlorophyll a, b and total (a+b), a/b and carotenoids ratio were obtained by collecting three disks of 26.6 mm in circumference and placed in amber glasses (10 mL), covered with aluminum foil and tape. adhesive, with 7 mL of DMSO (extractor reagent - dimethyl sulfoxide), incubated at 65ºC for two hours. After total chlorophyll extraction, the liquid was pipetted into Elisa plates, and reading was performed in a microplate reader, at chlorophyll wavelengths at 649 nm, 665 nm and 480 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. The levels of chlorophyll a, b and total and carotenoids were obtained by the formulas: Chl a (μL^{-1}) = 12.47*(665 nm) – 3.63*(649 nm). Chl a (g L^{-1}) = ((Chl a*(DMSO volume)/1000))/leaf disc leaf area (mm^2))*10000; Chl b (g L^{-1})= 25.06*(640 nm) – 6.5*(665 nm), Chl b = ((Chl b*(DMSO volume)/1000))/leaf area of the sheet (mm2))*10000; Chl a + b= 21.44*(649 nm) + 5.97*(665 nm). Chl a+b (g L^{-1}) = ((Chl a*(DMSO volume)/1000))/leaf disc leaf area (mm^2))*10000; the a/b ratio by dividing the values of a and b, carotenoids = (1000*(470 nm) – 2.14*(Chl a) – 70.16*(Chl b))/220, Carotenoid (g L^{-1}) = ((carotenoids*(DMSO volume)/1000))/leaf area of leaf disc (mm^2))*10000 adapted [19].

Net photosynthesis (A; µmol CO^2 m^{-2} s^{-1}), stomatal conductance (gs); mol H2O m^{-2} s^{-1}), ratio between CO^2 (Ci/Ca), internal concentration of CO^2 (Ci), quantum yield of photosystem I (FS1) and maximum quantum yield of photosystem II (FSII), relative rate of electron transport (ETR) were generated with an infrared gas analyzer (LI-6400, LI-COR, USA), equipped with an open-top chamber. These evaluations were performed on the color change of the berries.

The number of bunches and weight of bunches (kg) per plant were obtained by collecting all bunches from two plants, which were counted and weighed, and the average of these values was obtained. The moment of grape harvest for sampling was at the 120 days after bloom. The individual bunch weight (g) was estimated by the

total weight of bunches and divided by the number of bunches. Yield (kg ha^{-1}) was estimated by harvesting two plants, and then weighing the yield to the total number of plants in one hectare.

The design was in a completely randomized design, with three treatments and five replications per treatment, with two plants per replication. Significant means ($p<0.05$) were compared by the LSD test. The program for statistical analysis was SISVAR 2.0.

3. RESULTS AND DISCUSSION

A reduction of RFA was observed for plants under a plastic cover and anti-hail screen, in relation to uncovered plants, respectively, of 39% and 36% in the 2019/20 harvest, between uncovered plants and under anti-hail screen; the difference was 58% and 35% in the 2020/21 harvest, between uncovered plants and plants under plastic cover (Table 2). The greater reduction of RFA under a plastic cover in the second crop may be related to the accumulation of dirt and loss of transparency of the plastic placed in the cultivation line. Although the material is removed at the end of the harvest to increase its durability, the condition of accumulation of residues from the previous year is a relevant factor. Other studies carried out in Brazil also demonstrated the reduction of RFA under the plastic cover. Azevedo et al. [20] observed a decrease in the number of grape plants with the cover, at 50 cm above the plant canopy. Mota et al. [21], Cardoso et al. [22, 23], Chavarria et al. [24] also found a reduction in RFA under plastic cover. According to Chavarria et al. [25], the reduction of RFA can be 30% in covered vineyards, compared to the uncovered system. This condition is due to the imposition of the physical barrier imposed by the plastic, which resulted in a decrease in the availability of light [26]. According to Leitão et al. [12], the RFA was reduced by 40%, at 50 cm from the canopy, on vines under a plastic cover in the São Francisco river valley.

The quantity and quality of light incident on the canopy of plants greatly affects the development of vines, as they are directly linked to all physiological processes of plants [27]. The amount of available light is essential for the synthesis of photosynthetic pigments in plants, since the chlorophyll content of the leaves is directly linked to the reaction centers of the photosystems, a condition that leads to the growth and development processes of plants [28].

It was observed that the plastic cover was able to intercept the ultraviolet (UV) light incident on the canopy (Table 3). Lights in the blue, red, and far-red bands were also intercepted by the plastic cover, but at a lower intensity than the UV. Red light is another factor that slows down the leaf senescence process. The reduction of available red light in the canopy allows leaves to remain active for longer periods of time [28], as it presents late leaf senescence. The ratio of red and far-red (V/Vd) was higher for plants without the plastic cover. Plants under hail net had a lower V/Vd ratio, a condition that causes the plant to have an increase in vegetative growth, changing its characteristics to adapt to the light restriction imposed by the cover [24, 27, 29]. Plants subjected to shading have low respiratory rates and absorb available light, which makes them more efficient in terms of the use of radiation, a condition that reduces the waste of photons in the leaf, due to the smaller number of cells. According to Batista et al. [30], the quality of light available in plant canopy is directly linked to the production of structural genes that contribute to the adaptation of plants to shaded environments.

It was observed in Fig. 1 that the water potential of the plants was adequate for the development of the vine, in the three conduction systems, which must be between -0.2 and -0.6 MPa. This condition of no water deficit for plants under plastic cover is explained by the high rainfall that occurs in the region where the work was carried out.

Table 2. Photosynthetically active radiation (RFA; W m^{-2}) incident on the height of vine bunches of the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December, 2019 harvests /20 and 2020/21. Sao Joaquin, SC

Treatment	Crop 2019/20	Crop 2020/21
Uncovered	2317 a	1613 a
anti-hail screen	1492 b	1142 b
Cob. Plastic	1416 b	872 b
CV %	17,7	21,0

Means followed by the same letter, in the columns, did not differ by the LSD test (p<0.05)

Table 3. Light intensity at different wavelengths, and V/Vd ratio, at the height of vine bunches in the 'Chardonnay' cultivar, in an uncovered, covered cultivation system, with anti-hail screen, and with plastic cover, in December , 2019/20 harvests,. Sao Joaquin, SC

Radiation attributes	Uncovered	Screen	Plastic cover	CV %
Ultraviolet	76,7 a	76,6 a	12,1 b	11,3
Blue	27,8 a	21,2 b	16,2 c	13,0
Green	67,9 a	37,4 b	40,4 b	21,7
Red (V)	60,8 a	37,4 b	39,1 b	20,3
Far red (Vd)	37,3 a	27,5 b	26,7 b	15,1
V/Vd Ratio	1,63 a	1,29 c	1,47 b	5,92

Means followed by the same letter, in the lines, do not differ by the LSD test (p<0.05)

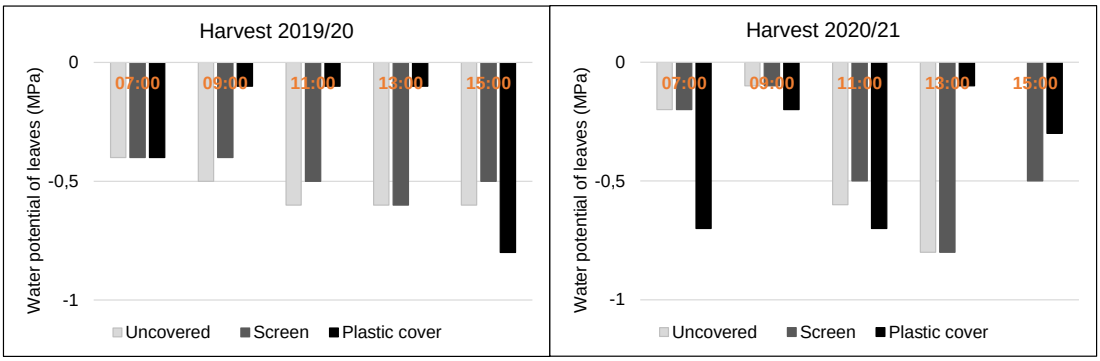

Fig. 1. Water potential of leaves in the cultivar Chardonnay, measured at three periods of the day (7:00, 9:00, 11:00, 13:00, 15:00 hours), in the month of December, in the 2019/2019 harvests 20 and 2020/21, in uncovered systems, under screen and plastic cover, in the city of São Joaquim, SC

Table 4. Gas exchange (gs) in vine bunches of the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December, 2018/19 and 2019/20 harvests São Joaquim, SC

Gas exchange	Uncovered	Screen	Plastic cover	CV %
Harvest 2018/219				
A (µmol $CO_2.m^{-2}.s^{-1}$)	13,5 ns	13,5	14,6	8,87
gs (mol $H_2O.m^{-2}.s^{-1}$)	0,19 ns	0,19	0,20	9,21
Ci/Ca	0,67 ns	0,68	0,66	7,50
Ci	254,9 ns	258,6	248,1	3,32
Fs	574,0 ns	545,5	585,4	8,64
Fv'/Fm'	0,52 ns	0,53	0,56	6,21
PhiPS2	0,32 ns	0,35	0,35	12,9
ETR (µmol $m^{-2}.s^{-1}$)	135,4 ns	144,4	152,7	10,5
Harvest 2020/21				
A (µmol $CO_2.m^{-2}.s^{-1}$)	14,5 ns	14,4	15,2	9,24
gs (mol $H_2O.m^{-2}.s^{-1}$)	0,23 ns	0,23	0,24	13,8
Ci/Ca	0,67 ns	0,67	0,67	5,32
Ci	267,1 ns	267,2	266,4	4,82
Fs	706,1 ns	703,3	700,0	28,3
Fv'/Fm'	0,52 ns	0,53	0,57	8,62
PhiPS2	0,29 ns	0,30	0,28	16,4
ETR (µmol $m^{-2}.s^{-1}$)	136,3 ns	149,3	154,4	16,2

*Means followed by the same letter, in the lines, do not differ by the LSD test (p<0.05). A – Net carbon assimilation rate. g – Stomatal conductance. Ci/Ca – ratio between indoor and ambient CO_2 concentrations. Internal ci-concentration of CO_2. Fs- photosystem I. Fv'/Fm'- maximum quantum yield of photosynthesis II. PhiPS2 - photosynthesis II. ETR – The relative rate of electron transport. *means followed by the same letter do not differ statistically by the LSD test (5% probability). *CV % - coefficient of variation.*

For the variables net photosynthesis (A), stomatal conductance (gs), CO_2 (Ci/Ca), CO_2(Ci), Photosystem I (FS I), the maximum quantum yield of photosystem II (PhiPS2), and relative rate of electron transport (ETR) there was no difference between treatments (Table 4). These results show that there is no damage to plants grown under a plastic cover in terms of photosynthetic capacity. The quality of light is directly linked to the photosynthetic processes of plants, an example of this condition according to Wang et al. [28] is that reduction in blue light delays the process of leaf senescence, as there is no degradation of chlorophyll present in chloroplasts. Plants are able to effectively compensate for the reduced availability of light for their development. This condition improves the CO_2 assimilation characteristics, supporting the transport of electrons and maintaining the efficiency of photosystem II. Plants can change their leaf morphology in terms of the arrangement of chloroplasts [31] and by changing the thickness of leaf tissues, responding according to the amount of light in the environment [26]. According to Da Silva et al. [17], the increase in temperature and reduction in relative humidity can provide a lower rate of net photosynthesis, a condition that directly reflects on the stages of plant flowering and berry maturation.

Chlorophyll a contents and chlorophyll a/b ratio in leaves were higher for plants under a plastic cover in the 2018/19 crop year. For chlorophyll b, the highest content was obtained in plants under hail net and plastic cover (Table 5). Plants with higher chlorophyll a/b ratios, according to Wang et al. [28] and Hairmansis et al. [32], present greater disorders in chloroplasts. This condition explains the need for adaptation of cell arrangements in leaves to maintain their photosynthetic capacity. The highest levels of chlorophyll b in shaded environments are related to the evaluation of plants and their adaptation to environmental changes [33]. In the 2019/2020 harvest year there was no difference between treatments for all variables related to chloroplast pigments. In the 2020/21 harvest year, there was a difference only for the chlorophyll a+b variable, which was higher in plants under the plastic cover, compared to plants under hail and uncovered screens. Kong et al. [34], in a study, carried out with the quality of light, temperature, and photosynthesis in cherry tomatoes, observed that plants subjected to higher temperatures had higher values of chlorophyll a, b and a+b. The data found in this work for the protected cultivation of vines corroborate those presented by Kong et al. [34]. Work carried out by Chavarria et al. [26] also showed higher levels of photosynthetic pigments in vines produced under the plastic cover. According to Streit et al. [31], plants subjected to high light intensities can undergo the process of photoinhibition, as a way of protecting the plant from excess light and, consequently, reducing chlorophyll levels. According to Wang et al. [26], plants subjected to a higher incidence of blue light tend to reduce the production of chlorophyll in the leaves, a condition observed in Table 5, which shows plants without cover present greater availability of blue light and, consequently, lower production of chlorophyll. The levels of carotenoids in the leaves did not differ between treatments (Table 5).

In the 2019/20 harvest, the cultivar Chardonnay presented leaf development (1), inflorescence appearance (5), flowering (6) and fruit development (7) were two days earlier in the plastic cover in relation to the screen, and seven days earlier than the overdraft. In the 2018/19 harvest, fruit maturation (8) occurred early for plants under the plastic cover.

This difference can be attributed to the increase in temperature under the plastic cover. A similar condition occurred for Alonso et al. [35], with seedless grapes produced in a greenhouse. Chavarria et al. [25] described the same behavior for 'Moscato Giallo' grapes under protected cultivation in the Serra Gaúcha, and it accelerated the sprouting process and allowed reaching the early maturation stage, considering that the period of regulation in the flowering and fruit ripening period, favors the ripening of grapes under a plastic cover in 10 to 50 days [36, 37]. For Salem et al. [38], seedless grapes produced under plastic cover showed anticipation of harvest from 17 to 22 compared to the uncovered system.

The increase in the average temperature under plastic cover resulted in the anticipation of some phenological stages (Table 6). The anticipation in the development stages of plants conducted under a plastic cover in relation to uncovered systems and under the screen in the 2019/2020 harvest is related to the occurrence of frost in November 2019. This caused the death of shoots, freezing of plant tissues, and plant defoliation by up to 90% in uncovered and under-screen systems (data not shown). This climatic phenomenon causes loss of tissue turgor, reduction in cell volume, cell dehydration, and

plant death [39]. Although the grapes reach the ripening point in advance in the plastic cover, when compared to other systems, this condition allows for greater permanence of the bunches in the field, providing a better quality of the berries, due to the accumulation of sugars and phenolic compounds, conditions that are influenced by the canopy temperature [24].

Table 5. Levels of photosynthetic pigments in vines in the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December, 2018/19, 2019/20 and 2020/21 harvests. Sao Joaquin, SC

Photosynthesizers pigments	Uncovered	Screen	Plastic cover	CV%
		Harvest 2018/19		
Chlorophyll a (g L^{-1})	2,50 c	3,14 b	3,23 a	2,02
Chlorophyll b (g L^{-1})	1,33 b	1,60 a	1,57 a	2,45
Chlorophyll a+b	3,83 b	4,74 a	4,80 a	1,41
a/b ratio	1,88 b	1,97 b	2,06 a	3,20
Carotenoids (g L^{-1})	0,55 ns	0,59	0,56	7,36
		Harvest 2019/20		
Chlorophyll a (g L^{-1})	1,93 ns	1,75	2,08	28,8
Chlorophyll b (g L^{-1})	1,17 ns	1,13	1,25	29,4
Chlorophyll a+b	3,09 ns	2,88	3,33	35,7
a/b ratio	1,66 ns	1,54	1,66	31,7
Carotenoids (g L^{-1})	0,38 ns	0,31	0,38	37,8
		Harvest 2020/21		
Chlorophyll a (g L^{-1})	1,35 ns	1,22	1,43	34,4
Chlorophyll b (g L^{-1})	0,81 ns	0,74	0,82	30,0
Chlorophyll a+b	2,16 ab	1,97 b	2,25 a	20,6
a/b ratio	1,66 ns	1,64	1,75	30,5
Carotenoids (g L^{-1})	0,29 ns	0,28	0,30	34,3

Means followed by the same letter, in the lines, do not differ statistically by the LSD test (5% probability). CV % - coefficient of variation

Table 6. Phenological stages (days after pruning to reach each stage), and vines in the 'Chardonnay' cultivar, in uncovered cultivation systems, covered with anti-hail screen and with plastic cover, in December, 2018/19 and 2019/20. Sao Joaquin, SC

Phenological stages	Days after pruning			
	Harvest 2018/ 2019			
	Uncovered	**Screen**	**Plastic cover**	**CV%**
0	25 ns	28	23	24,6
1	44 ns	45	38	20,7
5	57 ns	57	55	11,2
6	79 ns	76	76	10,0
7	135 ns	130	128	6,8
8	210 a	205 a	197 b	2,2
9	225 b	221 b	240 a	2,1
		Harvest 2019/2020		
0	52 ns	56	52	8,7
1	81 a	81 a	72 b	6,1
5	109 a	109 a	83 b	10,6
6	140 b	138 b	125 a	4,1
7	148 b	148 b	133 a	3,6
8	199 b	207 a	196 b	5,5
9	221 b	221 b	255 a	2,0

**(0)-budding, (1)-leaf development, (5)-inflorescence appearance, (6)-flowering, (7)-fruit development, (8)-fruit maturation, (9)-beginning of dormancy. *Evaluations carried out only in the 2018/19 and 2019/20 harvests. *Means followed by the same letter do not differ statistically by the LSD test (5% probability). *CV (%) - coefficient of variation*

Plants under plastic cover remained for longer periods with healthy and photosynthetically active leaves, which culminated in the delay of stage 9 (beginning of dormancy) in both agricultural seasons (Table 6). When there is no reduction in the chlorophyll content in the leaves, the photosynthetic rate does not show a drastic reduction [28]. According to Cardoso et al. [23] and Comarin et al. [40], the permanence of leaves in vines grown under plastic cover is longer than in uncovered plants. This condition can be attributed to the non-occurrence of leaf wetness and, consequently, lower incidence and severity of diseases in vine leaves (data not shown). According to Pedro Júnior and Hernandes [41], grape cultivars managed with plastic cover showed a reduction in the severity of fungal diseases and a decrease in plant defoliation.

There was no difference between treatments for the number of shoots per plant in 2018/19, 2019/20, and 2020/21 harvest years, and in the 2019/20 year, there was no difference for specific leaf area (AFE). The number of leaves per plant and the PF of leaves per plant were higher for plants treated with plastic cover in the three seasons. The AFE in the 2018/19 and 2020/21 harvests was higher in plants conducted under the plastic cover, differing from plants conducted in an uncovered system and under a hail net (Table 7). According to Salem et al. [38] and Chavarria et al. [25], vines under plastic cover have higher AFE. This condition is directly linked to the increase in temperature and the availability of water [42]. Another factor is the quality of light incident on the canopy since the greater vegetative growth under the plastic cover is related to the lower V/Vd ratio, as well as the greater availability of blue and red radiation, which falls on the canopy and favors the synthesis of pigments. photosynthetic agents [28].

Plants grown under plastic cover had a higher number of clusters per plant compared to plants in an uncovered system and under the screen, in the 2018/19 harvest. In the 2019/20 and 2020/21 harvest, plants under a plastic cover and anti-hail screen did not differ from each other, however, both covered environments provided a greater number of clusters per plant compared to the uncovered system. In the three seasons of study, the weight of bunches per plant and productivity were higher in plants under the plastic cover. The plants under the screen, and in the uncovered system, did not show differences between them. In the 2019/20 and 2020/21 crop years, the weight of individual bunches was higher for plants in a plastic cover system compared to the system under the screen and uncovered, which showed no differences between them. However, in the 2018/19 harvest year, plants under a plastic cover and under hail net did not show differences between them, and there was no difference between the screen cover and the uncovered system (Table 8). In the 2018/19 harvest, no differences were observed for plant bunch weight between plants grown under plastic cover and screen.

Table 7. Vegetative attributes measured in December, in 2018/19, 2019/20 and 2020/21 harvests, in uncovered systems, under screen and plastic cover in the 'Chardonnay' cultivar, in the city of São Joaquim, SC

Vegetative attributes	Harvest 2018/19			CV %
	Uncovered	Screen	Plastic cover	
Number of shoots plant^{-1}	26 ns	25	27	8,8
No. of leaves plant^{-1}	623 b	531 c	731 a	7,9
PF of leaves plant^{-1} (kg)	3,277 a	2,915 b	4,102 a	9,7
AFE (cm g^{-1})	14 b	15 b	18 a	11,0
Harvest 2019/20				
Number of shoots plant^{-1}	14 ns	14	15	7,8
No. of leaves plant^{-1}	399 b	381 b	472 a	7,3
PF of leaves plant^{-1} (kg)	1,223 b	1,332 b	1,793 a	8,1
AFE (cm g^{-1})	15 ns	18	16	21,0
Crop 2020/21				
Number of shoots plant^{-1}	26 ns	25	27	8,8
No. of leaves plant^{-1}	614 b	514 b	726 a	6,9
PF of leaves plant^{-1} (kg)	1,948 b	1,638 b	3,139 a	15,5
AFE (cm g^{-1})	15 b	15 b	22 a	3,5

**Number-number. *PF – fresh weight. AFE – specific leaf area. Means followed by the same letter on the collumn do not differ statistically by the LSD test (5% probability). CV % - coefficient of variation*

Table 8. Productive attributes of vines in the cultivar 'Chardonnay', in uncovered cultivation systems, covered with anti-hail net and with plastic cover, in December, 2018/19, 2019/20 and 2020/21 harvests. Sao Joaquin, SC

Productive attributes	Uncovered	Screen	Plastic cover	CV %
		Crop 2018/19		
No. of plant bunches^{-1}	31 b	32 b	43 a	11,1
Weight bunches kg plant^{-1}	3,9 b	4,6 b	6,4 a	15,0
bunch weight (g)	130,8 b	138,2 ab	145,3 a	6,77
Productivity (ton.ha^{-1})	11,221 b	13,460 b	18,482 a	15,0
		Crop 2019/20		
No. of plant bunches^{-1}	4,7 b	6,2 a	5,9 a	11,0
Weight bunches kg plant^{-1}	0,08b	0,08b	67,3a	52,0
bunch weight (g)	83,9 b	67,2 b	128 a	16,7
Productivity (ton.ha^{-1})	0,228 b	0,251 b	2,158a	56,0
		Crop 2020/21		
No. of plant bunches^{-1}	19 b	37 a	38 a	21,0
Weight bunches kg plant^{-1}	2,1 b	0,9 b	6,1 a	34,0
bunch weight (g)	111 b	112 b	159 a	8,9
Productivity (ton.ha^{-1})	6,104 b	2,441 b	17,449 a	34,2

Means followed by the same letter, in the lines, do not differ statistically by the LSD test (5% probability). CV % - coefficient of variation

The gain in the number of bunches per plant under the plastic cover, compared to the uncovered system, was 28%, 17%, and 50% in 2018/19, 2019/20, and 2020/21 crops, respectively. For plants conducted under anti-hail nets, the gains, in relation to the uncovered system, were 3%, 17%, and 29%, in 2018/19, 2019/20, and 2020/21 harvests, respectively. For the weight of bunches (g) plants under the plastic cover, in relation to uncovered, they showed an increase of 10%, 49%, and 30%, in 2018/19, 2019/20, and 2020/21 harvests, respectively. For the anti-hail net, gains were lower, 6%, 11%, and 0.9% compared to uncovered, in—2018/19, 2019/20, and 2020/21 harvests, respectively. The results of the present work corroborate those of Pedro Júnior and Hernades [41], who reported higher values for the number of bunches, the weight of bunches per plant, and individual weight of bunches in plants grown under the plastic cover.

These differences between the numbers of bunches can be attributed to the way of management in the pruning, conduction and thinning of bunches. Another important factor was the occurrence of late frost in November 2019, a condition that caused the death of shoots and defoliation in up to 90% in plants conducted in an uncovered system and under an anti-hail screen. This condition directly affected the productivity of the 2019/20 harvest, and provided a reduction in production in the 2020/21 harvest, as the physiological condition and the amount of reserve were compromised for the following year. The plastic cover provided productivity increases of 49%, 116% and 65%, compared to uncovered, in the 2018/19, 2019/20 and 2020/21 harvests, respectively. The anti-hail net increased productivity by 17%, 18%, 60%, compared to uncovered, in the 2018/19, 2019/20 and 2020/21 harvests, respectively.

The productivity gains are similar to the results found by Azevedo et al. [20] with grapes produced under the plastic cover in the São Francisco River Valley, and for grapes produced in Bento Gonçalves [40]. Pedro Júnior and Hernandes [41] also observed higher yields in grapes produced under plastic cover, in summer and winter crops. These authors attributed the productivity gains to the lower incidence of diseases and lower defoliation in the plants.

4. CONCLUSION

The objective of the work was "to evaluate the effect of protected cultivation on phenology, water relations, gas exchange, vegetative growth and production in 'Chardonnay' vine." Thus, it must conclude on how the plastic cover and the screen influenced phenology, water relations, gas exchange, vegetative growth and production in 'Chardonnay' vines. The use of plastic cover limits photosynthetically active radiation in grape plants, however, this condition did not alter gas exchange in vines.

Grapevines under plastic cover have higher levels of chlorophyll a, a+b and the a/b ratio.

The phenological stage of vines under plastic cover anticipates the maturation process of the grapes, and delays the senescence of the leaves.

Grape plants under cover have a higher number of leaves and specific leaf area when compared to other management systems.

Plants under plastic cover allow a greater number of clusters per plant, weight of clusters, and weight of clusters per plants larger than the screen and without cover.

Plants grown under plastic cover have 116% more productivity in years of adverse weather conditions (hail and frost).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Late summer pruning improves the quality and increases the content of functional compounds in Fuji apples

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ABSTRACT: The objective of this paper was to evaluate the effect of the time of summer pruning on the quality of Fuji apples, on the red color and functional properties, at harvest and after 3.5 months of cold storage. The treatments evaluated consisted of the control (without summer pruning) and summer pruning in December, January, and February. The percentage of fruit with more than 50% red color on the skin was 20.5% higher when summer pruning was performed later than when performed early. In general, summer pruning in January and February provided fruit with a relatively high content of anthocyanins (19 and 25.1 mg cyaniding 3-glycoside·100 g^{-1} FW, respectively), relatively high values of total phenolic compounds (370.1 and 438.7 mg EAG·100 g^{-1} FW, respectively) and total antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (8,097.5 and 8,089.2 µMol Trolox·100 g^{-1} FW, respectively) in the 2017/18 season. Summer pruning did not affect fruit quality after cold storage. Summer pruning performed in January or February increased functional compounds content and improved red color, while in February it increased flesh firmness.

Key words: *Malus domestica* Borkh., anthocyanins, phenolic compounds, antioxidant activity, human health.

INTRODUCTION

The physicochemical attributes of apples are important aspects in defining their monetary value and consumer acceptance of the product. Sugars, organic acids, and their relationship (ratio) are essential to fruit flavor (Petkovsek et al. 2007). However, the production of fruit without defects and with a high value and expressive red color is decisive for their classification in high-value categories, which impacts the profits of the producer and meets the preference of the consumer market (Fiovaranço and Lazzarotto 2012).

The regular consumption of apples improves human health, mainly by protecting against chronic diseases (Condezo-Hoyos et al. 2014). Polyphenols, found in apples, play an important role in the human health-promoting and anthocyanins, reduce the oxidation of low-density lipoprotein (LDL) cholesterol and decrease the development and progression of atherosclerotic lesions (Cardoso et al. 2011, Cory et al. 2018).

A few environmental factors, such as light, temperature, and humidity, and agronomic practices, such as irrigation, nutrition, thinning, and pruning, affect and change the quality attributes of apples during preharvest (Musacchi and Serra 2018). Highly dense orchards, with tall and vigorous plants, tend to have decreased light penetration in the canopy, especially in the lower parts and between plants, resulting in relatively low fruit yield and quality (Djordjević et al. 2020). Environments with high sunlight irradiation are favorable to the production of relatively large fruit with a high percentage of dry matter and red coloring (Musacchi and Serra 2018). Improving fruit quality by changing the canopy environment,

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especially regarding the amount of incident light, is among the benefits of summer pruning of apple trees (Cooley and Autio 2011). The greater availability of light in plants subjected to summer pruning allows for enhanced red coloring, anticipation of maturation, reduction in titratable acidity, and increased soluble solid contents of the fruit (Ashraf and Ashraf 2014).

Information relating to the time in which pruning is performed and its effects on the physicochemical and functional quality of apples is scarce. The time of summer pruning, in addition to influencing the physical-chemical characteristics, possibly has an influence on the functional quality of the fruits. Thus, the objective of this paper was to evaluate the effect of the time of summer pruning on the quality of Fuji apples, especially on the red color and functional properties.

MATERIAL AND METHODS

The experiment was carried out in an experimental orchard located at the São Joaquim Experimental Station, belonging to the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI) (28°17'39" S, 49°55'56" W; altitude of 1,415 m), São Joaquim, SC, Brazil, during the crop seasons of 2016/2017, 2017/2018, and 2018/2019. Apple trees of the Fuji Standard cultivar on Marubakaido rootstock with M9 filter were utilized. The orchard was established in 1999, with 4 m between rows and 1.5 m between plants.

The region's climate is humid mesothermal (Cfb), according to the Köppen-Geiger classification. In other words, the region has a constantly humid, temperate climate, with no dry season and cool summer. The average accumulation of temperatures of 7.2°C or less in the region is 900 hours. The soil of the experimental field was classified as Humic Cambisol, according to the Brazilian Soil Classification System (Alvares et al. 2014, Pasa et al. 2018). Figure 1 shows the data on climatic conditions, average temperature, thermal amplitude, rainfall, and hours of insolation from December to April in the three crop seasons evaluated.

Treatments consisted of different times of summer pruning (December, January, and February), performed on the 15th of each month, by removing whole branches in the upper, middle, and lower positions of the vegetative canopy, and without summer pruning (control). Vertical branches, and those that shaded the fruit, were removed by summer pruning. The experimental design used was a randomized blocks design with four replicates and an experimental unit composed of three plants.

Fruit of all treatments were harvested on April 26, 2017, April 11, 2018, and April 15, 2019, and transported to the laboratory for analysis of maturation and quality attributes. The following variables were evaluated: percentage of fruit with more than 50% red color, skin color (red color and base color), iodine-starch index, flesh firmness, soluble solids (SS), titratable acidity (TA), and the SS/TA ratio. In the 2016/17 and 2017/18 crop seasons, apple skins were sampled to analyze the contents of anthocyanins, total phenolic compounds (TPC), and total antioxidant activity (TAA; ABTS and DPPH methods). Fifty fruit per experimental unit were used to evaluate the percentage of fruit with more than 50% red color, while 20 fruit were used for the other evaluations. Thirty fruit per experimental unit of the 2016/12 and 2017/18 crop seasons were stored for 3.5 months (1 ± 0.2°C and relative humidity of 92 ± 4%) plus seven days of shelf life (23 ± 3°C and relative humidity of 60 ± 5%), simulating the marketing period. The apples were subsequently evaluated for flesh firmness, skin color (base color, changing from green to yellow), SS, TA, SS/TA ratio, and rot incidence.

Physicochemical attributes evaluation

- The percentage of fruit surface covered with red coloring was subjectively and visually assessed, counting number of fruit with a surface covered with more than 50% red color;
- Skin color was measured in terms of hue angle ($h°$) in the more and less red regions of the fruit (skin background color), using a colorimeter (Konica Minolta® CR 400, Tokyo, Japan);
- Flesh firmness was determined in two opposite regions of the equatorial portion of the fruit, with a small portion of the epidermis previously removed using a motorized penetrometer (Güss Manufacturing Ltd., Cape Town, South Africa) equipped with an 11-mm diameter tip and expressing the results in Newton (N);

- The iodine-starch index was determined by reacting the starch with a solution of 12 g of metallic iodine and 24 g of potassium iodide in 1 L of distilled water. The iodine solution was applied to a cut in the peduncular half of the equatorial region of the fruit, after which the color was compared (reaction of iodine with starch) considering a scale of 1-5, in which index 1 indicates the maximum starch content, and index 5 represents the fully hydrolyzed starch (Amarante et al. 2010);
- TA was determined in a 5-mL sample of fruit juice previously extracted from transversal slices taken from the equatorial region of the apples and chopped in an electric centrifuge. The chopped sample was diluted in 45 mL of distilled water, and titrated with 0.1 N sodium hydroxide solution until reaching pH 8.1, using an automatic titrator (Titro Line Easy®, Mainz, Germany). The results are expressed as % malic acid;
- The SS content was determined using a digital refractometer (Atago PR 201α, Tokyo, Japan), with results expressed in °Brix;
- The SS/TA ratio was calculated based on the values obtained for SS and TA.

Determination of the functional compounds

Obtaining the extracts

The extracts used to quantify the TPC and TAA in the fruit skin were obtained according to the methodology of Larrauri et al. (1997) modified by Rufino et al. (2007a). In centrifuge tubes (Falcon), 5 g of sample was weighed, 10 mL of methanol water solution (50:50, v/v) was added, and the mixture was homogenized with an ultra-turrax (SilentCruscher M, Heidolph, Germany) and kept at rest for 60 min at room temperature. The samples were centrifuged for 15 min at 4°C, and the supernatant was transferred to a 2-mL volumetric flask. Subsequently, 10 mL of acetone water solution (70:30, v/v) was added to the residue of the first extraction, followed by the extraction procedure above until obtaining the supernatant. Finally, the second supernatant was added to the first one, and the volume of the volumetric flask was adjusted to 25 mL with distilled water.

Determination of the total antioxidant activity

The TAA was determined using methodologies based on the ability of the extract to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid) (ABTS) radicals (Rufino et al. 2007a, Rufino et al. 2007b).

The DPPH method consisted of a radical prepared on the day of analysis by diluting DPPH (0.06 mM) in methanol. An aliquot of 0.1 mL of the extract and 3.9 mL of the DPPH solution was transferred to test tubes in triplicate. The mixture was homogenized, and the absorbance was quantified at a wavelength (λ) of 515 nm 30 min after the beginning of the reaction with the addition of the extract. The calibration curve was generated with standard Trolox solutions, and the results were expressed in antioxidant capacity equivalent to Trolox per 100 g of fresh mass (μmol Trolox·100 g^{-1} FW).

The ABTS method consisted of a radical solution prepared using an ABTS stock solution (7 mM) with potassium persulfate (140 mM), which was kept for 16 h at 20°C. Before the analysis, the radical solution was diluted with ethyl alcohol to an absorbance of 0.70 ± 0.05 at λ 734 nm. Aliquots of 30 μL were transferred to Falcon tubes containing 3 mL of the ABTS radical in triplicate, which were then homogenized. After 6 min of reaction, the absorbance was quantified at λ 734 nm. The calibration curve was established using standard Trolox solutions, with the results expressed in μmol Trolox·100 g^{-1} FW.

Determination of the total phenolic compounds

The TPC was determined by modifying the methodology described by Roesler et al. (2007). For this purpose, 2.5 mL of Folin-Ciocalteu water solution (30:70 v/v) and 0.5 mL of the extract were added to a tube (Falcon). The tube was shaken in vortex (Gehaka, AV-2, São Paulo, Brazil) and rested for 3 min. At the end of this period, 2 mL of a solution of sodium

carbonate (10%) was added to the tube and homogenized. The reaction time was 1 hour, after which the absorbance of each sample was determined at λ 765 nm. The calibration curve was established using gallic acid, and the results were expressed in mg of gallic acid equivalent per 100 g of fresh weight (mg EAG·100 g^{-1} FW).

Determination of the total content of anthocyanins

The total content of anthocyanins in the skin of the apples was determined according to the methodology of Fuleki and Francis (1968), with modifications. In tubes (Falcon), 2.5 g of plant tissue and 15 mL of solvent [ethanol (95%) and hydrochloric acid (1.5 N) (85:15, v/v)] were homogenized with ultra-turrax (SilentCruscher M, Heidolph, Germany) for approximately 1 min and stored for 34 hours (3-4°C). After incubation, the samples were centrifuged at 12,000 rpm for 15 min at 4°C. Later, 2 mL of the supernatant was transferred to a graduated tube, and the volume was adjusted to 50 mL with the extracting solvent. Analyses were performed at λ 535 and 700 nm, and the results were expressed in mg of cyanidin-3-glycoside per 100 g of fresh weight (mg cyanidin-3-glycoside·100 g^{-1} FW).

The data expressed as percentages were transformed into arc sin $\sqrt{x}/100$ and subjected to analysis of variance, and the interaction between treatments and crop season were also analyzed. When the treatment effect was significant, the treatment means were compared using the least significant difference test ($p < 0.05$) in each crop season in which there was a significant interaction or with average data from the crop seasons evaluated when there was no interaction.

RESULTS AND DISCUSSION

There was no interaction between treatments and crop seasons regarding iodine-starch index, SS content, and flesh firmness (Table 1). Summer pruning did not affect the iodine-starch index or SS content (Table 1). Contrary to the results obtained in this work, Robinson et al. (1983) reported greater starch degradation in apples exposed to a higher light intensity and attributed an increased fruit metabolism to this effect. Ikinci (2014), in turn, observed an inconsistent effect of summer pruning on the SS content in peaches, with alternating results throughout the evaluated seasons.

Table 1. Iodine-starch index, soluble solids, and flesh firmness of Fuji apples, at harvest, due to the time of summer pruning, in three crop seasons (2016/2017, 2017/2018, and 2018/2019). Mean data for three seasons.

Treatments	Iodine-starch index (1-5)	Soluble solids (ºBrix)	Flesh firmness (N)
Without summer pruning	4.5 a*	12.8 a	71.7 bc
Pruning in December	4.6 a	12.5 a	73.1 ab
Pruning in January	4.6 a	12.8 a	70.4 c
Pruning in February	4.7 a	12.6 a	74.0 a
CV (%)	4.3	3.8	3.4

*Means followed by the same letter in the columns do not differ by the least significant difference test ($p < 0.05$); CV: coefficient of variation.

Summer pruning in February provided greater flesh firmness at harvest than when pruning in January (Table 1). The relationship between light incidence and flesh firmness is highly variable. Studies have shown positive relationships between light incidence and flesh firmness in Fuji apples (Jung and Choi 2010). However, other studies have indicated that apples exposed to a higher light intensity tend to present less flesh firmness due to starch breakdown and increased respiratory metabolism (Robinson et al. 1983), which was not proven by this present study. According to Saure (1987), flesh firmness is a maturation attribute with variable behavior when subjected to summer pruning. However, in the present study, the effect was consistent throughout the three crop seasons evaluated.

The values of TA and the SS/TA ratio varied between crop seasons in response to the treatments applied (Table 2). TA was higher in apples harvested from plants of the control treatment in the 2016/2017 crop season and in fruit of plants submitted

to summer pruning in February in the 2017/2018 crop season. However, there was no difference between treatments for TA in the 2018/2019 crop season. Summer pruning also showed an inconsistent effect of TA for different peach cultivars, which varied according to the studied year (Ikinci 2014, Ikinci et al. 2014). However, the results are not conclusive, and they stated that there are studies that demonstrate that pruning increases the TA, those that have indicated the opposite effect, and others in which no effect was observed (Saure 1987). The influence of summer pruning on this attribute possibly depends on climatic conditions during fruit development.

Table 2. Titratable acidity, soluble solids/titratable acidity ratio (SS/TA), and skin background color (*h*°) in Fuji apples, at harvest, due to the time of summer pruning, in three crop seasons (2016/2017, 2017/2018, and 2018/2019).

Treatments	Titratable acidity (% malic acid)	SS/TA	Skin background color (*h*°)
2016/2017			
Without summer pruning	0.46 a*	27.1 c	101.5 a
Pruning in December	0.38 b	31.6 bc	104.8 a
Pruning in January	0.32 c	38.7 a	92.0 b
Pruning in February	0.34 bc	34.8 ab	98.7 ab
CV (%)	10.7	9.9	5.0
2017/2018			
Without summer pruning	0.35 b	41.6 a	108.4 a
Pruning in December	0.37 b	38.0 a	110.0 a
Pruning in January	0.36 b	39.3 a	108.9 a
Pruning in February	0.44 a	31.8 b	107.8 a
CV (%)	7.3	8.2	1.6
2018/2019			
Without summer pruning	0.39 a	30.3 a	107.1 a
Pruning in December	0.36 a	32.5 a	108.1 a
Pruning in January	0.37 a	32.9 a	104.2 b
Pruning in February	0.39 a	31.0 a	105.2 b
CV (%)	7.1	8.2	1.1

*Means followed by the same letter in the columns, for the same crop season, do not differ by the least significant difference test ($p < 0.05$); CV: coefficient of variation.

The SS/TA ratio was higher in fruit of plants submitted to summer pruning in January, presenting no significant difference from plants submitted to summer pruning in February in the 2016/2017 crop season. The 2017/2018 crop season showed the lowest SS/TA ratio in the fruits of plants submitted to summer pruning in February, with no difference between treatments in the 2018/2019 crop season. The results of the SS/TA ratio were determined by the variation in TA (Table 2), since the SS values did not differ between treatments (Table 1).

The values of skin background color (*h*° of the least red region) varied between crop seasons in response to the treatments applied (Table 2). The skin background color was less green (lower h° value) in fruit of plants submitted to summer pruning in January in the 2016/2017 crop season, showing no difference from those pruned in February of the same season and in January and February of the 2018/2019 crop season. However, there was no difference between treatments in the 2017/2018 season (Table 2). The final color of apples develops during the ripening process, in which chlorophyll degradation occurs in addition to the synthesis of secondary compounds (Musacchi and Serra 2018). The base color is considered a reliable indicator for evaluating apple ripeness (Saure 1987), for which the lowest *h*° of the base color obtained with summer pruning in the two crop seasons studied could indicate early fruit ripening. However, other maturation attributes, such as flesh firmness, SS content, and iodine-starch index, do not support this early maturation with summer pruning, regardless of the season.

There was no interaction between treatments and crop seasons for the development of red color at harvest. Summer pruning in January and February generally provided fruit with a more intense red color (lower *h*° value in the redder

region) and a higher proportion of fruit with > 50% of the surface-colored red (Table 3). Even plants pruned in December produced more fruit with this condition compared to plants without summer pruning. The red color of apples is an important factor in encouraging consumer purchase (Stanger et al. 2017). The plants pruned in February produced 37.6% more fruit with > 50% red color than the plants in the control treatment, which resulted in fruit with higher commercial value.

Table 3. Fruit coloring in the redder region (h°) and percentage of fruit with more than 50% of red coloring, at harvest, due to the time of summer pruning, in three crop seasons (2016/2017, 2017/2018, and 2018/2019). Mean data for three seasons.

Treatments	Redder region (*h*°)	Fruit with more than 50% of red coloring (%)
Without summer pruning	51.6 a*	40.5 c
Pruning in December	52.8 a	51.6 b
Pruning in January	41.8 b	56.8 ab
Pruning in February	44.3 b	64.9 a
CV (%)	13.1	22.2

*Means followed by the same letter in the column do not differ by the least significant difference test ($p < 0.05$); CV: coefficient of variation.

The red color in the skin of apples is the result of anthocyanin synthesis (Bae et al. 2006), which highly depends on light exposure (Jakopic et al. 2009). The content of anthocyanins was normally higher in fruit from plants submitted to summer pruning in January and February in the 2016/2017 and 2017/2018 crop seasons (Table 4). High-light intensity increases the synthesis of flavonoids, especially of the anthocyanin class, in some stages of fruit development, such as during ripening and harvesting (Zoratti et al. 2015). Therefore, pruning in January and February allowed for additional light to penetrate the canopy, improving fruit color. The control plants, for which no side branches or branches that shaded the fruit were removed, showed reduced light passage in the canopy, restricting the synthesis of anthocyanin pigments in the fruit. According to Ashraf and Ashraf (2014), summer pruning can improve light availability to fruit to develop a red color. Although the resumption of vegetative growth after pruning was not evaluated, it was possible to observe that plants pruned in December resumed their vegetative growth during the following months. However, in some cases, coloration can also be impaired since summer pruning can stimulate the growth of other vegetative buds, emitting new branches that shade the fruit (Schupp and Ferree 1988, Saure 1990), what may have occurred in this work for plants pruned in December.

Table 4. Content of total anthocyanins (TAN), total phenolic compounds (TPC), and total antioxidant activity (TAA; ABTS and DPPH methods) in the skin of Fuji apples, at harvest, due to the time of the summer pruning, in two crop seasons (2016/2017 and 2017/2018).

Treatments	TAN (mg cyanidin 3-glycoside·100 g^{-1} FW)	TPC (mg EAG·100 g^{-1} FW)	TAA (µMol Trolox·100 g^{-1} FW)	
			ABTS	DPPH
		2016/2017		
Without summer pruning	33.7 bc*	349.8 a	7,528.0 a	6,856.0 a
Pruning in December	24.4 c	363.0 a	7,367.0 a	9,214.0 a
Pruning in January	57.6 a	383.8 a	8,712.0 a	8,543.0 a
Pruning in February	42.5 ab	402.7 a	6,945.0 a	7,431.0 a
CV (%)	19.4	11.8	21.7	31.8
		2017/2018		
Without summer pruning	14.3 bc	253.4 b	3,350.0 ab	5,601.7 b
Pruning in December	13.5 c	224.4 b	2,844.4 b	5,643.3 b
Pruning in January	19.0 ab	370.1 a	3,198.2 ab	8,097.5 a
Pruning in February	25.1 a	438.7 a	3,977.8 a	8,089.2 a
CV (%)	12.6	15.2	11.4	15.0

*Means followed by the same letter in the columns, for the same crop season, do not differ by the least significant difference test ($p < 0.05$); CV: coefficient of variation; ABTS: 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl.

The TPC and TAA values varied between crop seasons in response to the treatments applied (Table 4). The 2016/2017 crop season showed no effect of the treatments on the TPC and TAA values (ABTS and DPPH methods) of the fruit skin. However, the plants pruned in January and February of the 2017/2018 crop season produced fruit with relatively high values of TPC and TAA (evaluated using the DPPH method) (Table 4). TPCs are influenced by environmental factors, especially light (Jakopic et al. 2009). Thus, as occurred with the accumulation of anthocyanins, summer pruning carried out in January and February possibly allowed for additional light to enter the canopy, resulting in higher TPC and TAA values in the fruit skin.

Polyphenols are the primary antioxidant compounds of many fruits and vegetables (Coklar and Akbulut 2017). The main groups of phenolic compounds in apples are phenolic acids, flavonoids, and anthocyanins (Ceymann et al. 2012, Jakobek et al. 2013). This explains the higher content of TAA (DPPH method) in the skin of the fruit of plants submitted to summer pruning in January and February, when higher values of TPC and anthocyanins were also observed.

In the 2016/2017 crop season, in which the fruit was harvested at the end of April (the longest maturation period), the thermal amplitude was higher, the rainfall was less frequent, and there was a higher number of hours of sunshine (Fig. 1). These factors contribute to the accumulation of color in the fruit, which may explain the lack of effect of summer pruning on phenolic compounds and antioxidant activity, as well as the higher content of these variables during this crop season than during the other crop seasons. The climatic data of the 2017/2018 crop season indicate that the opposite occurred in the previous season, when there was a lower average temperature, less thermal amplitude, less sunshine, and, in some months, higher rainfall. These conditions are unfavorable for the accumulation of color in the fruit, and therefore the effect of summer pruning was more evident in this crop season, indicating differences in the content of antioxidants and phenolic compounds.

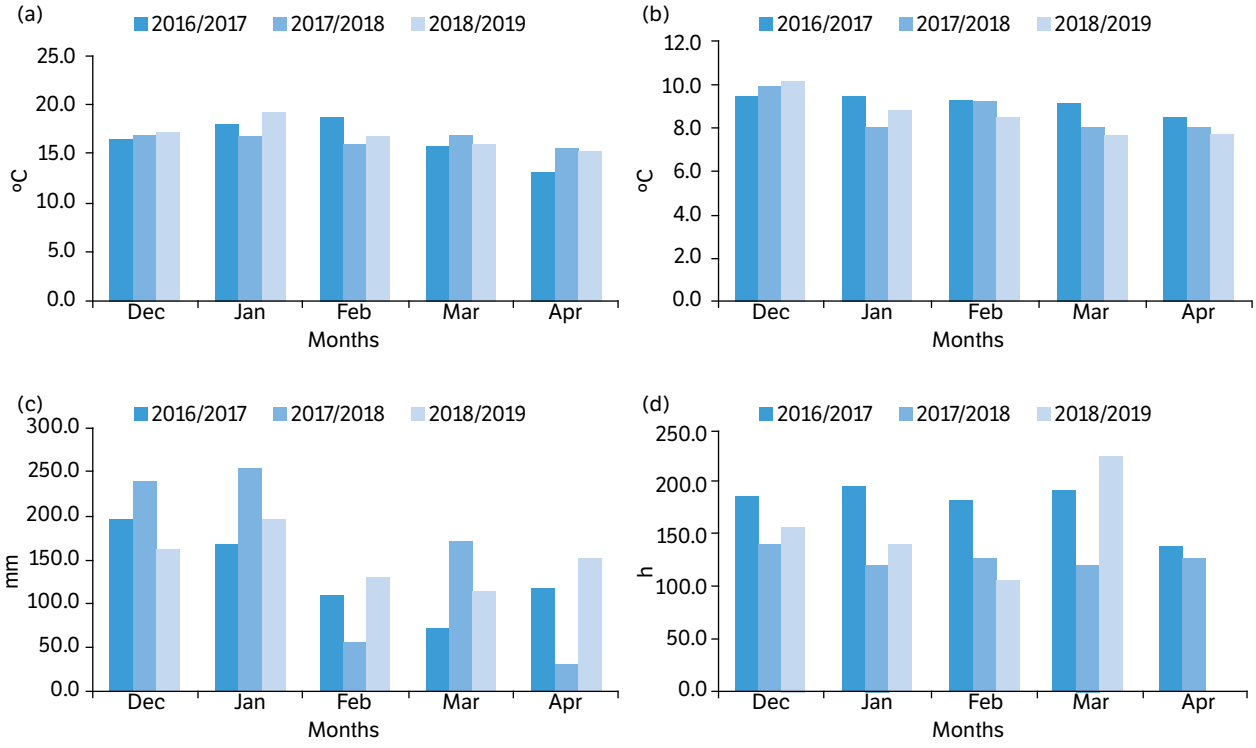

Figure 1. Climatic data on (A) average temperature, (B) thermal amplitude, (C) rainfall, and (D) insolation from the São Joaquim meteorological station (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, 2020).

The performance of summer pruning, evaluated after storage and compared to the control treatment (without summer pruning), did not influence the incidence of rot, yellowing of the fruit, flesh firmness, TA, SS content, and SS/TA ratio, regardless of the season (data not presented).

The optimal time for summer pruning is important considering its strong influence on the quality of the fruit produced. The results obtained show that summer pruning in January and February of Fuji Standard apple trees (a clone with fruit of reduced red coloring), with high vigor (orchards implanted several years ago on Marubakaido rootstock with M9 filter), may be a viable alternative to improve the quality of fruit at harvest, especially in harvests unfavorable to the development of the red color. As noted, the benefits from performing this practice at the appropriate time are not only in visual quality, but also in the functional quality of the fruit.

CONCLUSION

Summer pruning of Fuji Standard apple trees in January and February promoted the development of red coloration and increased the values of phenolic compounds and antioxidant activity in the fruit skin. Furthermore, summer pruning in February resulted in fruit with greater flesh firmness at harvest than that of fruit in the control group. On the other hand, summer pruning in December was ineffective for improving the color and functional properties of the fruit. Finally, summer pruning did not influence the quality of Fuji Standard apples in cold storage.

AUTHORS' CONTRIBUTION

Conceptualization: Lugaresi, A.; **Methodology:** Steffens, C. A., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Project administration:** Steffens, C. A., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Data curation:** Lugaresi, A., Souza, M. P.; **Funding acquisition:** Steffens, C. A., Amarante, C. V. T., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Formal analysis:** Lugaresi, A.; **Investigation:** Lugaresi, A., Steffens, C. A., Souza, M. P., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Supervision:** Steffens, C. A.; **Writing – original draft:** Lugaresi, A.; **Writing – review & editing:** Steffens, C. A., Souza, M. P., Amarante, C. V. T., Brighenti, A. F., Pasa, M. S., and Martin, M. S.

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Data will be available upon request.

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Cultivars performance and blackberry pruning management

Desempeño de cultivares y manejo de podas en mora

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Abstract

There was increased fruit consumption with potential health benefits in the last years, being blackberry one of these fruits. The objective of this work was to evaluate the performance of Blackberry cultivars and the influence of main stem management on BRS-Tupy cultivar. The first experiment assessed the cultivars: BRS-Tupy, Cherokee, Xavante, and Guarani; in the second experiment the evaluated prunings were: drastic winter pruning, 2, 3, and 4 main primocanes. The variables analyzed were: green and dry leaf mass, leaf area, number and average fruit mass, production per plant, average size and percentage of fruit moisture, and soluble solids. In relation to average weight, fruit size and yield, BRS-Tupy cultivar was superior in both cycles evaluated. As for soluble solids the Cherokee cultivar was superior, but only in the 2016/17 cycle. In the second experiment, in terms of number of fruits and production, pruning of 4 primocanes in the 2016/17 cycle was superior, but in the next cycle the pruning of 3 primocanes equaled it in the production variable. The drastic pruning in these variables is shown to be lower in the two cycles evaluated. Concerning the variable size of fruit, a significant difference was found only in the 2017/18 cycle, where drastic pruning presented smaller fruits. Thus, it can be concluded that, in general, BRS-Tupy cultivar presents the best productive results and that pruning with 4 primocanes initially stands out alone.

Keywords: climate, primocanes, production, *Rubus* sp., small fruits.

Resumen

El consumo de frutas con beneficios potenciales para la salud ha aumentado en los últimos años, siendo la mora (*Rubus* sp.) una de estas. El objetivo de este trabajo fue evaluar el desempeño de los cultivares de mora y la influencia del manejo de los tallos principales en el cultivar BRS-Tupy. En el primer experimento se evaluaron los cultivares: BRS-Tupy, Cherokee, Xavante y Guaraní; en el segundo experimento las podas evaluadas fueron: 0 tallos (poda drástica de invierno), 2, 3 y 4 tallos principales. Las variables analizadas fueron: masa foliar verde y seca, área foliar, número y promedio de masa frutal, producción por planta, tamaño y porcentaje de humedad de la fruta, y sólidos solubles. Con respecto al peso promedio, al tamaño del fruto y la producción a cultivar, BRS-Tupy fue mayor en los dos ciclos evaluados. En cuanto a los sólidos solubles, la variedad Cherokee fue superior en el ciclo 2016/17. En el segundo experimento, en términos de número de frutas y producción, la poda de 4 tallos en el ciclo 2016/17 fue superior; en el siguiente ciclo, la poda de 3 tallos fue igual. La poda drástica en estas variables se muestra inferior en los dos ciclos evaluados. En la variable de tamaño de fruta, se encontró una diferencia significativa solo en el ciclo 2017/18, donde la poda drástica mostró frutas más pequeñas. Con esto se puede concluir que existen diferencias entre los ciclos productivos evaluados, pero que, en general, el cultivar BRS-Tupy presenta los mejores resultados productivos y que la poda con 4 tallos se destaca inicialmente.

Palabras claves: clima, tallos, producción, *Rubus* sp., frutas pequeñas.

Introduction

The introduction of fruits into the diet, such as blackberry (*Rubus* sp.), either *in natura* or processed form has become an almost worldwide trend for beneficial effects on human health. The benefits are related to the antioxidants present in fruits, among which the most frequent are phenolic compounds that can sequester free radicals (Jacques & Zambiazi, 2011).

Blackberry is temperate fruiting, therefore, so that budding and flowering occur without anomalies, it is necessary that plants accumulate certain number of hours at low temperatures. Since the number of cold hours required is specific to each cultivar, when not achieved, it can affect the resultant productivity (Leonel & Mota-Segantini, 2015).

The phenological aspects of the blackberry may vary from cycle to production cycle, which depends on the fulfillment of cold hours required (Corrêa-Antunes, 2002). Consequently, there will be amendments in production. The latter demonstrates the importance of studying diversified cultivars in different regions and productive cycles.

The presence of thorns in cultivars make some cultural practices difficult, especially pruning (Campagnolo & Pio, 2012a). An alternative is pruning the primocanes close to the soil, called drastic pruning, or using cultivars without spines, such as the cultivar Xavante (Villa *et al.*, 2014). However, it should be noted that the number of main primocanes left after winter pruning influences the productive variables (Tullio & Ayub, 2013).

Therefore, the objective of this work was to evaluate the performance at four blackberry cultivars and the influence of the main primocanes number on BRS-Tupy cultivar in two production cycles.

Materials and methods

The experiments were done according to the geographical coordinates Latitude 27°07'11'' S, Longitude 52°42'30'' E, and altitude 610 m. The climate according to Köppen's classification is category C, subtype Cfa (Wet Subtropical Climate), and the local soil is classified as Dystropheric Red Latosol (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2004, p. 745).

The orchard was implanted in 2014 and evaluations occurred in the productive cycles of 2016/17 and 2017/18, second and third productive years, respectively. The cultural treatments adopted were the removal of weed plants when necessary, and the pruning as described below. Two experiments were evaluated.

Experiment 1

Four mulberry, BRS-Tupy, Cherokee, Xavante, and Guarani cultivars, each with three primocanes evaluated.

Experiment 2

The second experiment consisted of different numbers of evaluations at productive primocanes in the cultivar BRS-Tupy. The treatments were: drastic winter pruning (zero primocane), two, three and four productive primocanes selected in winter pruning.

The experimental design adopted was completely randomized, with five replicates, each replicate being represented by one plant. The spacing used was 3 meters between rows and 1.5 meters between plants (22.222 plants ha^{-1}).

Summer pruning was done by removing the primocanes that were productive, and by shortening those that grew 15 cm above the wire during the cycle. In the winter, pruning the number of productive primocanes per plant was selected; in the case of those that had received drastic pruning, all their aerial part was reduced to 10 cm in height. The other plants received the number of primocanes corresponding to their treatment; excess rods were reduced to 10 cm.

Vegetative and productive parameters were evaluated. Five leaves were collected by the middle third of each plant for mass measurement; in addition, the percentage of mass of leaf dry matter and leaf area was evaluated by using a digital leaf area meter.

The fruits were harvested every three days; counting to obtain the number of fruits and weighing to obtain average fruit weight and total production per plant was performed in all harvests. In addition to these variables, fruit size was evaluated with the aid of a 100 mL graduated column (± 1 mL), where the displaced volume of water after the addition of five fruits per harvest was divided by the number of fruits added. It was also evaluated the soluble solids content using a bench refractometer, and the moisture by drying the fruits in a forced air circulation oven.

The fruit harvest time was between the months of October to January in both production cycles. The harvest was carried out throughout the productive period of the plants every three days. Climatic data from the pruning period to the end of the harvest in the 2016/17 cycle and in the 2017/18 cycle are available in Figure 1(A) and (B), respectively.

The data obtained in each production cycle were evaluated separately in order to isolate the climatic factor. The data were submitted to analysis of variance, and when significant were compared by the Tukey test at 5 % probability.

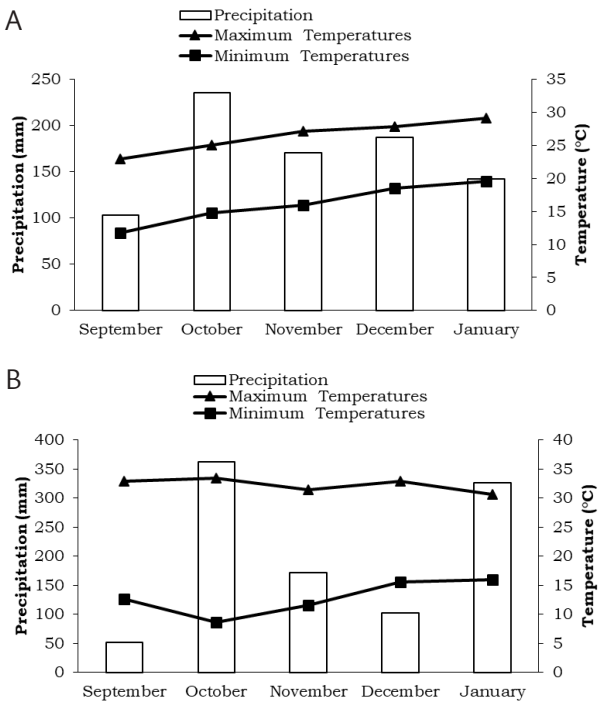

Figure 1. Climate data for the 2016/17 cycle (A) and for the 2017/18 cycle (B).

Results and discussions

Experiment 1

Concerning the mass of leaf dry matter variable, it was observed that in the 2016/17 production cycle the plants of the cultivar Cherokee presented lower dry mass accumulation in their leaves, and therefore, a greater amount of water (Table 1). In the 2017/17 cycle, the cultivar Xavante presented a lower mass of leaf dry matter but without significant difference of cultivar BRS-Tupy and Guarani.

For fresh leaf mass and average leaf area in the 2016/17 cycle, BRS-Tupy and Xavante plants presented the highest values. The latter, related to the largest fresh leaf mass, plants of these cultivars present a result of the larger leaf area. In the 2017/18 cycle these results were not repeated (Table 1).

In the 2016/17 production cycle the cultivar Xavante produced a lower number of fruits per plant. This also occurred in the 2017/18 cycle but there was no difference for the Cherokee cultivar (Table 2). Corrêa-Antunes *et al.* (2010) when evaluating blackberry cultivars in Pelotas-RS, found a higher number of fruits produced in comparison to the one found in this study. Under these conditions, BRS-Tupy plants produced 2878 fruits, cv. Guarani 3018 and cv. Cherokee 3283 fruits.

In the two productive cycles evaluated, BRS-Tupy cultivar was the one that produced fruits of higher average weight, in contrast to the Cherokee cultivar which was among the ones that produced lower fruits in both cycles (Table 2). A study by Ferreira *et al.* (2016) in Pelotas-RS, in different productive cycles, found average fruit mass ranging from 7-7.5 g in cv. BRS-Tupy, 4.8-3.7 g in cv. Guarani and the cultivar Xavante with fruits of 5.7-6.0 g. Values higher than those are found in this work.

The yield per plant was higher in the BRS-Tupy cultivar in the two cycles evaluated (Table 2); as a result, its large number of fruits and superiority in the average weight of the fruits. It can be noticed a production decrease in the cultivars of the cycle 2016/17 to 2017/18 which was variable in each cultivar. In the BRS-Tupy cultivar this decrease was 11.5 %; in cv. Cherokee 48.8 %; in cv. Xavante was 69.9 %, and in cv. Guarani was the lowest reduction (0.3 %).

Table 1. Vegetative parameters in different cultivars of blackberry.

Cultivars	Leaf Dry Matter (%)		Leaf Fresh Mass (g)		Mean Leaf Area (cm²)	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
BRS-Tupy	43.25 a*	58.61 ab	7.16 a	11.04 a	65.15 a	68.98 a
Cherokee	37.68 b	61.47 a	3.31 b	9.34 a	29.69 b	49.00 a
Xavante	46.10 a	53.50 b	7.68 a	10.75 a	62.61 a	68.65 a
Guarani	43.02 a	58.84 ab	3.75 b	10.34 a	35.57 b	67.49 a
CV (%)	4.55	6.58	19.20	13.47	19.00	18.55

*Values followed by the same letter in the column do not differ significantly (Tukey's test, P ≤ 0.05).

Table 2. Productive parameters in blackberry cultivars.

Cultivars	Number of fruits		Average Fruit Weight (g)		Yield (Kg)	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
BRS-Tupy	737.2 a*	828.2 a	6.69 a	5.28 a	4.96 a	4.38 a
Cherokee	671.8 a	402.8 b	3.73 c	3.15 c	2.52 b	1.29 c
Xavante	469.2 b	266.6 b	5.32 b	3.61 bc	2.52 b	0.96 c
Guarani	771.8 a	845.2 a	4.09 c	3.71 b	3.17 b	3.16 b
CV (%)	11.81	16.45	10.68	7.52	18.25	18.09

* Values followed by the same letter in the column do not differ significantly (Tukey's test, P ≤ 0.05).

Oliveira *et al.* (2017) evaluated some cultivars in an altitude region of the Minas Gerais State, among which there were. Tupy, Cherokee, and Guarani cultivars. In this case, the cultivar with greater production per plant was cv. Guarani. In addition, according to the same authors, the differences observed in Blackberry cultivars were related to the temperature variations of each region and the density of planting.

The data related to average size, moisture, and soluble solids can be observed in Table 3. Regarding the average fruit size, cv. BRS-Tupy presented larger fruits in relation to the other cultivars. In a study conducted by Campagnolo and Pio (2012b) in Marechal Cândido Rondon-PR, the cultivars that presented the largest fruit size were BRS-Tupy and Guarani.

Concerning moisture, it was observed that in the 2016/2017 cycle, the BRS-Tupy cultivar presented a higher percentage but without significant difference for the Xavante cultivar, which in turn did not differ from the other cultivars. In the later cycle, the superiority in moisture in the cultivar BRS-Tupy was fulfilled. These results agree with Hirsch *et al.* (2012), in where it is mentioned that blackberry fruits have high water content in fruits.

In the 2016/17 production cycle, cv. Cherokee presented 14 % more soluble solids in comparison to the average of the other cultivars, but in the following productive cycle, no significant difference between the cultivars was found. A data to be observed is that in the 2017/18 cycle the ºBrix content of fruits was lower than in the previous cycle.

A study conducted by Hirsch *et al.* (2012) in Pelotas-RS, found for cv. BRS-Tupy content of 10.1 ºBrix, 10.2 ºBrix in cv. Guarani and 8.4 ºBrix in cv. Cherokee. Caldas-MG Aymoto-Hassimotto *et al.* (2008) found values on soluble solids of 6.93 ºBrix in cv. BRS-Tupy and 9.23 for cv. Guarani. This difference can be explained by the fact that climate conditions may influence the chemical and fruit quality parameters of Berries, mainly due to the thermal difference and the intensity of solar radiation (Ali *et al.*, 2011; Caproni *et al.*, 2016).

Experiment 2

For the mass of leaf dry matter, it was observed that in the 2016/17 cycle, plants submitted to 2 main primocanes presented greater dry mass accumulation but without significant difference for those submitted to 3 primocanes, which did not differ from the plants submitted to the other treatments (Table 4). While in the 2017/18 cycle it was observed that, the higher the number of productive rods, the greater the dry mass accumulation in the leaves.

For the variable fresh leaf mass in the two productive cycles evaluated, plants submitted to drastic pruning presented greater leaf fresh weight. This result can be explained by the necessity of the plants to emit new primocanes and, according to Campagnolo and Pio (2012a), to possibly destine good part of their energies to form part of the area. This may have produced leaves with greater weight and larger size, as occurred in the 2017/2018 cycle.

The average leaf area of the plants was also influenced by the pruning applied in the two evaluated cycles: the higher the pruning intensity, the higher the leaf area of these plants.

In the productive variables (Table 5), the drastic pruning presented the lowest results. In the two cycles evaluated, the pruning of four primocanes revealed superiority in the number of fruits.

Table 3. Size, moisture and soluble solids fruits in blackberry cultivars.

Cultivars	Size (cm^3)		Moisture (%)		Soluble Solids (ºBrix)	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
BRS-Tupy	7.18 a*	6.06 a	85.77 a	84.04 a	10.38 b	6.89 a
Cherokee	4.73 b	3.61 b	84.04 b	79.97 b	11.88 a	8.15 a
Xavante	5.57 b	3.71 b	85.56 ab	80.96 b	10.45 b	8.33 a
Guarani	4.77 b	4.40 b	86.75 a	80.84 b	9.78 b	6.88 a
CV (%)	11.80	12.96	1.11	1.99	4.37	15.33

*Values followed by the same letter in the column do not differ significantly (Tukey's test, $P \leq 0.05$).

Table 4. Vegetative parameters in blackberry cultivar BRS-Tupy submitted to different numbers of primocanes.

Primocane Number	Leaf Dry Matter (%)		Leaf Fresh Mass (g)		Leaf Area Mass (cm^2)	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
Drastic (0)	40.16 b*	52.21 b	9.99 a	15.06 a	80.00a	102.72 a
2	43.80 a	57.71 ab	7.38 b	11.43 b	67.02 b	75.24 ab
3	43.25 ab	58.61 a	7.19 b	11.04 b	65.19 b	68.98 b
4	39.40 b	60.22 a	7.17 b	9.90 b	66.18 b	60.57 b
CV (%)	5.59	6.05	13.58	16.80	4.80	19.35

*Values followed by the same letter in the column do not differ significantly (Tukey's test, $P \leq 0.05$).

According to Broetto *et al.* (2009), whose study evaluated long pruning and short pruning, plants with long pruning showed a higher number of fruits, which can be explained by the fact that they possess a higher number of flower buds, agreeing with the results obtained in this experiment.

In the 2017/18 cycle, plants with drastic pruning produced a higher number of fruits compared to the first cycle. According to Campagnolo and Pio (2012a), this may be due to the maturity of the plants throughout the cycles. However, in both cases, this was not sufficient to reach the same productivity of plants that remained with a number of primocanes after winter.

For the average weight, the drastic pruning did not differ statistically in fruits of plants with two primocanes in the cycle 2016/17, but in the following cycle, the drastic pruning presented fruit with less weight. In a study conducted by Nogueira-Curi *et al.* (2015) in Lavras-MG, the fruits of cv. Tupy presented a fresh mass higher than that found in this study, 8.6 g and 6.8 g, in the respective cycles studied.

For the production per plant the quantity produced increased according to the number of plant primocanes, but in the cycle 2017/18, plants with three primocanes matched their production to plants with four primocanes. According to Ferreira *et al.* (2016), due to the larger leaf area, consequently, greater capacity to perform photosynthesis, plants with higher stem density obtain a higher production load.

In a study done by Villa *et al.* (2014), plants submitted to four primocanes began to have a reduced productive trend in relation to those submitted to three primocanes. However, in this case, the plants were in a higher population density, and this may be the reason why, with four primocanes, one can already perceive a productive decrease in the plants.

The greater production due to the increase of the primocanes number is related to the increase in the number of fruits in these plants. The fact that plants with drastic pruning produced the lowest values in the productive characteristics can be due to the fact that these plants, after winter, before starting the production, need to emit shoots, unlike the other treatments where their aerial part was previously defined previous cycle (Lugaresi *et al.*, 2018).

The results presented in Table 6 show that in the first productive cycle evaluated no significant difference was found for the average fruit size. However, in the later cycle, drastic pruning plants produced fruits of about 24 % smaller size. An explanation for this result could be that, as these plants in their second cycle produced more fruit than in the first one, part of their energy previously used in the formation of a smaller number of fruits, this time was applied in the production of more fruits but reducing their size.

The percentage of moisture decreased according to the number of primocanes present in the plants in the 2016/17 cycle. However, this fact did not occur in the following year, where no significant difference was found for the percentage of moisture.

Soluble solids content also changed between the two cycles. In the 2016/17 cycle, fruits of drastically pruned plants presented lower soluble solids content, but in the latter cycle, there was no significant variation in soluble solids of fruits from different prunings. According to Tullio and Ayub (2013), they found on average 5 ºBrix for cv. BRS-Tupy independent of the number of primocanes. According to the authors, these differences can be justified by

Table 5. Productive parameters Yield in blackberry cultivar BRS-Tupy submitted to different numbers of primocanes.

Primocane Number	Number of fruits		Average Fruit Weight (g)		Yield (kg)	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
Drastic (0)	175.0 c*	229.8 d	5.75 b	3.79 c	0.99 c	0.87 c
2	678.6 b	673.0 c	6.45 ab	4.56 b	4.39 b	3.07 b
3	737.2 b	828.6 b	6.69 a	5.28 a	4.96 b	4.38 a
4	932.2 a	931.8 a	6.73 a	5.01 ab	6.26 a	4.66 a
CV (%)	15.93	3.40	7.04	7.41	15.98	11.99

*Values followed by the same letter in the column do not differ significantly (Tukey's test, P ≤ 0.05).

Table 6. Quality parameters in fruits in blackberry cultivar BRS-Tupy submitted to different numbers of primocanes.

Primocane Numbers	Size(cm³)		Moisture		Soluble solids (°Brix)	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
Drastic	6.25 a*	4.64 b	87.86 a	80.79 a	8.86 b	7.46 a
2	6.90 a	6.13 a	86.19 ab	81.39 a	10.43 a	7.22 a
3	7.18 a	6.06 a	85.64 b	84.04 a	10.39 a	6.89 a
4	7.38 a	6.11 a	84.79 b	84.41 a	10.83 a	6.95 a
CV (%)	11.30	6.91	0.87	3.49	4.03	10.36

*Values followed by the same letter in the column do not differ significantly (Tukey's test, P ≤ 0.05).

the variations in the climatic conditions, as much to the regions, as from year to year.

Climatic variations, especially the volume of precipitation during the productive period, possibly influenced the productive results of the plants, especially the average fruit weight, yield, and fruit size. The lower puvliometric precipitation identified in the 2017/18 cycle may have influenced that, in general, these variables were lower in this cycle than in the first production cycle, in both experiments.

Conclusion

Under experimental conditions, in this region cv. BRS-Tupy is the most promising in terms of production and larger size fruit. Winter pruning where four primocanes remain in the cv. BRS-Tupy, at first, seems to be the most productive, with no loss in fruit quality. But with advancing cycles, pruning of three primocanes eventually equals. Drastic winter pruning affects production, it also interferes with fruits quality in some cycles, so it is not indicated.

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Germination and fungi incidence in Cerejeira-da-mata seeds at different fruit maturation levels and temperatures

Germinação e incidência de fungos em sementes de Cerejeira-da-mata em diferentes estádios de maturação dos frutos e temperaturas

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Abstract: The effect of different levels of physiological maturation of the fruit and temperature on the seed germination and incidence of fungi that are harmful to the seeds, becomes fundamental for the improvement of the propagation of different native fruit species. The objective of this work was to evaluate the effect of physiological maturation and temperature on the germination and fungi incidence in *Eugenia involucrata* DC. seeds. Three experiments were carried out. In the experiment I, the effect of four levels of physiological maturation was evaluated, visually determined based on the color of the fruit, and in the experiment II, was evaluated the effect of two levels of maturation and two temperatures (25 and 35 °C) on the germination and vigor of the seeds. In the experiment III, was investigated the incidence of fungi in the seeds at two temperatures (25 and 35 °C). The levels of maturation evaluated did not influence the percentage of germinated seeds, but they influenced seed vigor. The temperature of 25°C was suitable for seed germination. The fungi genera found on the seeds were *Trichoderma*, *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria, and Colletotrichum* with variable incidences depending on evaluated temperature.

Keywords: Cerejeira-do-Rio-Grande. *Eugenia involucrata*. Myrtaceae. Physiological maturation. Wild fruits.

Resumo: O conhecimento dos efeitos de diferentes níveis de maturação fisiológica do fruto e da temperatura sobre a germinação e incidência de fungos prejudiciais às sementes é fundamental para o aprimoramento das técnicas de propagação de diferentes espécies frutíferas nativas. Portanto, o objetivo desse estudo foi avaliar

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a influência do estádio da maturação fisiológica dos frutos e da temperatura sobre a germinação e incidência de fungos em sementes de *Eugenia involucrata* DC.. Foram conduzidos três experimentos, no primeiro avaliou-se o efeito de quatro estádios de maturação fisiológica distinguidos, visualmente, com base na coloração do fruto. No segundo, avaliou-se o efeito de dois estádios de maturação do fruto e duas temperaturas (25 e 35 °C) sobre o vigor e germinação das sementes. No terceiro, foi investigada a incidência de fungos nas sementes em duas temperaturas (25 e 35 °C). Os diferentes níveis de maturação fisiológica avaliados não interferem na porcentagem de sementes germinadas, contundo, influenciam o seu vigor. A temperatura de 25 °C é adequada para a germinação das sementes de *Eugenia involucrata* DC. Os gêneros de fungos identificados nas sementes foram *Trichoderma*, *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* e *Colletotrichum* apresentando variações na incidência de acordo com a temperatura avaliada.

Palavras-chave: Cerejeira-do-Rio-Grande. *Eugenia involucrata.* Myrtaceae. Frutíferas nativas. Maturação fisiológica.

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1 Introduction

Brazil stands out as one of the main centers of genetic diversity of wild fruit trees worldwide. Nevertheless, there is a lack technical information for most of these species. Wild fruits belonging to the family Myrtaceae are widely distributed throughout Brazil, constituting a source of germoplasm of enormous value (Lattuada *et al*., 2010). The Cerejeira-da-mata, *Eugenia involucrata* DC., belongs to the Myrtaceae family and is a native species of southern Brazil. It can be found from Minas Gerais to Rio Grande do Sul states in forest formations of the Atlantic complex, and in some regions of Paraná state (Donádio *et al*., 2004). Besides, *E. involucrata* also occurs in Mato Grosso do Sul, Minas Gerais and Goiás states, and in other countries of South America as Argentina, Uruguay, and Paraguay (Rodrigues & Carvalho, 2001).

E. involucrata is a native honey plant with great ecological importance disseminated by birds and useful in the recovery of degraded areas (Carvalho, 2008; Rodrigues & Carvalho, 2001), besides its landscape value due to the beauty of its flowers and stems (Lorenzi, 2002). In addition, this species has fruits that can be consumed fresh and processed to make sweets, jams, and liqueurs (Carvalho, 2008). In addition, has potential for pharmaceutical use, due its digestive, antidiarrheal and antirheumatic properties, and antifungal activity (Sardi *et al*., 2017; Toledo *et al*., 2020; Vechi *et al*., 2018). The wood is of good quality and can be used in civil construction, making agricultural tools, as firewood and for charcoal production (Carvalho, 2008). Due to its great economic and ecological importance, studies aimed at improving propagation of this wild fruit assume significant relevance (Avila *et al*., 2009).

Data regarding the physiological maturation of fruits and germination of seeds are crucial for the propagation of wild fruits, as they prescribe conditions for the collection and processing of seeds at appropriate times (Nogueira *et al*., 2013), providing for maximum physiological seed quality. Seed quality is also closely related to the presence of some fungal pathogens that may be associated with a decrease in germination rate and lower seedling development (Yorinori, 1982). Temperature is also an important factor that must be considered in the germination of the seeds, as it directly and indirectly influences the germination process (Huang *et al*., 2018).

Considering the above-mentioned factors, our objective with this work was to evaluate the effect of physiological maturation and temperature on the germination and fungal incidence in *Eugenia involucrata* DC. seeds.

2 Material e Methods

2.1 Experimental location

The experiments were conducted in the laboratory of plant biotechnology and propagation of the Federal University of the Southern Frontier - UFFS, Campus Chapecó-SC, using seeds from four individuals of *E. involucrata* species located in the municipality of Vitorino (26°15'31.9"S and 52°47'44.0"W), Paraná, Brazil.

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The fruits were collected and immediately taken to the laboratory for manual removal of the seed. After processing, the seeds were cleaned with sodium hypochlorite (2.5% active chlorine) at a concentration of 10% (v/v), for 10 minutes. Then, were placed in a sieve, leaving them in running water for three minutes. After that, experiments I e II carried out as follows:

2.2 Experiment I – Evaluation of the effects of physiological maturation of fruits on seed germination

Seeds were removed from fruits at four levels of physiological maturation, determined according to the coloring of the epicarp of the fruit: yellow with red spots (RS), bright red (BR), wine-colored (WC) and fruits that were on the ground (GR). The fruit coloration levels were determined based on the classification of Oro *et al*. (2012).

The seeds (25 seeds per box) were packed in transparent polyethylene boxes with dimensions of 11 x 11 x 3.5 cm (Gerbox®) containing a distilled water moistened substrate. The substrate was moistened whenever necessary. The substrate used was of vegetable origin for germination of tree species (Turfa Fértil®; conductivity: 1.5 ± 0.3 mS/cm; dry weight density: 280 kg/m³; pH: 5.5 ± 05; water retention capacity: 57%). The plastic boxes were placed in bio-oxygen demand incubators with 12-hour light cycles at 25 °C, to evaluate the seed germination parameters.

Emerging seedling counts were performed daily. Seedlings were considered emerged when they presented aerial parts and root systems with sufficient size for determination of normal seedlings, according to the rules for seed analysis (Brazil, 2009). The 'normal seedlings' were growing in tubes to confirm their normal development.

The germination was displayed in percentage of germinated seeds (%). The mean germination time and the germination speed index (GSI) were calculated according to the method established by Edmond and Drapala (1958) and Maguire (1962), respectively. The first count was performed on the 18th day after installing the experiment.

2.3 Experimental design and statistical analysis

The experimental design was completely randomized, with four treatments (different levels of physiological maturation: GR, WC, BR e RS) and five replicates of 25 seeds each. For the variable percentage of germination, the arcsine transformation $(x/100)^{0.5}$ was used and for the other variables the applied transformation was $(x+1)^{0.5}$. The data was submitted to Liliefors' normality test and analysis of variance (F test). When a significant difference was observed between the treatments the means were compared by Tukey´s test (p≤0,05). Sisvar version 5.6 (Ferreira, 2011) and SigmaPlot version 12.5 from Systat Software, Inc., San Jose California USA, were used for statistical analysis.

2.4 Experiment II - Influence of physiological fruit maturation and temperature on seed germination

The seeds used were removed from fruits with wine-colored epicarp (WC) and fruits that were on the ground (GR), according to the classification described previously (Experiment I). The seeds were cleaned and sowed in transparent polyethylene boxes with substrate, in the same conditions described in the experiment I, and placed in bio-oxygen demand incubators with 12-hour light cycles at 25 and 35 °C. The parameters evaluated were seed germination (%), the mean germination time (Edmond & Drapala, 1958), the germination speed index (Maguire, 1962) and the germination on first count (%). All the evaluations were carried out as described in the experiment I.

2.5 Experimental design and statistical analysis

The experimental design was completely randomized in 2 x 2 factorial arrangement with two levels of physiological maturation (WC and GR) and two temperatures (25 °C and 35 °C), with 5 replicates of 25 seeds each. For the variable percentage of germination, the arcsine transformation $(x/100)^{0.5}$ was used and for the other variables the applied transformation was $(x+1)^{0.5}$. The data was submitted to Liliefors' normality test and analysis of variance (F test). When a significant difference was observed between the treatments the means were compared by Tukey´s test (p≤0,05). Sisvar version 5.6 (Ferreira, 2011) and SigmaPlot version 12.5 from Systat Software, Inc., San Jose California USA, were used for statistical analysis.

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2.6 Experiment III – Evaluation of the influence of temperature on the incidence of fungi in seeds

To evaluate the incidence of fungi in seeds of *E. involucrata*, the seeds were subjected to superficial cleaning with 70% ethanol (30 sec), followed by 1% sodium hypochlorite (1 min) and three washes with distilled water (1 min each). Subsequently, the seeds were placed to dry on sterile filter paper at room temperature.

The seeds were packed in plastic transparent boxes (Gerbox®) with distilled water moistened germination paper (Germitest®) and incubated in bio-oxygen demand incubators at 25 ºC and 35 ºC, with a 12-hour light cycle for 7 days. In each box were placed 25 seeds, and the germination paper was moistened with distilled water daily. The method was the "*Blotter test*" with freezing (Brazil, 2009). To evaluate the incidence of fungi in the seeds, we used stereoscopic and optical microscopes. The fungi present in the samples were identified at the genus level, based on a specialized bibliography (Barnett & Hunter, 1998), determining the percentage of incidence of each fungal genus.

2.7 Experimental design and statistical analysis

The experimental design was completely randomized with 6 replicates of 25 seeds for each evaluated temperature (25 ºC and 35 °C). The data was submitted to the arcsine transformation $(x/100)^{0.5}$, and then to Liliefors' normality test. The fungal incidence was compared between temperatures for each genus by Student's t-test ($p \leq 0,05$). SigmaPlot version 12.5 from Systat Software, Inc., San Jose California USA, was used for statistical analysis.

3 Results and discussion

3.1 Experiment I

Seed germination (SG) was not influenced by the levels of physiological maturation of the fruits, with a mean of 89.25% of germinated seeds. However, the mean germination time (MGT), germination speed index (GSI) and germination at the first count (FCG) were influenced by the levels of physiological maturation of the fruits (Table 1).

Because seed germination was not influenced, we may infer that the complete formation of the seeds had already occurred at the maturation levels of the fruits evaluated in this study and that, therefore, only the vigor of the seedlings was altered. It is important to point out that the seeds collected from fruits on the ground were visually selected for the presence of insect or fungal damages, being discarded approximately 10% to 12% of the total number of seeds collected.

Table 1. Seed germination (SG), mean germination time (MGT), germination speed index (GSI) and first count germination (FCG) of *Eugenia involucrata* DC. seeds from fruits at different levels of physiological maturation in 12-hour light cycles at 25°C.

Levels of maturation[#]	SG (%)	MGT (Days)	GSI	FCG (%)
GR	89.5[ns]	23 b*	1.01 a*	20.8 a*
WC	87.0	26 a	0.80 b	4.8 c
BR	91.0	25 ab	0.98 a	12.0 bc
RS	90.0	23 b	1.06 a	19.2 ab
CV (%)	3.41	5.25	5.21	28.52

[#]Yellow with red spots (RS), bright red (BR), wine-colored (WC) and fruits that were on the ground (GR). ns: not significant. * Means followed by the same letter in the column do not differ significantly by Tukey's test ($p \leq 0.05$).

The effect of fruit physiological maturation on the germination of *E. involucrata* seeds was verified by Oro *et al*. (2012). The authors demonstrated that seeds removed from fruits on the ground presented the lowest percentage of germination, while seeds removed from wine-colored fruits exhibited the highest percentage of germination. This difference in seed germination in relation to our study may be related to the visual separation of damaged seeds from the fruits that were on the ground.

The mean seed germination time was highest for seeds from wine-colored fruits (26 days). The seeds from the fruits that were on the ground and fruits with a yellow coloration with red spots exhibited the shortest

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germination time (23 days). Seeds collected from fruits with bright red coloration showed intermediate germination time (25 days). The mechanism of endogenous plant hormonal regulation is supposed to be highly conserved in seed germination process. Genetic and physiological studies have shown the important roles of the phytohormones, abscisic acid (ABA), gibberellins (GAs), ethylene and brassinosteroids in seed germination process (Koornneef *et al*., 2002; Kucera *et al*., 2005; Xia *et al*., 2019). Therefore, the differences in mean germination time of the seeds may be attributed to the presence of substances that negatively modulate the germination process when the fruits reach maturation (wine-colored fruits). On the other hand, in the seeds from fruits that were in the ground these substances were possibly already in lower concentrations, and in the seeds from fruits visually at the beginning of maturation (yellow with red spots), there were still no substances formed or accumulated enough to interfere on the germination process.

Evaluating the mean germination time at various levels of physiological maturation of *E. involucrata* seeds, Oro *et al*. (2012), showed that seeds of fruits with green coloration (a maturation level not evaluated in the present study) exhibited the highest mean germination time (32 days), while the seeds removed from fruits with bright red color had the shortest mean germination time (27 days). In our study the seeds removed from fruits with bright red color did not present differences compared with seeds removed from fruits yellow with red spots, wine-colored fruits or fruits collected on the ground. The effect of various levels of physiological maturation of the fruits on the mean germination time of the seeds was also demonstrated by Gonçalves *et al*. (2015*)*, in pepper seeds (*Capsicum chinense* Jacq.) removed from fruits at five levels of physiological maturation. In that study, seeds from fruits with green color showed the highest mean germination time, and seeds removed from fruits with red-carmine color (the most advanced maturation level) had the shortest mean germination time.

We observed the shortest germination speed index in seeds removed from wine-colored fruits, being 21.56% lower than the other maturation levels evaluated. Seeds removed from fruits that were on the ground, bright red fruits and yellow fruits with red spots exhibited the highest germination speed index, with a mean of 1.02, not different from one another. Evaluating the effect of physiological maturation on the germination of *Luehea divaricate*, Schulz *et al*. (2017), observed that the germination speed index was highest in seeds obtained from fruits with green coloration than that of brown fruits. Brown-colored fruits corresponded to a stage of maturation more advanced than the that of fruits with green coloration. However, when the fruit reached the most advanced level of maturation evaluated, the germination speed index did not differ from that of other stages of maturation. In the present study, seeds removed from fruits collected on the ground showed the highest percentage of seeds germinated at the first count, with an increase of 8.8% and 16% compared to seeds from bright red and wine-colored fruits, respectively. However, seeds from fruits collected on the ground did not differ of the seeds from yellow-colored fruits and red spots, with a mean of 20% of germinated seeds at the first count.

Seed germination began on the 18th day and extended until the 58th day after the experiment implementation. The seeds from wine-colored fruits had the lowest germination percentages per unit of time (Figure 1). The same behavior was also observed for the germination speed index, mean germination time and percentage of seeds germinated at first count shown previously (Table 1). These results indicating curiously that the seeds show a reduction of vigor at this moment for some reason yet don´t know in this species.

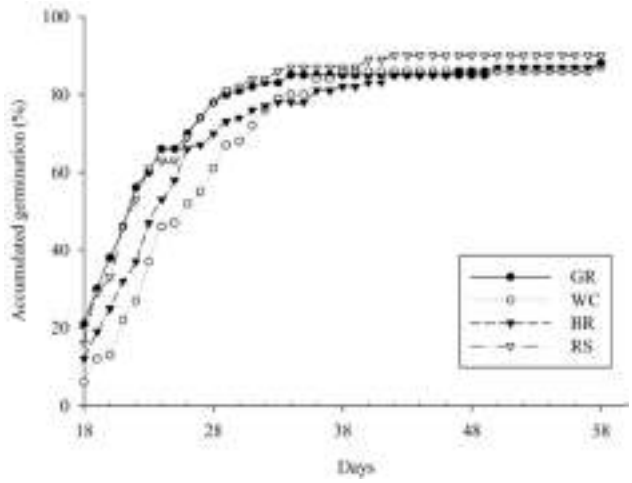

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Figure 1. Accumulted germination of *Eugenia involucrata* DC. seeds. Seeds removed from fruits yellow with red spots (RS), bright red (BR), wine-colored (WC), and fruits that were on the ground (GR).

Seed germination can occur normally even when they are not physiologically 'ready'. However, when this happens, there is the possibility of delayed seed germination and the formation of seedlings with low vigor (Carvalho & Nakagawa, 2012). This phenomenon was observed in our study, where only the vigor of the seedlings was influenced by the levels of physiological maturation of the fruit evaluated. The germinative process and initial seedling development, in addition to being influenced by environmental conditions, is controlled by the interaction of endogenous phytohormones, such as gibberellic acid (germination promoter) and abscisic acid (germination inhibitor). Recently, it was demonstrated that auxins acting negatively on germination. The phytohormones ethylene and brassinosteroids also influence the germination process (Finkelstein *et al*., 2008; Gupta & Chakrabarty, 2013; Koornneef *et al*., 2002; Kucera *et al*., 2005; Shu *et al*., 2016; Xia *et al*., 2019).Thus, it can be inferred that the variation of the germinative parameters related to the vigor of the seeds demonstrated in our study for the different levels of fruit maturation evaluated, may be related to fluctuations and/or interactions among phytohormones on germinative process.

The plant's environmental conditions at the time of seed formation can also influence the germination of the seeds, as demonstrated by Huang *et al*. (2018). Considering this fact, it may be inferred yet that some discrepancies in the results obtained in our study from those of other studies may be related to local and temporal variations in climatic conditions at the time of seed formation. In addition, one may mention the peculiarities of each species with respect to environmental and physiological conditions that modulate the germinative process.

3.2 Experiment II

The mean germination time (TMG), the germination speed index (GSI), and the percentage of seed germination in the first count (FCG) were influenced by temperature and physiological levels of fruit maturation. There was an interaction between temperature and physiological maturation levels of the fruit for the vigor of the seeds. However, seed germination (SG) was influenced only by temperature (Table 2).

Table 2. Seed germination (SG), mean germination time (MGT), germination speed index (GSI) and first count germination (FCG) of *Eugenia involucrata* DC. seeds from wine-colored fruits and fruits that were on the ground in two temperatures (25 °C and 35 °C) with 12-hour light cycles.

Temperature	Levels of physiological maturation[#]	
	GR	WC
	Seed germination (%)	
25 °C	89.5 aA[¥]	87.0 aA
35 °C	0.00 bA	0.00 bA
CV (%)	2.60	
	Mean germination time (Days)	
25 °C	23.5 aA[¥]	26.0 aB
35 °C	0.00 bA	0.00 bA
CV (%)	5.34	
	Germination speed index (GSI)	
25°C	1.01 aA[¥]	0.80 aB
35 °C	0.00 bA	0.00 bA
CV (%)	3.02	
	First count germination (%)	
25 °C	21.8 aA[¥]	5.8 aB
35 °C	0.00 bA	0.00 bA
CV (%)	48.41	

[#] WC: seeds removed from wine-colored fruits; GR: seeds removed from fruits that were on the ground.
[¥] Lowercase letters for comparison of means in the vertical (column) and uppercase letters in the horizontal (line). The means followed by the same letter did not differ significantly from each other by Tukey's test ($p \leq 0.05$).

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Seed dormancy is common in wild plants and ensures that seeds germinate only when environmental conditions are optimal. It thus is an adaptive trait in numerous seed-plant species, enabling wild plants to survive under stressful conditions in nature (Finkelstein *et al*., 2008; Shu *et al*., 2016). Temperature is among the principal factors influencing seed germination (Pereira *et al*., 2013), being able to alter the rate of water absorption and the biochemical reactions that determine the germination process (Carvalho & Nakagawa, 2012). In this study, the influence of temperature on the germination of *E. involucrata* seeds was clearly seen, because when they were exposed to a temperature of 35 °C there was total inhibition of germination (e.g.; secondary dormancy) at both fruit maturation levels evaluated.

Results demonstrating the negative influence of high temperatures on seed germination and emergence of seedlings were reported by Oliveira and Barbosa (2014) in their work with *Cedrela fissilis*. The authors demonstrated that germination was impaired with increasing temperature and that the lower germination percentage was observed when the seeds were exposed to 35 °C. The influence of temperature on seed germination was also verified by Gomes *et al*. (2016) in their study with *Eugenia involucrata*, *Campomanesia xanthocarpa*, *Acca sellowiana* and *Eugenia pyriformis*. The results obtained by these authors corroborate with those found in our study, demonstrating that *E. involucrata* seeds exhibited the highest seed germination at 25 °C. However, Lamarca *et al*. (2011) did not verify differences in seed germination of *E. involucrata* among the temperatures of 20, 25 and 30 °C, and the alternate temperatures of 20/30 and 20/35 °C.

The mean germination time at 25 °C was lower for seeds collected from fruits that were on the ground (Table 2), as demonstrated in experiment I, showing a reduction of 2.5 days in relation of the seeds removed from wine-colored fruits. The influence of temperature on mean germination time was also demonstrated in other studies. Nogueira *et al*. (2014), evaluating the effect of temperature and the presence or absence of light on the *Dalbergia cearensis* seeds, showed that the lowest germination time of the seeds occurred between 25°C and 30 °C.

The germination speed index at 25 °C obtained in our study was highest for fruits collected on the ground, with an increase of 20.8% when compared with seeds removed from wine-colored fruits. Lamarca *et al*. (2011) observed similar results, with the highest germination speed index of *E. involucrata* seeds at 25 and 30 °C. Significant differences were also observed by Nogueira *et al*. (2014), evaluating the effect of temperature and the presence and absence of light on the seeds of *Dalbergia cearensis*, where the highest germination speed index was detected at temperatures of 25 °C and 30 °C. On the other hand, Zucareli *et al*. (2015), evaluating the effect of temperature and light on the germination of *Passiflora incarnata* seeds, demonstrated that the highest values of germination speed index were obtained at 35 °C. These results indicating that different species exhibited distinctions with respect to the best temperature for seed germination, a factor that may be associated with the environmental conditions and physiological adaptation of each species.

The percentage of germination at the first count was 20% highest at 25 °C in seeds from fruits collected on the ground. Melo *et al*. (2017), evaluating the effect of different substrates and temperatures on the germination of *Eriotheca gracilipes* seeds, also demonstrated the influence of temperature on seed germination in all tested substrates. The authors demonstrated that the highest germination at first count occurred at 25 and 30 °C for *E. gracilipes*.

Seed germination can be influenced by external and internal factors. Internal factors include physiological dormancy and seed viability, among others. External factors include availability of water (in the seed and the substrate of germination), luminosity, the presence of gases (particularly O_2) and temperature (Carvalho & Nakagawa, 2012). In this study, it was possible to clearly demonstrate the effect of temperature on the germination of *E. involucrata* seeds. The effect triggered on *E. involucrata* seeds at 35 °C can be characterized as termo-dormancy, that is, inhibition of seed germination due to temperature. According to Huang *et al*. (2018), dormancy is dependent on the temperature at the time of seed formation in the plant ('maternal temperature'). Therefore, because *E. involucrata* is a native species of mild temperatures, it can be inferred that 35 °C prevented the germination of seeds of this species by creating an environment different from the favorable conditions for its development, like a survival strategy in stressful conditions.

3.3 Experiment III

Seed germination and the initial development of the seedlings are related to seed health. Some fungal pathogens can affect negatively seeds depending on environmental conditions. The primary factors for fungal

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development in seeds are temperature and humidity. Therefore, in our study, we evaluated the incidence of the fungi in *E. involucrata* seeds at 25 and 35°C.

The seed health test revealed the presence of six fungi genus in the *E. involucrata* seeds and the influence of temperature on their development (Figure 2). The following fungi genera were identified: *Trichoderma*, *Fusarium*, *Penicillium*, *Colletotrichum*, *Aspergillus*, and *Alternaria*. The fungi genera *Penicillium*, *Aspergillus* and *Colletotrichum* exhibited the highest incidence at 25 °C while, *Alternaria* was the unique fungi genus that exhibited the highest incidence at 35°C. The fungi genus *Trichoderma* showed non-temperature-dependence for its development in the *E. involucrata* seeds.

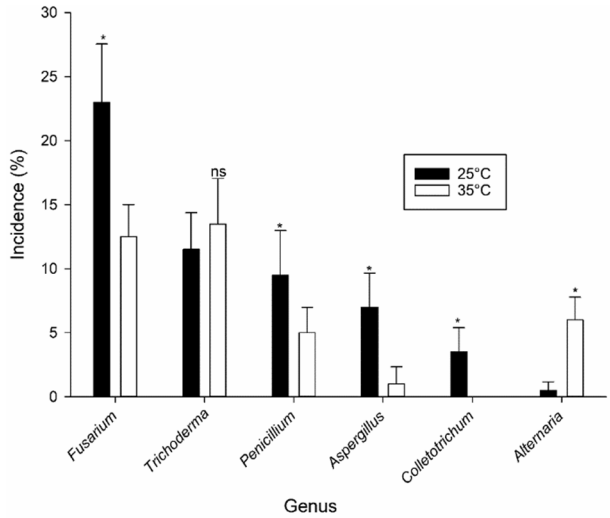

Figure 2. Incidence of fungi by genus (%) in *Eugenia involucrata* DC. seeds at 25 and 35°C with 12-hour light cycles. *Indicates significant differences in the fungi growing under the two temperatures by Student's t-test ($p \leq 0.05$). ns: not significant.

At 25 °C, the fungi genera *Fusarium*, *Trichoderma*, *Penicillium*, *Aspergillus*, *Colletotrichum*, and *Alternaria* were detected in 23%, 11.5%, 9.5%, 7%, 3.5%, and 1% of the seeds, respectively. At 35 °C, the fungi genera *Trichoderma*, *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus* were detected in 13.5%, 12.5%, 6%, 5% and 1% of the seeds, respectively. The fungi genus *Colletotrichum* did not develop at 35 °C.

Oliveira *et al*. (2011) evaluated the influence of various thermal treatments on fungi control in *Eugenia brasiliensis, Eugenia pyriformis* and *Eugenia uniflora* seeds, and observed the effect of temperature on the incidence of fungi in seeds. Various water temperatures were used, and various periods of time in which the seeds remained under these conditions. The authors demonstrated that the treatments tested modified the development of fungi genera *Cladosporium*, *Penicillium*, *Pestalotiopsis*, and *Alternaria* present in the seeds.

4 Conclusion

Considering the conditions under which our study was carried out, it is possible to point out:

The temperature of 25 °C is adequate for *E. involucrata* seed germination. The different levels of maturation evaluated do not affect the percentage of germinated seeds, however, influence their vigor. Seeds removed from yellow fruits with red spots and from fruits collected on the ground exhibited good germination and faster seedling development and may be used to the propagation of *E. involucrata*. Nevertheless, it is important to mention that the seeds removed from fruits collected on the ground should be inspected visually at the time of processing, so that it is possible to remove damaged seeds.

The influence of temperature on the fungi development in the seeds of *E. involucrata* can be clearly observed. However, the effective temperature for decreasing the fungi development inhibited the germination of the seeds, making it impossible to use this method as an auxiliary technique to control the health of the seeds. In addition, further studies should be carried out to identify which fungi genus are harmful and which ones are beneficial or neutral to the seed germination. The effect of the temperatures lower than 35 °C, where the seeds germinate and hinder the harmful fungi development may be investigated.

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PS2 SD P02

INFLUENCE OF ROOTSTOCKS ON NUTRITIONAL COMPOSITION OF PEAR FRUITS

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INTRODUCTION

Pear is a fruit of great importance for human nutrition due to the organoleptic characteristics and nutritional composition. Its cultivation in Uruguay generally uses rootstocks for adaptation to the soil, productiveness and precociousness, but rootstock selection may also influence fruit quality, with the ability to change its chemical composition.

The objective of this work was to evaluate the influence of rootstocks on some physicochemical and nutritional values of pear cv. Williams, grafted on six rootstocks (EMC, BA29, OHxF333, OHxF40, OHxF69 and Adams) in Uruguayan production conditions.

MATERIALS AND METHODS

The experimental design was a randomized complete block design with four replicates located in an orchard at INIA-Las Brujas Experimental Station. At harvest, twenty fruits were sampled per replicate, conditioned in a cold chamber and then at room temperature until consumer maturation conditions. Soluble solids content (SS), titatrable acidity (TA), color and texture were evaluated and then fruit peel and pulp were separated for determination of ascorbic acid (Vitamin C) and minerals contents.

The data collected were submitted to analysis of variance (ANOVA) and when significant, were compared by the Tukey test at 5% probability, by the statistical program InfoStat.

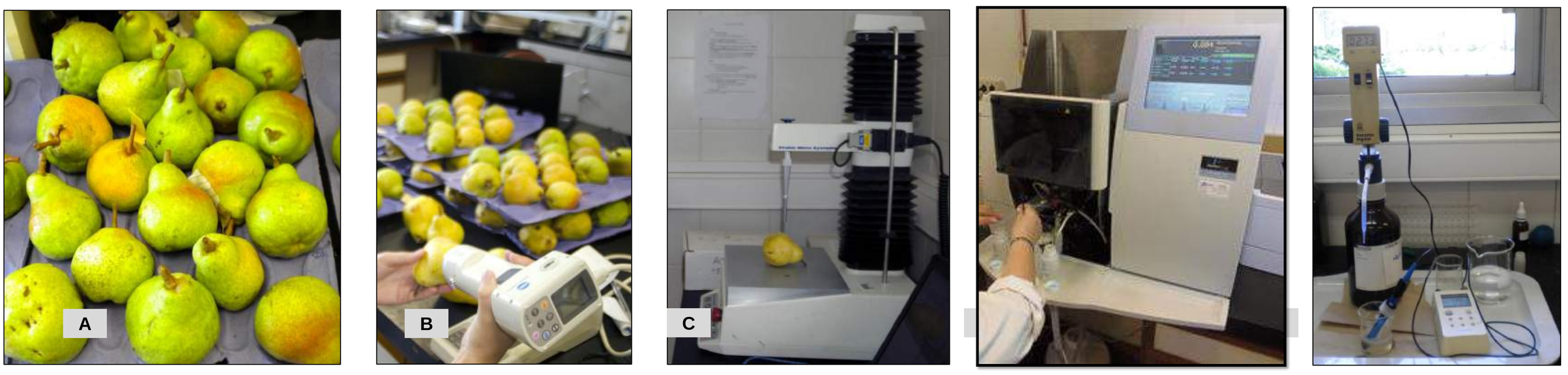

Figure 1: Pear fruits at temperature of 20ºC (A), color analysis (B), texture (C), minerals (D) and titratable acidity (E) determinations.

RESULTS AND DISCUSSION

The results obtained are expressed in Tables 1, 2 and 3. As a results, in relation to the soluble solids, titratable acidity, color and texture, no differences were found between the rootstocks. For ascorbic acid content, no significant differences between rootstocks were found, but the concentration was higher in the fruit peel than pulp. The highest phosphorus content was found in the pulp, but calcium and magnesium contents were higher in the peel. The OHXF 40 and OHXF 69 rootstocks provided higher phosphorus and zinc contents and the boron content was higher in fruits of plants grafted on OHXF 40 rootstock.

Table 1: Soluble solids content (ºBrix), titratable acidity (g malic acid per 100 mL), color and texture (hardness lb and adhesiviness lb.sec) in pear fruits grown with different rootstocks.

Rootstock	Soluble solids	Titratable acidity	Color			Texture	
			L*	a*	b*	Hardness	Adhesiveness
EMC	13,1 a*	0,36 a	68,0 a	-4,4 a	46,9 a	3,71 a	0,13 a
OHXF333	13,6 a	0,36 a	69,7 a	-3,3 a	48,7 a	3,38 a	0,17 a
OHXF 69	12,7 a	0,35 a	67,0 a	-3,4 a	45,4 a	3,70 a	0,17 a
BA 29	12,9 a	0,34 a	67,7 a	-3,2 a	46,6 a	4,57 a	0,17 a
Adams	12,4 a	0,33 a	67,2 a	-2,9 a	46,6 a	4,14 a	0,14 a
OHXF 40	12,0 a	0,32 a	66,5 a	-3,5 a	45,9 a	4,63 a	0,19 a
CV	5,75	6,84	2,32	36,22	2,57	20,85	26,50

*A verages followed by the same letter in column, do not differ from each other by the Tukey test at 5% probability.

Table 2: Effect of rootstock on mineral content (P, Ca, Mg and B expressed in%, Zn in mg kg of fruit) and vitamin C (mg ascorbic acid per 100 g of fruit) on Williams pear fruits on average concentration in the shell and pulp.

Rootstok	Minerals					Vitamin C
	P	Ca	Mg	Zn	B	
EMC	0,08 b*	0,13 a	0,05 a	10,63 b	12,75 bc	4,91 a
OHXF 333	0,09 b	0,10 a	0,04 a	9,00 b	13,50 b	5,31 a
OHXF 69	0,10 a	0,14 a	0,05 a	13,63 a	13,63 b	4,96 a
BA 29	0,08 b	0,18 a	0,05 a	9,63 b	12,63 bc	4,54 a
Adams	0,08 b	0,18 a	0,05 a	10,25 b	11,88 c	5,21 a
OHXF 40	0,10 a	0,18 a	0,05 a	10,75 ab	15,00 a	5,05 a
CV (%)	10,90	36,4	25,46	17,87	5,96	13,64

*A verages followed by the same letter in column, do not differ from each other by the Tukey test at 5% probability.

Table 3: Effect of the fruit part on the mineral content (P, Ca, Mg and B expressed in%, Zn in mg kg of fruit) and vitamin C (mg ascorbic acid per 100 g of fruit) on average concentration in different rootstocks.

Part of the fruit	Minerals					Vitamin C
	P	Ca	Mg	Zn	B	
Peel	0,08 b*	0,18 a	0,06 a	11,08^{ns}	13,43^{ns}	6,5 a
Pulp	0,10 a	0,12 b	0,04 b	10,21	13,03	3,5 b
CV (%)	10,9	36,4	25,46	17,87	5,96	13,64

*Averages followed by the same letter in column, do not differ from each other by the Tukey test at 5% probability.

CONCLUSIONS

Only the contents of some minerals are affected by the use of different rootstocks under Uruguayan conditions, but only when rootstocks and canopy both are *Pyrus*. Therefore, it is possible to choose rootstocks that are more convenient in terms of productivity but which also provide a higher index of minerals to the fruits.

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POSTHARVEST TECHNOLOGY
Article

Treatment with nitric oxide preserves the quality of cold stored 'Cripps Pink' apples

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ABSTRACT: The objective of this work was to evaluate the effect of pre-storage fruit treatment with nitric oxide (NO) on the quality maintenance of cold stored 'Cripps Pink' apples. The following treatments were evaluated: 0 $\mu L \cdot L^{-1}$ of NO (control); 10 $\mu L \cdot L^{-1}$ of NO for 2 h; 10 $\mu L \cdot L^{-1}$ of NO for 4 h; 10 $\mu L \cdot L^{-1}$ of NO for 8 h; and 20 $\mu L \cdot L^{-1}$ of NO for 2 h. Fruit treated with NO, regardless of concentration and application time, had a greener skin background color than the control, especially those treated with 10 $\mu L \cdot L^{-1}$ for 8 h. After 7 days of shelf life (23 ± 3 °C/relative humidity, RH, of 65 ± 5%), fruit treated with 10 $\mu L \cdot L^{-1}$ for 8 h and 20 $\mu L \cdot L^{-1}$ for 2 h had lower respiration rate and a greener skin background color than the control. Fruit treated with NO, regardless of concentration and application time, had a lower incidence of flesh browning after storage. The use of NO (10 $\mu L \cdot L^{-1}$ applied for 8 h and 20 $\mu L \cdot L^{-1}$ applied for 2 h) pre-storage maintains the quality of 'Cripps Pink' apples, because it delays skin yellowing and reduces flesh browning. Nitric oxide has no effect on the maintenance of flesh firmness in 'Cripps Pink' apples.

Key words: *Malus domestica*; Pink Lady; physiological disorder; flesh browning; skin background color.

INTRODUCTION

'Cripps Pink' apple is one of three varieties that can be marketed as Pink Lady. Such fruits must meet a minimum standard of quality, which includes having more than 40% (in Asia > 40% and < 60%) of the surface covered with a strong pink to shiny red color, flesh firmness of at least 6.5 $kg \cdot cm^{-2}$ (± 64 N), soluble solids (SS) of at least 13 °Bx, and, on average, only 1% of fruits with internal defects (Pink Lady 2020).

'Cripps Pink' apples produced in South America are exported as Pink Lady to various countries on many continents, and can take, from harvest, up to 70 days to arrive at their destination. During this period, the apples are kept under refrigeration, during which fruit ripening and loss of quality, reduction of flesh firmness, skin yellowing and development of skin greasiness and flesh browning can occur. The apples are picked at the limit of the flesh firmness desirable, according to the quality standards, especially in orchards covered by an anti-hail net, where the development of the required red coloration is delayed. It is well established that flesh firmness at harvest is strongly correlated with storage potential, since fruits harvested with a lower flesh firmness tend to have less storage potential. So, it is essential to identify technologies complementary to refrigeration to allow a greater delay in ripening and to maintain quality for as long as possible.

Fruit ripening involves alterations initiated by ethylene. Thus, controlling ethylene during storage, transport and commercialization is essential (Keller et al. 2013). Some consolidated technologies are available for this purpose, for example using ethylene absorbers (Keller et al. 2013) and fruit treatment with 1-MCP (Williamson et al. 2018).

Ethylene absorption is mainly carried out using products based on potassium permanganate impregnated in materials, normally a mineral, of high surface area. The main disadvantages of this technology are related to the regenerative nature and the significant generation of by-product (residue), as well as the larger amount of product needed to effectively keep a low concentration of ethylene in the environment (Keller et al. 2013).

The 1-MCP treatment has shown excellent results in the maintenance of apple quality in cold storage (Amarante et al. 2010; Hackbarth et al. 2017; Williamson et al. 2018). However, this technology is expensive and has several disadvantages, such as its negative impact on the aroma of the fruit (Thewes et al. 2015) and is not effective on control postharvest fruit decay.

Nitric oxide (NO) is a reactive nitrogen species that is naturally produced in live cells and is involved in the regulation of many processes during plant development (Kolbert et al. 2019; Manjunatha et al. 2010; Palma et al. 2019). The exogenous application of NO, at low concentrations, delays the maturation process (Abdollahi et al. 2013; Mukherjee 2019). Its effect on maturation and senescence can be attributed, at least in parts, to the reduction of the synthesis of ethylene through the inhibition of the enzymes acid 1-aminocyclopropane-1-carboxylic (ACC) synthase and ACC oxidase (Manjunatha et al. 2012; Palma et al. 2019). Nitric oxide can also help maintain the postharvest quality of fruits through the control of oxidative stress via induction of enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and the suppression of lipoxygenase (LOX) (Manjunatha et al. 2010; 2012; Palma et al. 2019), being able to prevent the manifestation of physiological disturbances. However, this effect of NO is dependent of its concentration and the species of fruits (Manjunatha et al. 2010; Palma et al. 2019). Moreover, NO reduces the incidence of postharvest rot (Abdollahi et al. 2013; Palma et al. 2019). Nitric oxide gas treatment could be an option for maintenance the quality of 'Cripps Pink' (or Pink Lady) apples during storage and transport to distant markets.

The objective of this study was thus to evaluate the effect of treatment with NO (dose and application time) on the maintenance of the quality of cold stored 'Cripps Pink' apples.

METHODS

The experiment was carried out with 'Cripps Pink' apples harvested in 2018/2019, from a commercial orchard located in the municipality of Vacaria, RS (50°42" W; 28°33' S; 955 m of altitude), southern Brazil. The treatments evaluated were of 0 $\mu L \cdot L^{-1}$ of NO (control); 10 $\mu L \cdot L^{-1}$ of NO for 2 h; 10 $\mu L \cdot L^{-1}$ of NO for 4 h; 10 $\mu L \cdot L^{-1}$ of NO for 8 h; and 20 $\mu L \cdot L^{-1}$ of NO for 2 h. These NO concentrations were chosen because of results obtained in other studies with various types of fruits. The highest concentration was applied only during 2 h because, in theory, a higher concentration requires a shorter treatment time, and it was not possible to test the combination of all factors due to the limited amount of fruit and infrastructure available for this study.

For the application of NO, gas from a high-pressure cylinder was used (nitric oxide 1000 $\mu L \cdot L^{-1}$ + N^2 balance). Nitric oxide was added to the interior of air-tight chambers with a volume of 450 L, until it reached the concentrations required for each treatment (10 and 20 $\mu L \cdot L^{-1}$), with the fruits being exposed to the treatment, for the required time in an environment with 1 kPa of O_2 + ≤ 0.1 kPa of CO_2. The treatments were done in this environment (with low O_2) to prevent the reaction of NO with O_2 (forming NO_2), and thus guaranteeing its effectiveness (Liu 2016).

After treatment, the apples were kept for 70 days under cold storage (1.5 ± 0.2 °C and relative humidity, RH, of 94 ± 2%), simulating the period of transport for 'Cripps Pink' apple for exportation, followed by 7 days of shelf life (23 ± 3 °C and Relative Humidity of 65 ± 5%).

At harvest, the apples were evaluated for skin background color, percentage of red color, flesh firmness, titratable acidity (TA), SS and iodine-starch index. After storage, at the time of chamber opening, ethylene production and respiration rates, skin background color, greasiness and incidence of rot and cracks were evaluated. After 7 days of shelf life, in addition to the same attributes evaluated at the time of chamber opening, the variables flesh firmness, TA, SS and flesh browning incidence were evaluated.

To quantify ethylene production (ηmol $C_2H_4 \cdot kg^{-1} \cdot s^{-1}$) and respiration (ηmol $CO \cdot kg^{-1} \cdot s^{-1}$) rates, the apples in each replicate were packed in 4.1 L containers, with an air-tight seal, according to Brackmann et al. (2017). After 30 min, a plastic syringe of 1.0 mL was used to collect three samples of air from the atmosphere of the free space from these containers. These samples were then injected into a gas chromatographer (Clarus 580, Perkin Elmer, USA), equipped with a Porapak N column of 3 m length (80–100 mesh) and a flame ionization detector. The temperatures of the column, the detector and the injector were 70, 250 and 130 °C, respectively. The flows of nitrogen, hydrogen and synthetic air were 70, 30 and 300 $mL \cdot min^{-1}$, respectively. The respiration rate was quantified using an electronic gas analyzer (O_2 and CO_2) (Schelle, Germany), with the circulation of CO_2 in a closed circuit.

The iodine-starch index (scale of 1–5, where index 1 indicates the maximum starch content, and index 5 represents totally hydrolyzed starch) was determined by cutting the fruit through the equatorial region, and immersing the cut surface of the stem half in iodine solution (12 g metallic iodine + 24 g potassium iodine/1 L of distilled water) (Amarante et al. 2010).

The flesh firmness (N) was determined in two opposing regions, in the equatorial portion of the fruit, with a small portion of the skin previously removed, with the aid of a motorized penetrometer (Güss Manufacturing Ltd., South Africa), with an 11 mm diameter tip (Amarante et al. 2010).

The percentage of red coloring on the fruit was determined visually by examining the fruit surface, estimating the total area of the fruit that was covered by red color (Amarante et al. 2009). The background color of the skin was measured in terms of lightness (L), chroma (C) and angle hue (h°), in an area with the least red coloration, with the aid of a colorimeter (CR 400, Konica Minolta, Japan) (Brackmann et al. 2017).

The TA (% of malic acid) was determined in a sample of 5 mL of juice extracted from transverse slices taken from the equatorial region of the apples and shredded in an electric centrifuge. This sample was diluted in 45 mL of distilled water and titrated with an automatic titrator (Titro Line Easy, Schott Instruments, Germany), with a sodium hydroxide solution of 0.1 N until pH 8.1 (Amarante et al. 2010).

The SS content (%) was determined with a digital refractometer (PR 201α, Atago, Japan), using the juice extracted from the fruit as mentioned for TA (Amarante et al. 2010). The SS/TA ratio was then calculated.

The incidence (%) of rot, cracks and flesh browning was evaluated by counting the number of apples with these symptoms (Brackmann et al. 2017). To evaluate flesh browning, the apples were cut transversely in the equatorial region and analyzed for the presence of a diffuse brown color in the flesh.

The rate of skin greasiness was evaluated using methodology described by Yang et al. (2017), subjectively by rubbing fruit against the hand and scoring the degree of greasiness as none (0), slight (1), moderate (2), or severe (3).

The experimental design was entirely randomized. Each treatment was composed of five replicates, with each experimental unit composed of 30 fruits. The values in % were transformed by the formula arcsine $[(x+0.5)/100]^{1/2}$ prior to analysis. The data was submitted to variance analysis and the averages of the treatments were compared by LSD test ($p < 0.05$).

RESULTS AND DISCUSSION

At harvest the fruit presented the following attributes: flesh firmness of 82.9 N, TA of 0.48%, SS of 12.4%, iodine-starch index of 4.0, 55% of the surface of the fruit with a strong pink to red color, and the h°, C and L of background color of the skin were 103.7, 42.6 and 73.5, respectively.

At the time chamber opening, after 70 days of storage, the fruit treated with 10 $\mu L \cdot L^{-1}$ of NO for 4 h presented the lowest ethylene production rate, but without differing significantly from the 10 $\mu L \cdot L^{-1}$ of NO for 8 h treatment. However, after 7 days of shelf life, the ethylene production rate did not differ between treatments (Table 1). The effect of NO on ethylene biosynthesis is due to the stoichiometric reduction of ACC to 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC) and to the formation of a stable ternary complex "ACC — ACC oxidase — NO", which hinders the oxidation of ACC to ethylene (Manjunatha et al. 2012). In 'Galaxy' apples there was no effect of NO on the ethylene production after storage in controlled atmosphere (CA) and plus 7 days of shelf life (Brackmann et al. 2017), as in most of the treatments evaluated in the current study.

Table 1. Ethylene production (ηmol $C_2H_4 \cdot kg^{-1} \cdot s^{-1}$) and respiration (ηmol $CO \cdot kg^{-1} \cdot s^{-1}$) rates of 'Cripps Pink' apples stored during 70 days (1.5 ± 0.2 °C and RH of 92 ± 4%), at the time of chamber opening, and plus 7 days of shelf life (23 ± 3 °C and RH of 65 ± 5%), in function of the nitric oxide application (concentration/application time) at the beginning of the storage.

Nitric oxide (concentration/ application time)	Ethylene production rate	Respiration rate	Ethylene production rate	Respiration rate
	Time of chamber opening		7 days of shelf life	
0 $\mu L \cdot L^{-1}$	1.54a	160.4a	3.30a	114.4a
10 $\mu L \cdot L^{-1}$/2 h	1.89a	144.8ab	3.16a	124.7a
10 $\mu L \cdot L^{-1}$/4 h	1.14b	132.4b	3.08a	118.1a
10 $\mu L \cdot L^{-1}$/8 h	1.47ab	160.1a	3.43a	95.9b
20 $\mu L \cdot L^{-1}$/2 h	1.56a	144.5ab	3.00a	97.3b
CV (%)	15.2	10.0	10.8	16.1

Note. *Average followed by different letters in the columns differ between themselves by the test LSD ($p < 0.05$). CV: coefficient of variation.

The respiration rate measured at the time of chamber opening was lower for fruit treated with 10 $\mu L \cdot L^{-1}$ of NO for 4 h than fruit control and those fruit treated with 10 $\mu L \cdot L^{-1}$ of NO for 8 h, but did not differ from that fruit treated with 10 and 20 $\mu L \cdot L^{-1}$ of NO for 2 h. After plus 7 days of shelf life, respiration rate was lower in fruit from the 10 $\mu L \cdot L^{-1}$ of NO for 8 h and 20 $\mu L \cdot L^{-1}$ of NO for 2 h than other treatments (Table 1). Other studies observed that treatment with NO reduces the respiration rate of 'Galaxy' apples (Brackmann et al. 2017) and plums (Singh et al. 2009). Nitric oxide reduces cellular respiration through the reversible inhibition of cytochrome oxidase (Pandey et al. 2019).

The values of L and C for the background color of the skin did not differ between treatments. However, the h° value at chamber opening was higher in fruit treated with NO than fruit control, independent of the concentration and treatment time. After plus 7 days of shelf life, apples treated with 10 $\mu L \cdot L^{-1}$ of NO for 8 h and 20 $\mu L \cdot L^{-1}$ of NO for 2 h had a higher h° value than control and 10 $\mu L \cdot L^{-1}$ of NO for 2 h treatments (Table 2). A higher h° value in the apple skin represents less yellow coloration, which indicates a lower degree of matureness, since the skin color is a trustworthy indicator of fruit maturation (Saure 1987). In 'Packhams Triumph' pears, a lower degree of yellowing of the skin was observed following the application of NO (Hendges et al. 2016). The lower h° value observed in the fruit control group after 70 days of storage and plus 7 days of shelf life visually indicated more mature fruit. The greener skin background color in NO-treated fruit can be attributed to a higher chlorophyll content. In broccoli, NO delayed the loss of chlorophyll a (Eum et al. 2009). Nitric oxide could be a protective molecule, preserving the chloroplast membrane against the toxicity of reactive oxygen species (Lazalt et al. 1997).

Table 2. Attributes of skin color of 'Cripps Pink' apples stored during 70 days (1.5 ± 0.2 °C and RH of 92 ± 4%), at the time of chamber opening, and plus 7 days of shelf life (23 ± 3 °C and RH of 65 ± 5%) in function of the nitric oxide application (concentration/application time) at the beginning of the storage.

Nitric oxide (concentration/ application time)	L	C	h°	L	C	h°
	Time of chamber opening			7 days of shelf life		
0 $\mu L \cdot L^{-1}$	73.9a	39.9a	91.8c	71.7a	42.0a	84.3c
10 $\mu L \cdot L^{-1}$/2 h	74.7a	41.1a	96.9ab	73.5a	42.9a	86.5bc
10 $\mu L \cdot L^{-1}$/4 h	73.2a	40.1a	97.1ab	72.0a	42.5a	89.5ab
10 $\mu L \cdot L^{-1}$/8 h	75.1a	41.5a	99.6a	72.8a	43.2a	90.4a
20 $\mu L \cdot L^{-1}$/2 h	75.4a	40.9a	95.5b	73.1a	43.7a	90.0a
CV (%)	2.5	5.5	2.4	2.5	2.6	2.6

Note. *Averages followed by different letters in the columns differ between themselves by the LSD test ($p < 0.05$). CV: coefficient of variation.

There was no difference between treatments in flesh firmness, after 70 days of storage plus 7 days of shelf life (Table 3). In 'Galaxy' apples there was also no observed effect of the application of NO on the maintenance of flesh firmness after storage (Brackmann et al. 2017). However, in 'Feicheng' peaches (Sun et al. 2011; Zhu et al. 2006), 'Amber Jewel' plums (Singh et al. 2009) and 'Packhams Triumph' pears (Hendges et al. 2016), NO treatment led to higher flesh firmness after storage. This divergence of results between studies could be due to the different species of fruit, as well as to the experimental procedures adopted. The results show that NO is not effective at maintaining the flesh firmness of 'Cripps Pink' apples.

Table 3. Flesh firmness, titratable acidity (TA), soluble solids (SS), SS/TA ratio and flesh browning incidence in 'Cripps Pink' apple stored during 70 days (1.5 ± 0.2 °C and RH of 92 ± 4%) plus 7 days of shelf life (23 ± 3 °C and RH of 65 ± 5%), in function of the nitric oxide application (concentration/application time) at the beginning of the storage.

Nitric oxide (concentration/ application time)	Flesh firmness (n)	TA (%)	SS (%)	SS/TA ratio	Flesh browning (%)
0 $\mu L \cdot L^{-1}$	66.3a	0.454a	15.0a	33.1b	62.1a
10 $\mu L \cdot L^{-1}$/2 h	66.5a	0.432ab	14.6ab	33.9ab	10.1b
10 $\mu L \cdot L^{-1}$/4 h	66.1a	0.445a	14.9ab	33.4ab	6.2b
10 $\mu L \cdot L^{-1}$/8 h	66.3a	0.414b	14.5ab	35.0a	13.8b
20 $\mu L \cdot L^{-1}$/2 h	64.4a	0.426ab	14.3b	33.6ab	11.6b
CV (%)	4.6	4.4	3.2	3.7	48.7

Note. *Averages followed by different letters in the columns differ between themselves by the LSD test ($p < 0.05$). CV: coefficient of variation.

TA was higher in fruit from the control group and in those treated with 10 $\mu L \cdot L^{-1}$ of NO for 4 h, but did not differ between fruit treated with 10 and 20 $\mu L \cdot L^{-1}$ of NO for 2 h (Table 3). In 'Amber Jewel' plums and longans (Duan et al. 2007) NO positively affected on maintenance of TA (Singh et al. 2009). However, in the present study there was no observed positive effect of NO on the maintenance of TA, as also observed in 'Galaxy' apples (Brackmann et al. 2017). Soluble solids were lower in the fruit treated with 20 $\mu L \cdot L^{-1}$ of NO for 2 h than fruit control (Table 3). However, all the treatments resulted in fruits with rates of SS above the minimum limit desired (13%) and, therefore, 'Cripps Pink' apples can be commercialized as 'Pink Lady' (Pink Lady 2020). The SS/TA ratio was higher, when compared to the control, in apples treated with 10 $\mu L \cdot L^{-1}$ of NO for 8 h (Table 3). This result can be explained mainly by the values of TA, which were lower apples treated with 10 $\mu L \cdot L^{-1}$ of NO for 8 h and higher in the control. In line with the quality standards required for 'Pink Lady' (Pink Lady 2020), SS is more relevant than TA, because 'Cripps Pink' apple has an elevated TA, which is maintained during storage (Table 3). Therefore, 'Cripps Pink' apples with an elevated SS/TA ratio can be considered more desirable.

All treatments with NO, independent of the concentration and the duration of exposure, reduced flesh browning after 70 days of cold storage plus 7 days of shelf life (Table 3). Flesh browning is due to oxidative stress that cause damage to cellular membranes and subsequent loss of cellular compartmentalization. Studies have shown that NO can reduce oxidative stress by inducing enzymes such as SOD, POD and CAT, and suppressing LOX (Manjunatha et al. 2010; 2012). Pristijono et al. (2006) reported the reduction of flesh browning with the application of NO in minimally processed 'Granny Smith', 'Royal Gala', 'Golden Delicious', 'Sundowner', 'Fuji' and 'Red Delicious' apples. The authors suggested that NO modulates the oxidative activity of the enzyme polyphenol oxidase and other oxidative enzymes, minimizing the browning reactions.

There were no differences between treatments in incidences of rot and fruit cracking or the skin greasiness (Table 4).

The results demonstrate that treatment with NO can help maintain the quality of 'Cripps Pink' apples after harvest, mainly because of the reduction in yellowing of the skin and flesh browning incidence, as well as the higher SS/TA ratio. The effects were most obvious with the lowest concentration applied for the longer application time (10 $\mu L \cdot L^{-1}$ of NO for 8 h) and with the highest dose evaluated (20 $\mu L \cdot L^{-1}$ of NO for 2 h) (Tables 2 and 3). Some studies have demonstrated the positive effect of NO with a combination of lower concentrations of NO and shorter application, than shown in the present study, for example in 'Amber Jewel' plums with 10 $\mu L \cdot L^{-1}$ for 2 h (Singh et al. 2009), 'Feicheng' peaches with 10 $\mu L \cdot L^{-1}$ for

1 h (Sun et al. 2011), and 'Pajero' strawberry with 10 $\mu L \cdot L^{-1}$ for 2 h (Soegiarto and Wills 2006). These differences can be attributed to the rate of absorption of NO, which varies according to the product (Manjunatha et al. 2010).

Table 4. Rot and fruit cracking incidences and skin greasiness in 'Cripps Pink' apple stored during 70 days (1.5 ± 0.2 °C and RH of 92 ± 4%), at the time of chamber opening (CO), and plus 7 days (7D) of shelf life (23 ± 3 °C and RH of 65 ± 5%), in function of the nitric oxide application (concentration/application time) at the beginning of the storage.

Nitric oxide (concentration/ application time)	Rot incidence (%)		Fruit cracking incidence (%)		Skin greasiness (1–3)	
	CO	7D	CO	7D	CO	7D
0 $\mu L \cdot L^{-1}$	2.5a[s]	2.5a	0.0a	0.0a	1.9a	2.0a
10 $\mu L \cdot L^{-1}$/2 h	0.0a	2.5a	1.3a	1.3a	2.1a	2.2a
10 $\mu L \cdot L^{-1}$/4 h	5.0a	5.0a	0.0a	0.0a	2.2a	2.3a
10 $\mu L \cdot L^{-1}$/8 h	0.0a	0.0a	0.0a	0.0a	2.2a	2.2a
20 $\mu L \cdot L^{-1}$/2 h	1.3a	1.3a	1.3a	1.3a	2.1a	2.3a
CV (%)	65.1	46.1	55.6	55.6	17.4	14.6

Note. *Averages followed by different letters in the columns differ between themselves by the LSD test ($p < 0.05$). CO: time of chamber opening. 7D: 7 days of shelf life. CV: coefficient of variation.

Nitric oxide was applied at low O_2 (1 kPa), as recommended by Liu (2016). However, according to Soegiarto and Wills (2004), short-term treatment with low concentrations of NO is able to extend the postharvest life, because NO at low concentration, such as those used in this work, has a relatively slow rate of degradation in air. For example, Soegiarto et al. (2003) found that the degradation of 30 $\mu L \cdot L^{-1}$ NO in air was much lower than expected with a retention of two-thirds of the original NO after 2 h of treatment. It is possible that 20 $\mu L \cdot L^{-1}$ NO for 2 h can be applied in air with positive results in relation to maintaining quality.

CONCLUSION

The use of NO (10 $\mu L \cdot L^{-1}$ applied for 8 h and 20 $\mu L \cdot L^{-1}$ applied for 2 h) pre-storage maintains the quality of 'Cripps Pink' apples, because it delays skin yellowing and reduces flesh browning.

Nitric oxide has no effect on the maintenance of flesh firmness in 'Cripps Pink' apples.

AUTHORS' CONTRIBUTION

Conceptualization: Steffens C. A.; **Methodology:** Steffens C. A., Miqueloto T., Fernandes R. C., Demari C. K. P., Anami J. M. and Lugaresi A.; **Investigation:** Steffens C. A., Miqueloto T., Fernandes R. C., Demari C. K. P., Anami J. M. and Lugaresi A.; **Writing – Original Draft:** Steffens CA., Miqueloto T., Fernandes R. C., Demari C. K. P., Anami J. M., Lugaresi A. and Amarante C. V. T.; **Writing – Review and Editing:** Steffens CA., Miqueloto T., Fernandes R. C., Demari C. K. P., Anami J. M., Lugaresi A. and Amarante C. V. T.; **Funding Acquisition:** Steffens C. A. and Amarante C. V. T.; **Supervision:** Steffens C. A.

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Data will be available upon request.

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ORIGINAL ARTICLE

Genetic diversity of the genus *Prunus* based on per se evaluation of peach clonal rootstocks

Diversidade genética do gênero Prunus *com base na avaliação per se de porta-enxertos clonais de pessegueiro*

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Enxertia

ABSTRACT: The multivariate analyses application and genetic divergence quantification can provide parameters that aid the selection of superior rootstocks. The objective of this study was to evaluate the technical feasibility of using the genetic diversity of the genus *Prunus* as a clonal rootstock of the peach cultivar 'BRS-Libra'. The experimental orchard used was part of a national network for evaluation of rootstocks for prunaceous trees, under the general coordination of *Embrapa Clima Temperado*. The design was randomized and data were collected during the 2016/2017 production cycle. Physical analyses (trunk section area, average fruit mass), chemical analysis (soluble solids), and quantification of estimated productivity of the plants were performed. The data were subjected to the Shapiro-Wilk normality test at a 5% probability level, after which the grouping of the rootstocks was performed using the Unweighted Pair Group Method Using an Arithmetic Average (UPGMA) hierarchical method. The grouping resulted in the formation of five groups. The cultivars of group I, Mirabolano 29C and Marianna 2624, showed graft incompatibility with the evaluated cultivar scion. Similarly, cultivars of group II showed characteristic symptoms of graft incompatibility, resulting in poor development. Group III and IV were composed of cultivars that showed low and medium vigor, with good characteristics for use in orchards with high density, while the cultivars of the group V stood out because they showed high vigor, recommended for orchards with low density. The use of various rootstocks influenced the behavior, vigor and fruit production of the BRS Libra cultivar, with genetic divergence.

RESUMO: *A aplicação de análises multivariadas e quantificação da divergência genética fornecem parâmetros que favorecem a seleção de porta-enxertos superiores. O objetivo deste trabalho foi avaliar a viabilidade técnica do uso de parte da diversidade genética do gênero* Prunus *como porta-enxerto clonal do pessegueiro 'BRS-Libra'. O pomar experimental faz parte de uma rede nacional de avaliação de porta-enxertos para prunáceas, sob a coordenação geral da Embrapa Clima Temperado. O delineamento experimental foi em blocos ao acaso e a coleta de dados ocorreu no ciclo produtivo 2016/2017. Foram realizadas análises físicas (área de secção do tronco, massa média de frutos) e química (sólidos solúveis), além da quantificação da produtividade estimada das plantas. Os dados obtidos foram submetidos ao teste de normalidade de Shapiro-Wilk, ao nível 5% de probabilidade, sendo posteriormente realizado o agrupamento dos porta-enxertos através do método hierárquico UPGMA. A realização do agrupamento resultou na formação de cinco grupos. As cultivares do grupo I, Mirabolano 29C e Marianna 2624, apresentaram incompatibilidade de enxertia com a cultivar BRS-Libra. Semelhantemente, as cultivares do grupo II apresentaram sintomas característicos de incompatibilidade de enxertia, resultando em pouco desenvolvimento. Os grupos III e IV são compostos por cultivares que apresentaram baixo e médio vigor, com boas perspectivas de uso para formar pomares em alta densidade, enquanto as cultivares do grupo V destacaram-se por apresentar alto vigor, recomendadas para pomares de baixa densidade. O uso de diferentes porta-enxertos influencia no comportamento da cultivar BRS-Libra no vigor e produção de frutos, havendo divergência genética entre eles.*

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1 Introduction

Peach cultivation has been prominent during the last decade and is now considered the third largest temperate fruit crop in the world, after apple and pear (Roknulazam et al., 2019). However, in Brazil, production of the genus *Prunus* remains low. Rio Grande do Sul state is the largest producer of peaches in Brazil. However, in 2014, Rio Grande do Sul productivity achieved 9.78 t ha^{-1}, being below the world average (14.26 t ha^{-1}) (Mayer et al., 2019). In 2016, the Brazilian peach and nectarine production involved 17,283 hectares, with annual production of 191,855 tons. By contrast, the world's top leader, China, produced 838,768 hectares and 14,469,004 tons (64.35% greater than that of Brazil) (FOOD AND AGRICULTURE ORGANIZATION, 2018).

Among the aspects that contribute to the low Brazilian production are the use of low-quality nursery trees, with rootstock propagated by seeds, as well as the use of mixtures of industrial-grade pits that do not provide quality material for rootstock function.

Various factors directly or indirectly affect the success of peach cultivation. An important one is the selection of rootstocks that are more adapted to the conditions of environment. Each rootstock has a variable influence on the results depending to the vigor of the plant, as well as yield and physicochemical characteristics of the fruit (López-Ortega et al., 2016; Szymajda et al., 2019.).

The application of multivariate analyses and quantification of genetic divergence can provide parameters that aid the selection of superior rootstocks. Several techniques of multivariate statistics, including analysis of the main components, canonical variables and hierarchical grouping methods, have been used in studies of genetic divergence (Cruz et al., 2012). However few studies, such as Bakht et al. (2013) and Menegatti et al. (2019), have been developed to quantify the genetic dissimilarity among rootstocks of the genus *Prunus.* The objective of this work was to evaluate the technical feasibility of exploiting the genetic diversity of the genus *Prunus* as clonal rootstocks for the peach cultivar BRS-Libra.

2 Materials and Methods

The experiment was conducted in a peach orchard in the Experimental Area of the Fronteira Sul Federal University, Chapecó campus. The orchard is located at latitude 27°07'06"S, longitude 52°42'20" W, altitude 605 meters. The local climate, According to the classification of Köppen, the climate of the region is classified as C, subtype Cfa (Subtropical climate), with cold, humid winters and moderate, dry summers. The soil is classified as Rhodic Hapludox.

The orchard is part of a national network for evaluation of rootstocks of prunaceous trees, under the general coordination of *Embrapa Clima Temperado*. The planting occurred in 2014 with a spacing of 5 m between rows and 2 m between plants (5 m × 2 m), totaling 1,000 plants ha^{-1}. The cultivar scion was BRS-Libra, grafted on 24 different cultivars of clonal rootstocks (Table 1), propagated by herbaceous cuttings. As a control, we used own-rooted nursery trees (without rootstock) from cv. BRS-Libra. The nursery trees were originated from *Embrapa Clima Temperado* live germplasm bank, Rio Grande do Sul, Brazil.

Table 1. Identification and species of the 24 rootstocks used for peach cv. BRS-Libra with control (self-rooted plants)
Tabela 1. Identificação e espécies de 24 porta-enxertos sob a cultivar copa BRS-Libra, com testemunha (plantas autoenraizadas)

Treatments	Specie	Origin
Tsukuba-2	*Prunus persica*	Japan
Genovesa	*Prunus salicina*	Pelotas-RS, Brazil
Clone 15	*Prunus mume*	Jaboticabal-SP, Brazil
Nemared	*Prunus persica*	California, The United States
Tsukuba-1	*Prunus persica*	Japan
Barrier	*Prunus persica* × *Prunus davidiana*	Italy
Ishtara	*(Prunus cerasifera* × *Prunus salicina)* × *(Prunus cerasifera* × *Prunus persica)*	France
Cadaman	*Prunus persica* × *Prunus davidiana*	France
Capdeboscq	*Prunus persica*	Pelotas-RS, Brazil
BRS Libra Autoenraizado	*Prunus persica*	Pelotas-RS, Brazil
De Guia	*Prunus persica*	Pelotas-RS, Brazil
Rosaflor	*Prunus persica*	Pelotas-RS, Brazil
G × N.9	*Prunus persica* × *Prunus dulcis*	Unknown
Flordaguard	*Prunus persica* × *Prunus davidiana*	Florida, The United States
Rigitano	*Prunus mume*	Jaboticabal-SP, Brazil
Tardio-01	*Prunus persica*	Pelotas-RS, Brazil
P. *mandshurica*	*Prunus mandshurica*	Pelotas-RS, Brazil

Table 1. Continuation...
Tabela 1. Continuação...

Treatments	Specie	Origin
Mirabolano 29C	*Prunus cerasifera*	Unknown
Tsukuba-3	*Prunus persica*	Japan
Okinawa	*Prunus persica*	Okinawa, Japan
Santa Rosa	*Prunus salicina*	California, The United States
México Fila 1	*Prunus persica*	Mexico
Marianna 2624	*Prunus cerasifera* × *Prunus munsoniana*	California, The United States
I-67-52-4	*Prunus persica*	The United States
GF 677	*Prunus persica* × *Prunus amygdalus*	France

The experimental design was randomized with 25 treatments (24 rootstocks + own-rooted trees control) and four replicates, each plot consisting of one plant. The orchard was arranged in six rows, using the four central rows and the two on the edges considered as borders. The plants were managed in the form of a "Y", without irrigation.

Data collection occurred during the production year 2016/17. The analyzed variables were: a) trunk section area (cm^2), measured five centimeters above and five centimeters below the grafting point with the aid of a digital caliper; b) average mass of fruits (g), obtained by random harvesting of 20 fruits of each plant, weighed with a gram digital scale with accuracy of two decimal places; c) soluble solids (°Brix), determined with juice of 10 random fruits per plant, using a digital refractometer (Instrutherm®, RTD-95); d) estimated productivity per hectare (tons ha^{-1}).

The normality of the data was verified by the Shapiro-Wilk test, at a 5% probability level. When necessary, the Box-Cox transformation was used, represented by lambda (λ). Once the mathematical assumption was met, analysis of variance (ANOVA) was performed, and means were compared using the Scott-Knott test, at 5% probability.

The similarity between the cultivars was calculated using the Jaccard index with the Vegan package (Oksanen et al., 2017) based on data collection from analyzed variables. From the similarity matrix data, the rootstocks were grouped using the hierarchical method of UPGMA (Unweighted Pair Group Method Using an Arithmetic Average). The univariate and multivariate analyses were performed using program R.

3 Results and Discussion

The results for the Shapiro-Wilk normality test are presented in Table 2. We observed that the test was not significant for the analyzed variables, suggesting that the data were normally distributed.

As seen in Table 3, the Mexico Fila 1 and I-67-52-4 treatments stood out from the others, in that they were statistically superior for four of the five variables evaluated. By contrast, the treatments 'Mirabolano 29 C' and 'Marianna 2624' had low performance for the analyzed variables.

Table 2. Shapiro-Wilk test values for normality of the data obtained from the evaluated variables

Tabela 2. Valores do teste de Shapiro-Wilk para normalidade dos dados obtidos a partir das variáveis avaliadas

Variables	W	P
TSA above the grafting point (cm2)	0.99209	0.8279*
TSA below the grafting point (cm2)	0.99207	0.8264*
Average mass of fruits (g)	0.97506	0.0747*
Soluble solids (°Brix)	0.91914	0.4036*
Estimated productivity (tons ha-1)	0.97869	0.1051*

W values indicate normality, *P* values indicate confidence level with which one can reject the null hypothesis of normality, *TSA* trunk section area, *not significant at 5% probability.

The UPGMA grouping suggested the formation of five groups, based on cutoff of 0.2, (Figure 1). We observed that group V contained most of the treatments (11) followed by group III, with six treatments, group II and IV, both with three treatments each, and finally, group I with only two of the 25 treatments evaluated in the study.

Group I, comprising 'Mirabolano 29C' and 'Marianna 2624' treatments, consisted of dead plants. The cause of the mortality of these cultivars was the incompatibility with the peach tree, manifested five months after planting the nursery trees. Both rootstocks are recommended and used for plum cultivation (*Prunus domestica*). In the present study, the incompatibility of grafting, expressed through incomplete attachment at the point of grafting between the scion and the rootstock, was also observed in the initial phase of the cultivation of the plants (five months).

In an evaluation of grafting compatibility in genus *Prunus spp.*, Rodrigues et al. (2001) associated the incompatibility of grafting of cultivars Marianna 2624 and Mirabolano 29C with the highest activity of peroxidase and concentration of phenols in relation to the peach plants grafted on these rootstocks. That contributed to a difference in lignin content among species and led to a lack of vascular connections at the point of grafting. There was also incompatibility of translocated type grafting between these rootstocks and cultivar BRS-Kampai (Neves et al., 2017; Raseira et al., 2014).

Group II consisted of *P. mandshurica*, Genovesa and Santa Rosa. The plants grafted on these rootstocks had low vegetative and reproductive development throughout the evaluated cycle. The Genovesa cultivar produced only 30% of the estimated average yield of groups III, IV and V. In addition, at the end of the cycle, all the replicates of this cultivar died, demonstrating incompatibility of grafting from the third year of cultivation.

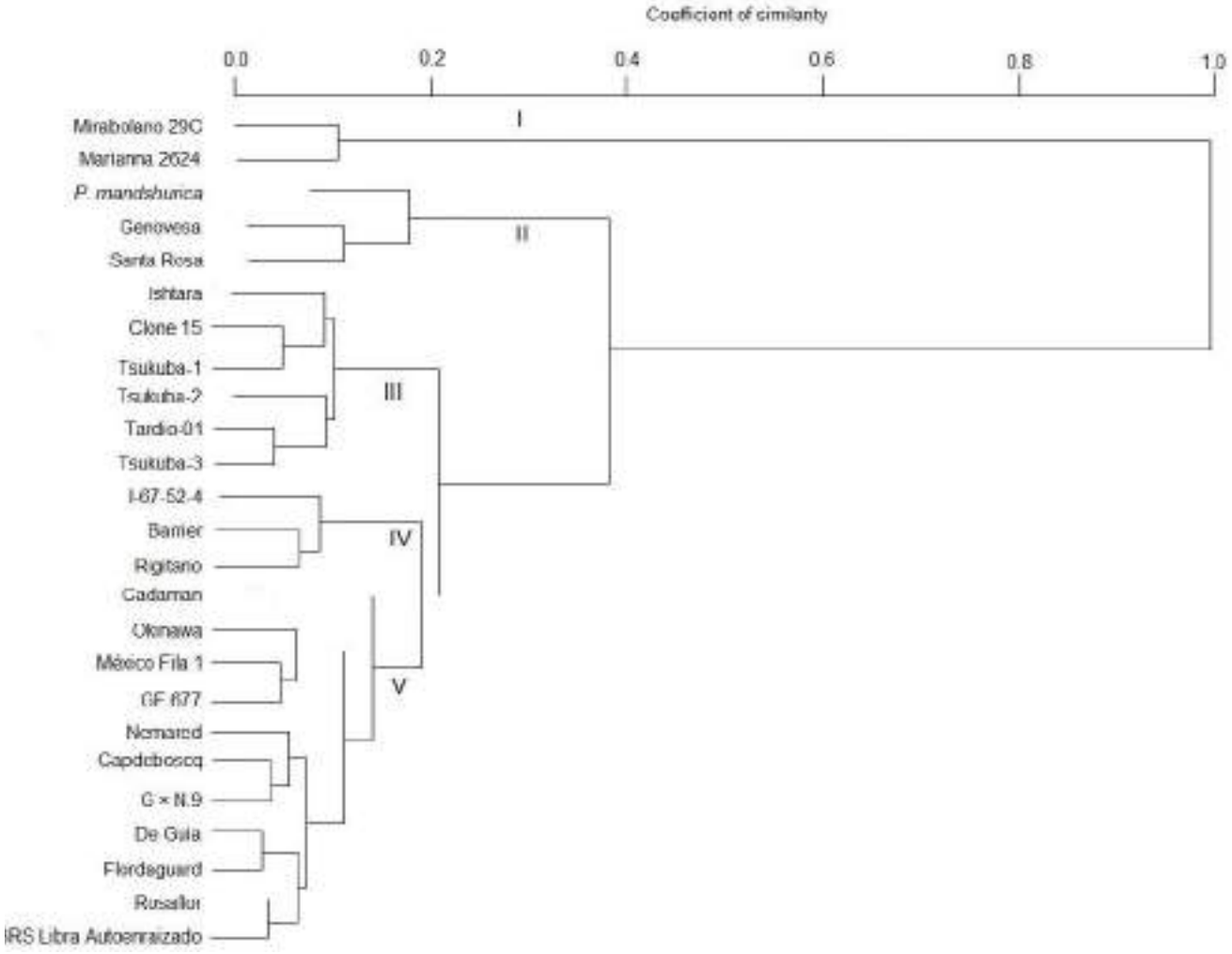
In similar fashion, the other cultivars of group II (*P. mandshurica* and Santa Rosa) showed characteristic symptoms of grafting incompatibility, similar to those observed for the Genovesa cultivar during the production cycle up to eight months after planting with leaf shrouding, necrosis and death of lateral branches and excessive thickening at the point of grafting.

Table 3. Means and standard deviations for the variables evaluated in 'BRS-Libra' peach cultivar on different rootstocks and in own-rooted plants
Tabela 3. Médias e desvio padrão para as variáveis avaliadas na cultivar de pêssego 'BRS-Libra' sob diferentes porta-enxertos e plantas autoenraizadas

Treatments	EP (tons ha^{-1})	SS (°Brix)	FM (g)	SAA (cm^2)	SBA (cm^2)
Tsukuba-2	16.74±4.20b*	9.57±0.88a	94.03±3.88b	16.95±3.20d	17.47±2.38b
Genovesa	4.70±0.64c	8.04±0.57b	48.39±2.80c	20.03±3.98c	25.66±7.07a
Clone 15	14.48±5.23b	10.07±2.34a	113.02±26.48a	20.39±2.29c	22.49±1.87b
Nemared	18.01±6.48a	8.70±0.75b	109.24±21.93a	27.97±5.16b	35.85±5.90a
Tsukuba-1	14.45±2.7b	10.10±0.67a	99.44±15.30b	20.44±2.80c	20.56±1.76b
Barrier	13.53±4.90b	9.53±0.73a	130.24±7.54a	25.24±4.15b	28.97±8.07a
Ishtara	8.70±2.51c	9.16±1.22b	111.74±32.47a	14.02±1.10d	17.25±1.73b
Cadaman	18.09±2.63a	8.70±0.53b	80.11±7.34b	24.64±2.01b	29.65±6.03a
Capdeboscq	15.78±3.30b	10.66±0.50a	106.94±9.32a	25.28±4.46b	28.44±5.46a
Autoenraizado	24.45±5.35a	8.59±0.85b	112.87±25.53a	27.75±4.83b	31.21±5.43a
De Guia	20.45±1.64a	9.05±0.97b	101.45±7.19b	30.55±4.09a	35.72±4.95a
Rosaflor	20.24±6.40a	8.39±1.57b	108.56±13.37a	27.07±3.60b	32.55±6.93a
G × N.9	14.29±3.42b	9.44±1.24a	103.75±4.36b	25.66±5.36b	32.60±8.11a
Flordaguard	19.89±5.86a	8.04±1.95b	105.59±18.40b	32.86±5.09a	36.31±3.65a
Rigitano	11.94±2.32b	10.33±0.86a	124.99±10.75a	21.51±2.09c	23.23±3.21b
Tardio-01	14.85±2.27b	9.48±0.34a	112.28±4.28a	19.02±1.44c	20.33±1.79b
P. mandshurica	5.22±1.21c	9.47±0.64a	60.23±4.48c	13.22±1.87d	16.29±2.82b
Mirabolano 29C	0.00±0.00d	0.00±0.00c	0.00±0.00d	2.82±0.58e	7.23±0.64C
Tsukuba-3	17.37±3.42a	9.46±0.70a	103.68±18.30b	17.41±1.74d	19.86±5.04b
Okinawa	14.21±3.57b	9.96±0.70a	90.30±3.25b	27.69±4.05b	33.78±5.24a
Santa Rosa	4.39±0.39c	10.85±2.09a	50.28±15.03c	19.05±4.26c	24.45±4.93b
México Fila 1	19.28±2.83a	9.90±0.95a	99.51±10.47b	32.36±3.35a	37.64±8.07a
Marianna 2624	0.00±0.00d	0.00±0.00c	0.00±0.00d	6.05±0.71e	5.12±1.34C
I-67-52-4	20.31±5.52a	9.80±1.13a	124.96±20.17a	28.54±2.22b	34.40±3.77a
GF 677	12.34±4.93b	9.03±0.23b	95.86±9.61b	21.75±4.13c	36.63±5.67a
CV (%)	16.85	8.69	13.47	10.13	9.89

EP estimated productivity, *SS* soluble solids, *FM* mean fruit mass, *SAA* trunk section area above the grafting point, *SBA* trunk section area below the point of grafting
*Equal letters in the column do not differ statistically from one another by the Scott-Knott test, 5% probability.

Figure 1. Dendrogram of genetic similarity between rootstocks of the genus Prunus, engrafted with the cultivar BRS-Libra scion, generated by the UPGMA method from the Jaccard index. Cutoff = 0.2
Figura 1. Dendrograma de similaridade genética entre porta-enxertos do gênero Prunus, enxertados sob a cultivar copa BRS Libra, gerado pelo método UPGMA a partir do índice de Jaccard. Ponto de corte = 0,2



Good graft compatibility is a requirement for selecting appropriate rootstocks for agronomic use (Emmanouilidou & Kyriacou, 2017). In this regard, the cultivars of group II presented few factors that would favor their inclusion in cultivation systems.

On the other hand, as seen in Table 2, cv. Santa Rosa, also in group II, stood out in terms of soluble solids content, with an average value of 10.85 ± 2.09 °Brix. Soluble solids contribute to a variety of fruit quality characteristics, including flavor, texture and nutraceutical properties. According to Crisosto & Crisosto (2005), the degree of consumer acceptance was closely related to the content of soluble solids.

Group III included the cultivars Ishtara, Clone 15, Tsukuba-1, Tsukuba-2, Tardio-01 and Tsukuba-3. These cultivars showed productivity 15% lower than the average of groups IV and V.

Besides it had lower productivity, the group included plants that presented with less vigor, expressed as smaller trunk section area. De Rossi et al. (2004) also observed lower vigor in plants grafted on Tsukuba-1. Based on this observation, in addition to a lack of signs of incompatibility, cultivars of this group, due to their lower vigor, could be candidates for use in densely planted orchards, possibly requiring less intervention with pruning. Thus, the lower productivity observed in the plants of group III could be compensated for by the greater number of plants that they allow to be cultivated in the area, as well as by the lower labor requirement for the pruning management.

Group IV (I-67-52-4, Barrier and Rigitano) presented higher average fruit mass, 21% higher than the mean of groups III and V. This group showed lower productivity and vigor than did group V and showed higher productivity than did group III. The characteristics that highlight the importance of the cultivars of this group are the tolerance to nematodes of the genus *Meloidogyne*, conducive to the improvement of fruit quality, low vigor and ease of propagation by herbaceous cuttings (Couto et al., 2004; Pereira et al., 2007).

In contrast to the other groups, group V encompassed plants showing higher productivity and greater vigor. The cultivars of this group included Cadaman, Okinawa, Mexico Fila 1, GF 677, Nemared, Capdeboscq, G × N.9, De Guia, Flordaguard, Rosaflor and own-rooted BRS-Libra. Although these plants were more productive, they were more vigorous. Consequently, rootstocks of this group, as well as the own-rooted plants, required more manpower in terms of pruning and management, possibly increasing production costs.

Similar results with the Okinawa cultivar were reported by Mayer & Pereira (2006), who analyzed the vigor of peach clones propagated by herbaceous cuttings, confirming that the Okinawa cultivar presented larger trunk diameter and greater growth of the main branches, indicating high vigor and less possibility of success in the formation of orchards of high density. Varago et al. (2017) reported that cv. Cadaman initially showed high vigor, however with similar decreases from the fourth to the fifth productive cycle. The same authors reported that the cultivar Nemared provided vigorous plants with good productivity, as observed in the present study.

Based on the similarity coefficient, it can be seen that group IV and V were the most similar to each other (0.1), while group I was the most distinct (1.0) in relation to the others, with respect to graft incompatibility. Similarly, group II differed from group III (0.4) while the latter group was distinguished from group IV (0.2) with respect to the scale of genetic dissimilarity projected in the dendrogram (Figure 1).

Although the cultivars of group V were more productive, they showed more vigor. Consequently, rootstocks from this group required more manpower in terms of pruning and management, possibly increasing production costs. On the other hand, the cultivars of group III, although less productive, were less vigorous and required less expense in terms labor for their management. In this way, the choice of rootstock becomes fundamentally important for planning units of production.

4 Conclusion

We conclude that the use of various rootstocks influenced the behavior of the BRS – Libra cultivar in terms of vigor and fruit production. The cultivars of group V, showing greater vigor, gave good potential for use in low planting density orchards, while the cultivars of group III, composed of rootstocks with lower vigor, presented potential for use in orchards of medium-high density.

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Influência da cultivar e do manejo de poda sobre os aspectos fenológicos da amoreira-preta

Influence of cultivar and pruning management on the phenological aspects of blackberry

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Resumo. Dentre outros fatores, o clima, e o manejo, também podem afetar o desenvolvimento e o comportamento da amoreira-preta, proporcionando diferentes resultados sobre as cultivares. Portanto o objetivo com este trabalho foi avaliar o desempenho fenológico de cultivares de amoreira-preta e plantas conduzidas com diferentes números de hastes. Foram realizados dois experimentos, o primeiro constitui-se da avaliação de quatro cultivares de amoreira-preta: BRS Tupy, Cherokee, BRS Xavante e Guarani, para o segundo experimento, com a cultivar BRS Tupy, foram avaliados diferentes números de hastes: poda drástica de inverno, duas, três e quatro hastes primárias conduzidas também no sistema tradicional. Os aspectos fenológicos avaliados nos dois experimentos foram: início, fim e duração da floração e da colheita. Diante dos resultados obtidos percebe-se uma grande diferença entre os ciclos produtivos em relação ao período de floração e colheita, sendo mais extenso no primeiro ciclo. Os aspectos fenológicos das cultivares testadas são afetados pelos fatores climáticos observados durante as avaliações, apresentando assim variações entre os dois anos. Durante o primeiro ciclo, somente as plantas submetidas à poda drástica não conseguiram atingir o mesmo período de produção que plantas submetidas aos demais tratamentos. Entre as cultivares testadas 'BRS Tupy' e 'Cherokee' mantiveram os maiores períodos de colheita, mesmo apresentando uma grande variação entre os ciclos. Plantas jovens da cultivar BRS Tupy apresentam período de colheita restrito.
Palavras-chave: *Rubus* sp., floração, colheita.

Abstract. Among other factors, climate and management may also affect the development and behavior of blackberry, providing different results on cultivars. Therefore the objective of this work was to evaluate the phenological performance of blackberry cultivars and plants conducted with different numbers of primocane. Two experiments were carried out, the first one consisted of the evaluation of four blackberry cultivars: BRS Tupy, Cherokee, BRS Xavante and Guarani, for the second experiment, with the cultivar BRS Tupy, different numbers of primocane were evaluated: drastic pruning of winter, two, three and four primary primocane also conducted in the traditional system. The phenological aspects evaluated in the two experiments were: beginning, end and duration of flowering and harvest. Considering the results obtained, a great difference can be observed between the productive cycles in relation to the period of flowering and harvest, being more extensive in the first cycle. The phenological aspects of the tested cultivars are affected by the climatic factors observed during the evaluations, thus presenting variations between the two years. During the first cycle, only plants subjected to drastic pruning did not reach the same production period as plants submitted to other treatments. Among the tested cultivars 'BRS Tupy' and 'Cherokee' they maintained the highest harvesting periods, even though they presented a great variation between the cycles. Young plants of the cultivar BRS Tupy present a restricted harvest period.
Keywords: *Rubus* sp, flowering, harvesting

.Introdução

Por ser uma cultura oriunda de clima frio, o cultivo da amoreira-preta (*Rubus* sp.) no Brasil se destaca, principalmente, em regiões de clima temperado. O aumento progressivo na produção e consumo dessa fruta se deve aos avanços das técnicas de manejo, na pós-colheita e na descoberta dos benefícios que o seu consumo pode gerar para a saúde (Curi, 2012).

Recentes descobertas apontam que frutas de amoreira-preta apresentam propriedades nutracêuticas, principalmente pela presença de antocianinas e flavonoides (Curi, 2012). Além disso, segundo Fu et al. (2015) o consumo de frutas com elevados níveis de antocianinas, flavonoides e ácidos fenólicos, são boas fontes de antioxidantes naturais.

Fatores genéticos, ambientais, climáticos, de manejo e tratos culturais influenciam os aspectos fenológicos de espécies e cultivares do gênero *Rubus* (Attílio, 2009). O clima, em especial, é um fator importe por gerar uma grande influência no desenvolvimento da cultura (Ferreira et al., 2016).

Além disso, a produtividade e qualidade dos frutos altera-se de uma forma negativa ou positiva, devido a alteração no acúmulo térmico necessário para um desenvolvimento adequado (Martins, 2015). Isso remete a importância de estudar métodos alternativos de poda em cultivares implantadas em diferentes locais, a fim de verificar seu comportamento perante as condições edafoclimáticas regionais.

Em cultivos convencionais, no primeiro ano após o plantio a poda das hastes é realizada no período de dormência das plantas, fazendo-se um desponte para estimular a emissão dos ramos secundários de produção. Durante este procedimento, deve-se ter o cuidado de reduzir a altura das hastes de modo que fiquem com 15 cm acima da linha de arame para facilitar o amarrio das mesmas. No período de verão após a colheita, elimina-se todas as hastes que produziram e desponta as hastes do ano que irão produzir no próximo ciclo (Antunes et al., 2004).

A poda é uma operação importante a ser realizada na cultura, no entanto é uma prática complicada de ser efetuada, pois naturalmente as plantas da grande maioria das cultivares apresentam espinhos. Uma das alternativas para facilitar o manejo seria efetuar a retirada de toda a parte aérea da planta após o inverno. Porém, segundo Villa et al. (2014) plantas conduzidas desta forma, ao emitirem novas brotações acabam destinando prioritariamente suas reservas para a formação da sua nova parte aérea, e não para a formação das frutas.

Diante deste contexto, o objetivo com este trabalho foi avaliar por dois ciclos consecutivos, o desempenho fenológico de cultivares de amoreira-preta conduzidas com diferentes números de hastes e intensidade de poda.

Métodos

O presente trabalho foi realizado em Chapecó, Santa Catarina em um pomar de amoreira-preta localizado a Latitude de 27° 07' 11" S, Longitude 52° 42' 30" E e altitude de 610 m. O clima segundo a classificação de Köppen é categoria C, subtipo Cfa (Clima Subtropical Úmido). O solo do local é classificado como Latossolo Vermelho Distroférrico (EMBRAPA, 2004).

As condições de temperatura máxima, mínima e também de precipitação durante a condução do trabalho estão apresentadas na Figura 1. Pode-se observar que no segundo ciclo produtivo o inverno foi um pouco mais rigoroso do que no primeiro, os meses de Maio, Junho, Julho, Agosto e Setembro de 2016 apresentaram temperatura médias mínimas menores que no ano de 2015. Além disso, com exceção do mês de Agosto, o ano de 2016 teve uma precipitação superior a 2015. Essa diferença climática entre os anos pode culminar em variações fenológicas entre os ciclos produtivos.

O pomar conduzido em sistema tradicional de espaldeira, foi implantado no ano de 2014, com espaçamento de 1,5 m entre plantas e 3 m entre linhas, somando um total de 2.222 plantas ha $^{-1}$. As avaliações foram realizadas nos dois primeiros anos, correspondente aos ciclos produtivos de 2015/16 e 2016/17.

O delineamento utilizado nos dois experimentos foi inteiramente casualizado, com cinco repetições. No primeiro experimento avaliou-se quatro cultivares (BRS Tupy, Cherokee, BRS Xavante e Guarani), todas conduzidas com três hastes. No segundo, avaliou-se a cv. BRS Tupy conduzida com diferentes números de haste e intensidade de poda (zero, duas, três e quatro hastes). Considerou-se como tratamento zero, a poda drástica onde todas as hastes foram cortadas a 10cm do solo, nos demais tratamentos as hastes foram despontadas 15cm acima da última linha de arame do sistema de condução.

A poda foi realizada em Setembro de 2015 e Agosto de 2016, conforme o início da brotação em cada ciclo. Neste período foi selecionado o número de hastes por planta de acordo com os tratamentos.

As avaliações de floração seguiram a metodologia proposta por ANTUNES (2000), o início de floração (IF) e o término da floração (TF) foram considerados quando as plantas apresentavam 5% e 90% de suas flores abertas, respectivamente. A diferença de dias entre IF e TF foi definida como duração da floração (DF). As avaliações de início, término de duração de colheita (IC, TC, DC) seguiram a mesma metodologia utilizada para avaliar a floração.

Os dados obtidos foram avaliados pelo teste F, e somente quando significativos fora submetidos a análise de variância pelo teste de Tukey a 5% de significância.

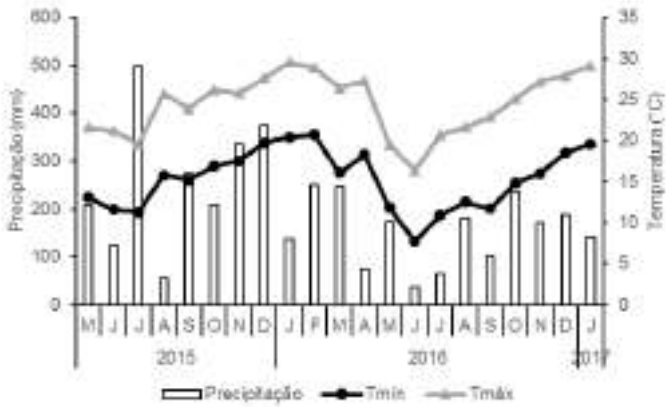

Figura 1. condições de precipitação e temperatura máxima (Tmáx) e temperatura mínima (Tmín) durante a condução do experimento. Chapecó, SC, 2018.

Resultados e discussões

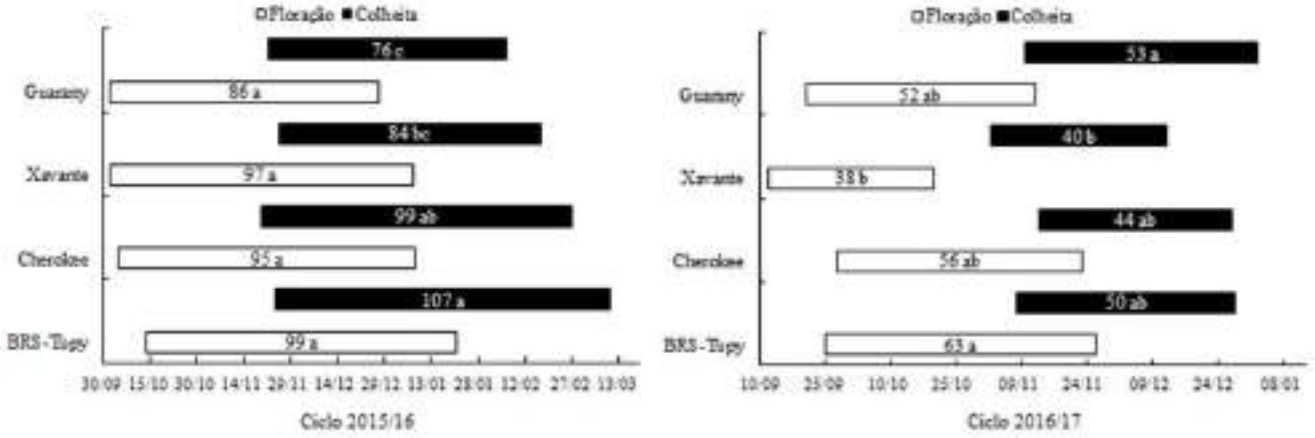

Figura 2. Avaliação de início, término e duração de floração e colheita de diferentes cultivares de amoreira-preta no Oeste catarinense no ciclo produtivo de 2015/16 e 2016/17. Chapecó, SC, 2018.

Experimento 1

De acordo com os resultados das variáveis fenológicas do ciclo produtivo 2015/16, observa-se que houve de certa forma uniformidade entre as cultivares quanto ao início da floração, que ocorreu na primeira quinzena de Outubro de 2015, finalizando na segunda quinzena de janeiro, não havendo, portanto, diferença significativa entre as cultivares (Figura 2).

A cv. BRS Xavante apresentou o menor ciclo de floração, iniciando na primeira quinzena de Setembro e finalizando logo após a segunda quinzena de Outubro. Já as cultivares BRS Tupy e Cherokee prolongaram sua floração por mais um mês. O período florífero da cv. BRS Xavante teve uma duração de 38 dias, porém com diferença significativa apenas para a cv. BRS Tupy, que apresentou 63 dias de floração (Figura 2).

Quando comparado os dois ciclos produtivos, percebe-se que há uma redução do período de floração no segundo ciclo. O qual pode está relacionado ao clima e as características genéticas inerentes de cada cultivar. Pois segundo Antunes et al. (2010), as variações nos aspectos fenológicos podem estar relacionadas à genética específica de cada cultivar e aos fenômenos climáticos durante o período, como temperatura e fotoperíodo, os quais interferem diretamente na brotação e floração das plantas.

Em estudos conduzidos por Campagnolo & Pio (2012a), foi constatado que o início de floração nas cultivares Cherokee, Guarani, BRS Tupy e BRS Xavante ocorreu no mês de Setembro em Marechal Cândido Rondon – PR, e o final da floração no mês de Dezembro a início de Janeiro, no primeiro ciclo produtivo em 2009/10.

A quantidade de horas frio acumulada pode influenciar e assim gerar variações nas características fenológicas de floração, entre regiões e ciclos de cultivo, resultado das variações de temperatura (Oliveira et al., 2017). Um outro fator relevante é a data de elevação das temperaturas, que também pode interferir no desenvolvimento das plantas (Botelho, et al. 2009).

Analisando o ínicio do período de colheita, verifica-se uma determinada homogeneidade entre as cultivares no ciclo 2015/16, o qual ocorreu entre 20 a 25 de Novembro. Com relação ao término, a cv. Guarani foi a primeira a finalizar, no início de Fevereiro, enquanto a colheita da cv. BRS Tupy se

estendeu até a primeira quinzena de março, apresentando-se assim como a de ciclo mais longo diferindo das cultivares BRS Xavante e Guarani, sendo estas, 21 e 29% inferiores, respectivamente (Figura 2).

Para o mercado frutícola, a maior amplitude do período de colheita é um fator positivo, pois, favorece a disponibilidade de frutas ao mercado consumidor por mais tempo, promovendo o escalonamento e a comercialização da produção.

Já no ciclo 2016/17, a produção ocorreu no período de Novembro a Janeiro, gerando um período florífero e de colheita antecipados e reduzidos, quando comparado ao ciclo anterior. A precocidade de colheita é fator importante na produção de frutíferas, pois neste período há pouca disponibilidade da fruta no mercado.

Neste mesmo ciclo, a cv. BRS Xavante apresentou duração de colheita de apenas 40 dias sendo 7,5% inferior a cv. Guarani. Na Figura 2 observa-se que ocorreu um intervalo de tempo entre o final da floração e início da colheita na cv. BRS Xavante, que pode está relacionado ao tempo de formação das frutas, que é superior ao total de floração.

Com relação ao período de colheita, Hussain et al. (2016), ao conduzirem experimentos em Londrina – PR, com as cultivares BRS Tupy e BRS Xavante obtiveram 49 e 51 dias de duração respectivamente. Campagnolo & Pio (2012a) também relatam que a duração da colheita no primeiro ciclo produtivo em estudos conduzidos no município de Marechal Cândido Rondon – PR foi inferior em todas as cultivares testadas, vindo a inverter no segundo ciclo.

Essa diferença encontrada entre as regiões, os ciclos de cultivos e as cultivares pode ser explicada em função das condições de solo e de clima sendo esta última relacionada a temperatura, fotoperíodo e a precipitação. Fatores que podem acelerar ou retardar a maturação das frutas. Segundo Campagnolo & Pio (2012a), cada cultivar é diferente em necessidade de temperaturas, por isso podem ser verificadas diferenças entre uma mesma cultivar e em locais diferentes.

Além disso, o acúmulo de horas frio e a precipitação podem ter influenciado essa diferença encontrada entre os diferentes anos. Como o pomar não é irrigado, as plantas dependem somente da água da chuva, portanto a falta dela em alguns períodos críticos pode acarretar em diferenças entre os anos.

Experimento 2

Em relação aos resultados fenológicos do ciclo 2015/16 nos diferentes tipos e intensidade de poda na cv. BRS Tupy verificou-se que o início da floração ocorreu entre 10 a 18/10 e finalizou-se entre os dias 06/12 a 21/01. O menor período de floração foi em plantas em que realizou-se a poda drástica de inverno, prolongando-se em média por mais 38 dias nos demais tratamentos (Figura 3).

No segundo ciclo produtivo (2016/17), o período total de floração foi em média de 54 dias, sem diferença estatística entre as podas. Observa-se uma precocidade de aproximadamente 3 a 4 semanas no início de floração quando comparado ao ciclo anterior de 2015/16, terminando no final do mês de Novembro.

Segundo Broetto et al. (2009) ao avaliar a poda longa e a poda curta em plantas de amoreira, atribuíram o atraso do início da floração a localização das gemas floríferas que floresceriam primeiro terem sido eliminadas com a poda drástica, demandando um período extra para as gemas vegetativas se diferenciarem em gemas floríferas.

Campagnolo & Pio (2012b), quando realizaram a poda drástica de inverno na cv. BRS Tupy em Marechal Cândido Rondon – PR em 05 de julho, obtiveram resultados superiores em relação à duração de colheita. É provável que estes resultados tenham sido conseguidos em função da antecipação da poda, prática inviável de se executar na região em que foi instalado este experimento, em função da alta incidência de geadas.

O início da colheita no primeiro ciclo produtivo ocorreu entre 23 e 27 de Novembro. A última colheita nas plantas com poda drástica foi realizada em 11 de Janeiro, com duração total de 48 dias em média. Já para as plantas com dois, três e quatro hastes primárias, a duração foi superior a 100 dias de colheita (Figura 3).

A poda drástica realizada em amoreira-preta pode prejudicar a produção e o armazenamento de fotoassimilados, que estão diretamente relacionados a diferenciação floral e a produção (Villa et al., 2014). E isso pode explicar o fato de plantas com poda drástica de inverno apresentaram menor período de colheita.

No ciclo 2016/17, na primeira quinzena de Novembro iniciaram a colheita nas plantas que receberam poda de dois, três e quatro hastes. Nas plantas com poda drástica de inverno a colheita iniciou na segunda quinzena do mês, em resposta a floração que também se apresentou tardia (Figura 3). O final das colheitas aconteceram do fim do mês de Dezembro à início de Janeiro.

O resultado do curto período de floração e colheita na poda drástica de inverno explica-se, principalmente pelas plantas terem destinado suas reservas para a formação da parte aérea, portanto possuíam menor quantidade de reservas disponíveis para a frutificação (Campagnolo & Pio, 2012b), (Villa et al., 2014).

Ao contrário do ciclo anterior, em 2016/17 não encontrou-se diferença significativa para a duração da colheita. Entretanto, deve-se salientar que a poda drástica de inverno acabou retardando o início de colheita.

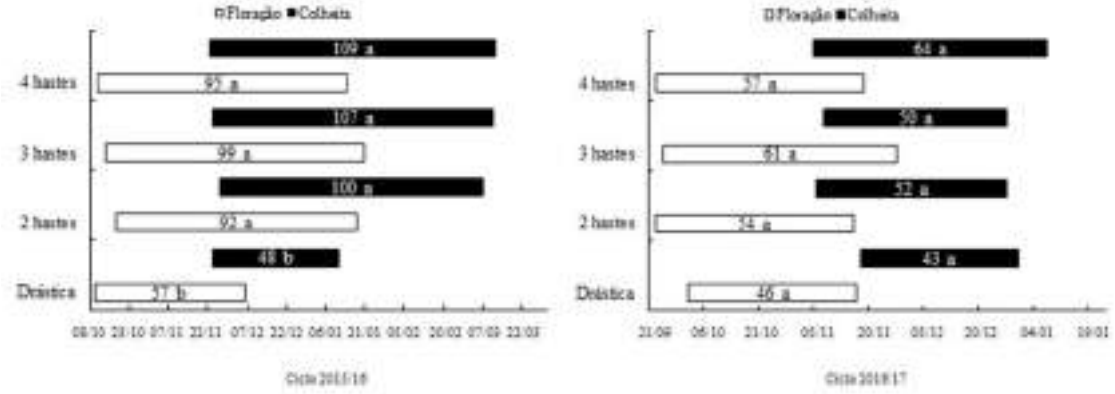

Figura 3: Avaliação de início, término e duração de floração e colheita de diferentes números de hastes em amoreira-preta no Oeste catarinense no ciclo produtivo de 2015/16 e 2016/17. Chapecó, SC, 2018.

Conclusões

As condições climáticas influenciam as características fenológicas das cultivares nos diferentes ciclos avaliados.

Entre as cultivares testadas a cv. BRS-Tupy e a Cherokee mantiveram os maiores períodos de colheita, mesmo apresentando grande variação entre os ciclos.

A cv. Xavante apresenta boa precocidade de colheita, no entanto o período de colheita curto, reduzindo a disponibilidade das frutas ao mercado.

O manejo de poda drástica de inverno em plantas de amoreira-preta jovens, provoca redução no período de floração e colheita para a cv. BRS Tupy.

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Initial productive development of peach trees under modern training systems

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ABSTRACT

Orchards implanted under high density show highest production and precocious harvest. However, few studies in Brazil have sought to maximize production through the variation of training systems. Therefore, the objective was to evaluate the initial development of cultivars 'Barbosa' and 'Chiripá' under different planting densities and modern training systems. The experiment was evaluated during two productive cycles (2015/16 and 2016/17). In the 'Vase' system, a spacing of 3.5 m was used, in the 'Y-shape' 1.5 m was used, and in the 'Central Leader' system, a spacing of 0.8 m was used between plants. The spacing between rows was equal to 5 m. The two cultivars ('Barbosa' and 'Chiripá') were evaluated for vegetative, phenological development and productive traits. The Capdeboscq rootstock, from seedlings, was used for the two cultivars evaluated. Regarding to the vegetative development, as increase the plant density, like in 'Central Leader' training system, the pruned green mass and the canopy dimension decrease. With regard to phenology, there is no change between the systems evaluated. However, for the productive components, the 'Central Leader' system was more productive, in addition to maintaining the quality of the fruit, when compared to other systems and training. Consequently, it is concluded that the training system in 'Central Leader ' has been showing good vegetative and productive results in the first two years of evaluation. More evaluations are necessary for greater precision regarding the productive potential over the years.

1. Introduction

The cultivation of peach (*Prunus persica* (L.) Batsch) undergoes a constant transition and remodeling in its cultivation system. Currently, with a greater proportion, there are commercial orchards with low planting density and open training systems (Hansen et al., 2018). To a smaller extent, some orchards have a higher planting density and modern management systems, aiming mainly at increasing production by area and reducing labor (Schupp and Baugher, 2011).

High density planting crops have high yield (Glenn et al., 2011) and precocious harvest (Robinson et al., 2012), besides allowing the mechanization of some activities (Schupp and Baugher, 2011). According to Pasa et al. (2017), the quality of the fruit does not change when comparing low and high planting density systems. However, Bussi et al. (2015) found improvements in quality, in addition to higher productivity in high density planting systems. In addition, high density perpendicular systems reduce the incidence of brown rot (*Monilinia* spp.) in fruits (Bussi et al., 2015).

However, peach cultivars available on the market express their own performance and characteristics when managed under different training systems (Zec et al., 2016; Hansen et al., 2018). Few studies in Brazil have sought to maximize production through the variation of training systems. In this regard, the aim of this study was to evaluate the initial development of the peach cultivars 'Barbosa' and 'Chiripá' in modern plant training systems combined with planting densities.

2. Material and methods

In the spring of 2014, peach cultivars 'Barbosa' and 'Chiripá' were planted in an orchard located at a latitude of 27° 7′ South, Longitude 52° 42′ West and 605 m altitude above sea level. The local climate according to Köppen is Humid Subtropical and the soil is classified as

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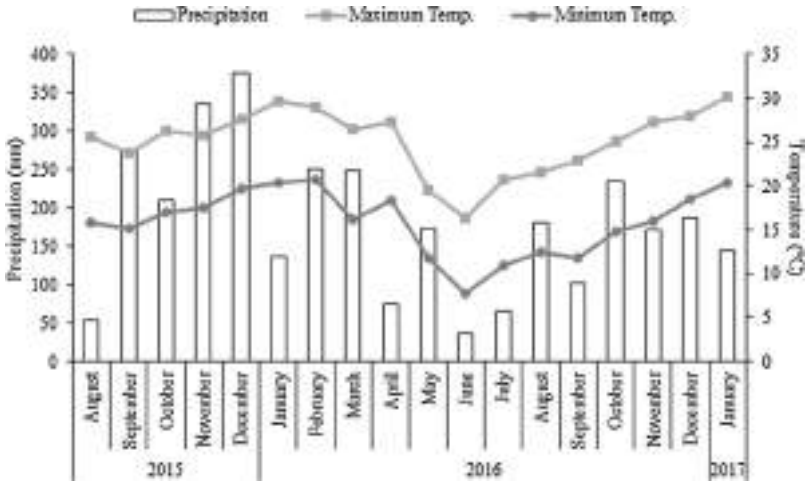

Fig. 1. Temperature and precipitation conditions that occurred during the trial. Adapted from INMET - Instituto Nacional de Meteorologia (2020).

Red Latosol Distroferric. Temperature and precipitation conditions are presented in the Fig. 1.

The plants were conducted in three different training systems and planting densities. The spacing between rows was equal to 5 m, varying the spacing between plants according to the training system. In the 'Vase' system, a spacing of 3.5 m was used, in the 'Y-shape' a spacing of 1.5 m was used and in the 'Central Leader' system (CL) 0.8 m. These systems are equivalent to 571, 1333 and 2500 plants ha^{-1}, respectively. 'Vase' training was used in the guard rows in addition to the control plots, since this is the traditional plant training system.

Pruning and thinning of the fruits were performed in accordance with the recommendations for each cultivar and the training system adopted (Pereira and Raseira, 2014a, 2014b; Raseira et al., 2014). The 'Capdeboscq' rootstock, from seedlings, was used for the two cultivars evaluated. The experiments were conducted in a randomized complete block design, with three treatments, 'Vase', 'Y-shape' and 'CL' and three replicates, with the experimental unit consisting of five plants. The peach cultivars were evaluated independently according to the training systems.

The evaluations were conducted during the production cycles of 2015/16 and 2016/2017. The analyzed variables were separated into vegetative, phenological and productive components. Vegetative evaluations: a) trunk diameter (mm), measured 10 cm above the grafting point; b) canopy size (CS, cm^3), measured using formulas for each training system, 'Y-shape' CS = (W.h.((e1 + e2)/2)), 'Vase' and 'CL': CS = (W.E.h), being equatorial width of the plant (W), height of the canopy, measured from the first insertion of the main branches to the apex (h), canopy thickness on the right side (e1), canopy thickness on the left side (e2), transverse width of the plant (E); c) rate of increase in canopy size (%); d) accumulated mass of branches removed with pruning (kg $plant^{-1}$).

For the phenological evaluation the following variables were considered: a) flowering, being beginning (10 % of open flowers), full (50 % of open flowers) and end of flowering (10 % of the flowers still had petals); b) harvest, with the start and end dates of the harvest; c) harvest duration, difference in the number of days between the beginning and end of harvest; d) total cycle, being the number of days between the beginning of flowering and the end of the harvest. Finally, regarding the productive components, the following were evaluated: a) number of fruits per plant; b) equatorial diameter (mm); c) average fruit mass (g); d) soluble solids (°Brix); e) production per plant (kg $plant^{-1}$); f) estimated productivity (t ha^{-1}).

The collected data were submitted to analysis of variance by the F test. When significant, Tukey's test was applied, with $P \leq 0.05$ of significance. The phenological results were submitted to descriptive analysis, regardless of the means test.

3. Results

Under the conditions of the experiment, the cultivars showed different growth vigor hence not started fruit production in the same year. The cultivar (cv.) 'Barbosa' presented production in the second year of cultivation (2015/16). However, cv. 'Chiripá' presented less and slower vegetative growth compared to cv. 'Barbosa', consequently cv. 'Chiripá' showed production only in the third year (2016/17), being possible to evaluate the vegetative development only in the second year of cultivation.

For evaluations of vegetative development, it was observed in relation to the trunk diameter that the training systems had no different significant effect between the two cultivars in either of the productive cycles evaluated. On average, the cultivars 'Barbosa' and 'Chiripá', in the second cycle, had a diameter of 71.1 and 43.6 mm, respectively.

Regarding the canopy size (Fig. 2), it is observed for cv. 'Barbosa',

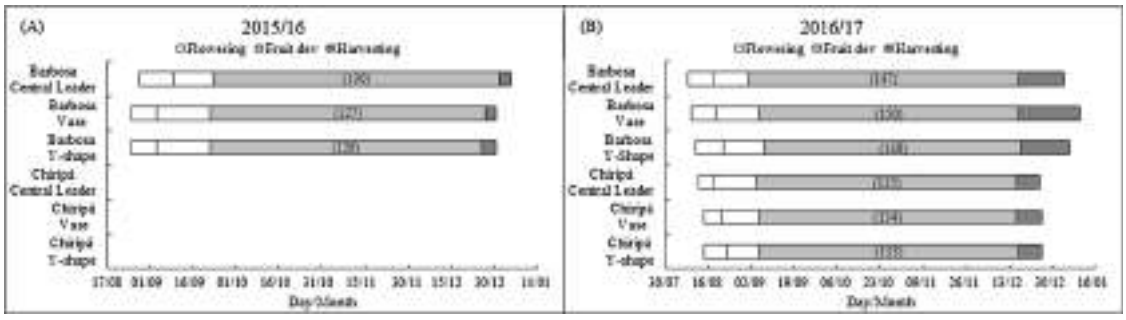

Fig. 2. Canopy size (CS), Canopy size increase rate (CSIR) and accumulated mass of branches removed with pruning (AMBP) for different training systems in peach cultivars 'Barbosa' (A and B) and 'Chiripá' (C and D) in the productive cycles of 2015/16 and 2016/17. Lower case letters in the bars and upper case letters in the row, different from each other by Tukey's test at $P \leq 0.05$.

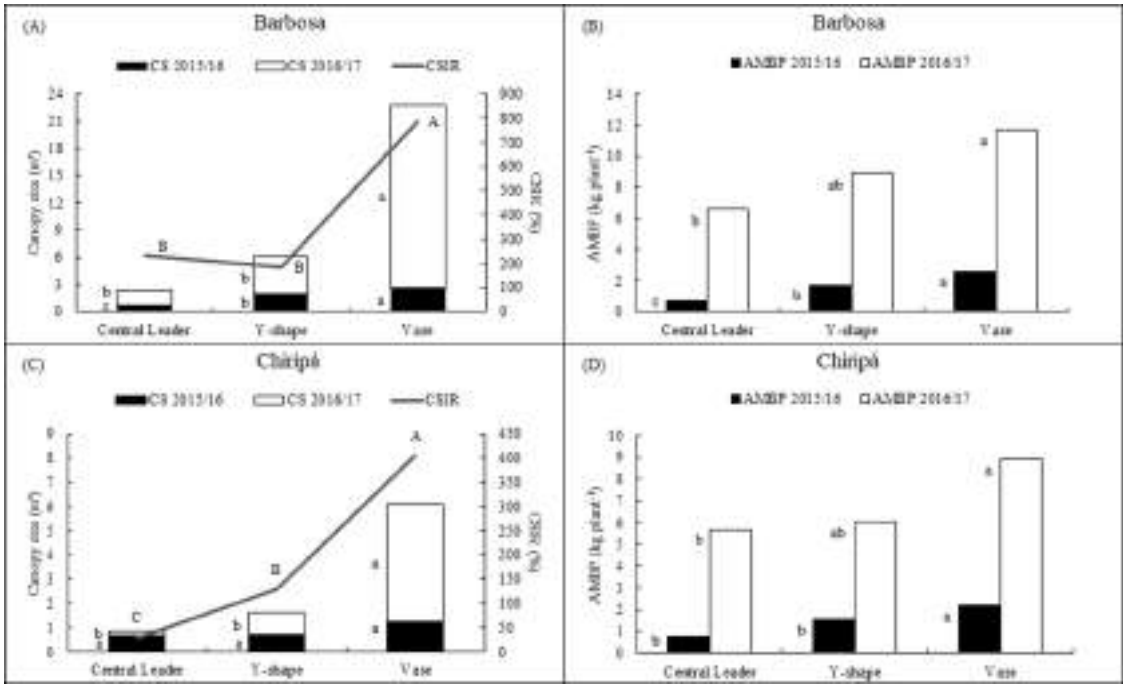

Fig. 3. Flowering period, fruit development and harvest for different training systems in peach cultivars 'Barbosa' and 'Chiripá' in the productive cycles of 2015/16 (A) and 2016/17 (B). The number in parenthesis total number of days of the reproductive cycle. The bar dividing the flowering period indicates full bloom stage.

the 'Vase' training system in the 2015/16 cycle, was 27 % bigger than 'Y-shape' and this 36 % bigger than 'CL', which had 0.72 m^3. In the 2016/17 cycle, the 'Vase' system had 22.7 m^3, five times larger when compared to the other systems. For cv. 'Chiripá', a difference was observed only in the second cycle, in which the 'Vase' system had 6.0 m^3, four times the size of the other systems tested.

For the rate of increase in the canopy size (Fig. 2), it was observed in cv. 'Barbosa', that the 'Vase' training system showed an increase of 787 % in relation to its initial size. For the cv. 'Chiripá', it was observed an increase rate of 406 % for 'Vase', followed by 'Y-shape' and 'CL', 130 % and 30 %, respectively, in relation to its initial size.

For the variable accumulated green mass of branches removed with pruning (Fig. 2), a similar behavior was observed in the two years and in the two cultivars evaluated. It was found a linear increase in green pruned mass from the 'CL' training system to the 'Vase' training system.

Regarding the phenological variables (Fig. 3), the cv. 'Barbosa' for the first cycle (2015/16) and the two peach cultivars for the second cycle (2016/17), did not show changes in their phenological development, even varying the training system and planting density. On average cv. 'Barbosa' presented a total cycle of 127 and 148 days for the first and second cycle, respectively. For cv. 'Chiripá', a cycle of 134 days was observed in the 2016/17 cycle.

For the productive components (Fig. 4), regarding production per plant, the training systems for cv. 'Barbosa', showed a difference only in the second evaluation cycle. In this cycle, the system 'Vase' showed 43.9 kg $plant^{-1}$, and twice more productive than the other training. The cv. 'Chiripá' resembling, presented a production of 9.6 kg plant-1 for the 'Vase' system, being twice higher than the 'Y-shape' system. Regarding the number of fruits per plant, results similar to production per plant were observed, which are correlated.

Regarding the estimated productivity, the opposite behavior than for production was observed (Fig. 4). The 'CL' system with the highest planting density, showed the highest productivity. For cv. 'Barbosa', this system presented 1.0 t ha^{-1} in the first productive cycle, being three times higher than 'Vase'. For the second cycle (2016/17), 'CL' presented 41.8 t ha^{-1}, with the other systems reaching only 62 % of this productivity. Likewise, for cv. 'Chiripá', the 'CL' training system presented 17.9 t ha^{-1} and the other systems only 32 % of this productivity.

Concerning the quality of the fruits, there was no change between the training systems tested in the two peach cultivars. On average, the

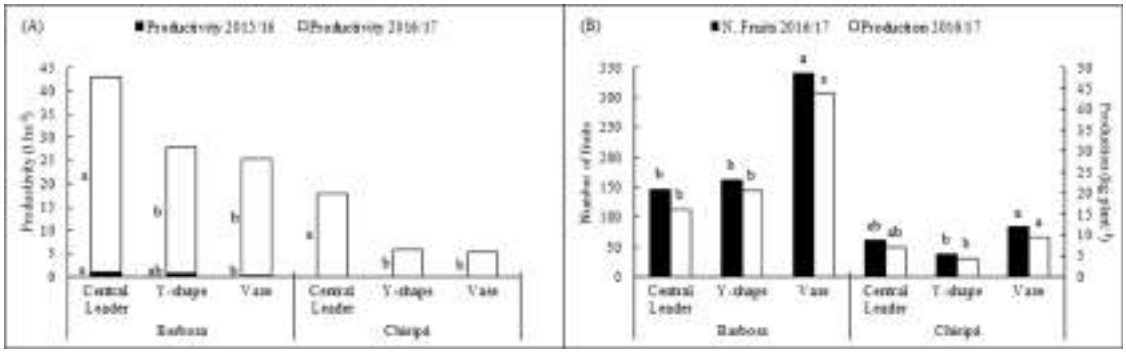

Fig. 4. Estimated productivity (t ha^{-1}) (A), production (kg $plant^{-1}$) and number of fruits per plant (B) in different training systems in peach cultivars 'Barbosa' and 'Chiripá' in the productive cycles of 2015/16 and 2016/17. Lower case letters in the columns and upper case letters in the row, different from each other by Tukey's test at $P \leq 0.05$.

cultivars 'Barbosa' and 'Chiripá' showed 10.7 and 11.1°Brix of soluble solids, 63.3 and 60.3 mm in diameter, and 129.7 and 119.3 g of average fruit mass, respectively.

4. Discussion

With regard to vegetative growth, the vigor among the different training systems and planting densities did not change in neither of the cultivars tested. Therefore, it can be inferred that there is no competition between the trees under the different the training systems, while according to Layne et al. (2002), there is a reduction in trunk diameter in crops with high planting density, because there is greater competition for light, water and nutrients. According to Caruso et al. (2001) low density systems invest more than 95 % of their annual dry matter gain in vegetative and root growth, resulting in higher trunk diameter growth.

Due to this high vegetative growth, open systems such as 'Vase', with low planting density, show a bigger canopy size (Hamana et al., 2016) and a greater rate of increase in size. Otherwise, plants conducted in 'CL' occupy less air space (Marini et al., 1995), providing, according to Stassen (2014), less base shading, less incidence of thief branches and better distribution of production throughout the entire plant.

It was verified in the 'CL' system, tested in cv. 'Chiripá', a stability in vegetative growth, that is, it did not show an increase in the canopy size as observed in the other systems. When stability is achieved, all net photosynthesis rate synthesized during the previous cycle is used for the proper bud formation, production and fruit quality of the subsequent cycle.

Related to the high growth rate of the canopy of open systems, there is higher export of green mass from the plant. Mainly due to the higher pruning intensity for proper plant formation (Caruso et al., 2001), in addition to favoring the entry of light into the interior of the plant, providing greater physical-chemical quality to the fruits (Kumar et al., 2010). As for modern systems such as 'CL', there is low demand for formation pruning, providing lower materials exported from the plant, even if summer pruning is essential for this system (Stassen, 2014).

Even if the training systems, combined with planting densities showed changes in the canopy size, growth rate and intensity of pruning in tested cultivars, the plant phenology did not change. Therefore, under the conditions of the experiment, the training does not interfere with precocity or delay the harvest time.

Furthermore, when the cv. 'Barbosa' was conducted under the 'CL' and 'Y-shape' training systems it showed the same canopy size increase rate and the same intensity of pruning for both training systems. However, the cv. 'Barbosa' showed greater yields under the 'CL' training system than under 'Y-shape' training system because these systems are related to different planting density. Peach trees under high density usually produce greater yields than peach trees under low density (Grossman and DeJong, 1998; Marini and Sowers, 2000).

In relation to productive variables, open systems at low planting density showed a high amount of fruits per plant, compared to 'CL' at high density. However, when this load is estimated by area, open systems do not maintain the same rates. This is directly related to the planting density used, which for open systems such as 'Vase', contains only 571 plants per hectare, different from 'CL', which contains 2500 plants per hectare.

According to Grossman and DeJong (1998) and DeJong et al. (1999) the 'Y-shape' training system showed greater yields than 'Vase' system. However, under the conditions of the experiment, this difference was not observed in any cultivars. Although the 'Y-shape' training system showed less fruits number and less yield per plant than 'Vase' system its major plant density resulted in a similar, but not greater, yield than 'Vase' training system.

In addition to presenting higher productivity, the 'CL' system maintains the quality of the fruit, whether in size or sugar content. According to Pasa et al. (2017) there is greater profitability using modern plant training systems, because the quantity of fruit is higher and the quality is maintained in this system.

5. Conclusion

The conclusion is that the training system 'Central Leader' has a higher fruit yield and better vegetative performance for both 'Barbosa' and 'Chiripá' cultivars. Phenological development and fruit quality are not affected by training systems. More evaluations are necessary for greater precision regarding the productive potential over the years.

CRediT authorship contribution statement

Alison Uberti: Conceptualization, Data curation, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Alice Silva Santana:** Data curation. **Adriana Lugaresi:** Data curation. **Jean do Prado:** Data curation. **Bachelor Louis:** Data curation. **Richardson Damis:** Data curation. **Doralice Lobato de Oliveira Fischer:** Funding acquisition, Resources, Supervision, Methodology. **Clevison Luiz Giacobbo:** Funding acquisition, Resources, Supervision, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CONDUCTION SYSTEMS IN FIG TREE WITH TWO CULTIVARS, EVALUATING FRUITS QUALITY AND YIELD

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ABSTRACT - The fig tree is rustic and one of the fruit species with great economic importance, because it adapts easily to different climate and soil types. The objective of this study was to evaluate the influence the branches number and the fig tree cultivar on yield and fruit quality. The orchard implanted with two cultivars, Roxo de Valinhos and Pingo de Mel, and conducted with different numbers of productive branches, being: 16, 24 and 32. The utilized lineation was completely randomized, with three replicates, each replicate being composed of four plants. The analyzed variables were: branches length (cm), number of fruits per plant, number of fruits per branch meter, yield of mature fruits (Kg ha^{-1}), yield of green fruits (Kg ha^{-1}), accumulated productivity (Kg ha^{-1}), fruit size (cm^3), average fruit weight (g), soluble solids (°Brix) and fruit dry matter (%). The daily elongation rate and the mean length of the branches did not influence the different treatments. For productivity, noticed superiority on cultivar Roxo de Valinhos, with the higher the number of productive branches in the plants. Fruit quality, relative to soluble solids and dry matter, also did not influence the treatments. Fruits in larger caliber and size found from plants of the cultivar Roxo de Valinhos. Both for the production of fruits for fresh consumption and for the industrialization it is recommended the conduction with greater number of productive branches being between 24 and 32, for the greater productive yield.
Keywords: *Ficus carica* L., number of branches, Roxo de Valinhos, Pingo de Mel.

SISTEMAS DE CONDUÇÃO DE FIGUEIRA COM DUAS CULTIVARES, AVALIANDO QUALIDADE DOS FRUTOS E PRODUTIVIDADE

RESUMO - A cultura da figueira é rústica e uma das espécies frutíferas de grande importância econômica, pois adapta-se facilmente a diferentes tipos de clima e solo. Objetivou-se com este estudo avaliar a influência do número de ramos e da cultivar de figueira na produtividade e qualidade dos frutos. O pomar foi implantado com duas cultivares, Roxo de Valinhos e Pingo de Mel, e conduzido com diferentes números de ramos produtivos, sendo: 16, 24 e 32. Utilizou-se o delineamento experimental inteiramente casualizado, com três repetições, sendo cada repetição composta por quatro plantas As variáveis analisadas foram: comprimento de ramos (cm), número de frutos por planta, número de frutos por metro de ramo, produtividade de frutos maduros (Kg ha^{-1}), produtividade de frutos verdes (Kg ha^{-1}), produtividade acumulada (Kg ha^{-1}), tamanho de fruto (cm^3), peso médio de fruto (g), sólidos solúveis (°Brix) e massa seca dos frutos (%). A taxa de alongamento diário e o comprimento médio dos ramos não foram influenciados pelos distintos tratamentos. Para a produtividade observou-se superioridade da cultivar Roxo de Valinhos sendo maior quanto maior o número de ramos produtivos nas plantas. A qualidade dos frutos, relativa a sólidos solúveis e massa seca, também não sofreu influência dos tratamentos. Os frutos de maior calibre e tamanho foram encontrados em plantas da cultivar Roxo de Valinhos. Tanto para a produção de frutos para consumo in natura como para a industrialização recomenda-se a condução com maior número de ramos produtivos sendo entre 24 e 32, pelo maior rendimento produtivo.
Palavras-chave: *Ficus carica* L., número de ramos, Roxo de Valinhos, Pingo de Mel.

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INTRODUCTION

The fig tree (*Ficus carica* L.) is a rustic plant, and of great economic importance, due to its appearance and flavor, it can be consumed fresh consumption or industrialized, presenting in its nutritional composition numerous health benefits, since it is rich in minerals and vitamins (DIAS, 2014). Besides being a crop that adapts easily to different types of climate and soil (ANDRADE et al., 2014).

The quality of fruits and vegetables is expressed by a set of sensory properties such as appearance (color, size, shape and defects), texture (firmness, hardness) and flavor (sweetness, acidity, taste and aroma), nutritional and multifunctional value (CHITARRA and CHITARRA, 2005). Soluble solids content is directly related to taste and is a fundamental parameter for fig quality (LEÃO et al., 2006). The wholesale market price for figs is mainly given by color, size and appearance (CHALFUN, 2012).

There are hundreds of fig tree cultivars described in the literature, with different agronomic and pomological characteristics (BISI et al., 2016). However, still there is no large number of rustic cultivars adapted to the Brazilian conditions of cultivation (SILVA et al., 2017).

For commercial cultivation, currently there is cultivate only Roxo de Valinhos cultivar, which hav high economic value and is characterized by rusticity, vigor and productivity. With good adaptation to different types of climate, and still supporting the drastic pruning system (KOTZ et al., 2011). The cultivar Pingo de Mel is not considered a cultivar in high rusticity when compared to Roxo de Valinhos, however, it is characterized by producing very sweet fruits, demonstrating high vigor and productivity, with good adaptation to the drastic pruning system (CHALFUN, 2012).

The distribution ratio of photoassimilates in the plant is through the source-sink relatioships, in which the source organs (mainly leaves) produce the photoassimilates as a result of photosynthesis, and these can be stored in some other reserve organs, such as roots, meristems and fruits, thus being called sink. The balance between production and demand for photoassimilates is important for improving production, and this is possible through proper distribution of the source-sink relationship (DUARTE and PEIL, 2010). Cultural management such as pruning, for example, affects the balance between vegetative and reproductive growth of the plant by the distribution of resources such as carbohydrates (MYERS, 2003).

For plants that produce in branches of the year, such as the fig tree, pruning is decisive, being that fruiting occurs in flowers that grow on branches sprouted in spring and bloom abundantly (FACHINELLO et al., 2009). For a commercial system in which drastic pruning is used, the number of branches varies between 12 and 36, depending on the training systems, and the purpose of production (fresh consumption market or industry) (Dias, 2014). On this, the objective of this work was to evaluate the influence of the number by branches on two fig tree cultivars, fruit yield and quality.

MATERIAL AND METHODS

The experiment was done in 2015, in fig tree orchard with one year of implantation, located at latitude 27° 07’ 11” S, longitude 52° 42’ 30” E and altitude of 610 m. The soil analysis in the area showed the following characteristics: pH 5.8, 3.5% organic matter, P content of 5 mg dm^{-3}, K 72 mg dm^{-3}, Ca 5.8 cmolc dm^{-3} and Mg of 2.9 cmolc dm^{-3}. Two cultivars, Roxo de Valinhos and Pingo de Mel, used in planting spacing of 5 m x 2 m (rows x plants) and were conducted with different numbers of branches, from the fruiting pruning. The plants, in the evaluated cycle, were in their first year of cultivation, so it was still running in the pruning formation, aiming at the end of the third year to reach the desired number of branches for each treatment.

Winter pruning or fruiting pruning was done on August 22nd, 2015, and in mid-September there was a strong frost that caused damage to some shoots. Climate data concerning the period driving period are available in Figure 1. The utilized lineation was completely randomized, with three replicates, each replicate being composed of four plants, in a 2 x 3 bifactorial scheme, two cultivars (Roxo de Valinhos and Pingo de Mel), three different numbers of branches (16, 24 and 32 branches $plant^{-1}$).

The variables analyzed were: the branches length, considering the base by branch to the tip of the apical bud of the same branch, the measurements were carried out weekly, being the beginning of the emission of fruits and the end of the productive cycle, number of fruits: determined by the final count of fruits per plant (at 119 days), yield of mature fruits, yield of green fruits, accumulated productivity: obtained by summing between mature fruits and green fruits (expressed in kg ha^{-1}). Then fruit size: measured with the aid of a pachymeter, average fruit weight, soluble solids (°Brix): determined by reading in a digital refractometer, fruit dry matter: determined by oven drying with forced air ventilation, heated to 65°C±2 until constant weight.

The data were submitted to analysis of variance by the F test and compared to each other by the Tukey test, at the 5% level of significance, and the analyzes performed through the WinStat statistical program (MACHADO and CONCEIÇÃO, 2005).

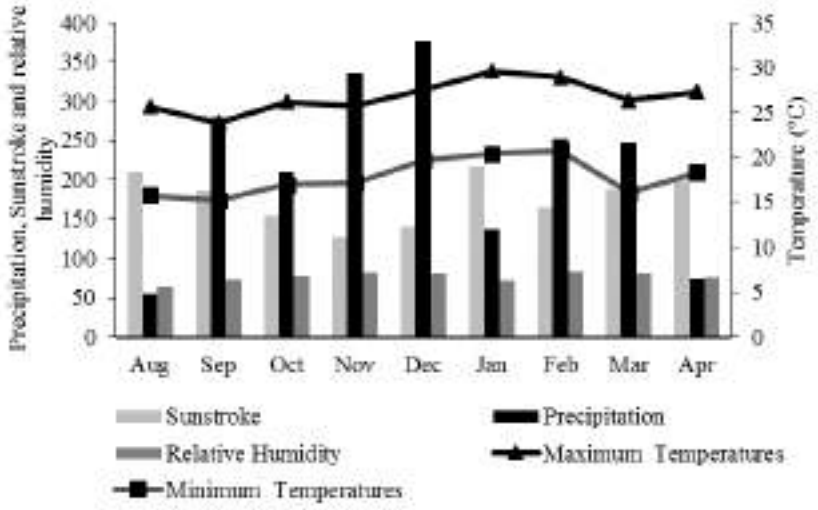

FIGURE 1 - Climatic data in the conduction period of the experiment. National Institute of Meteorology, Brazil (INMET) (2017).

RESULTS AND DISCUSSION

As it is shown in Figure 2, the average daily stretching rate of branches, adapted as described by Chapman and Lemaire (1993), depending on the cultivars and the days of evaluation, that the interaction between them occurred, and the plants of the cultivar Roxo de Valinhos showed a growth of 14% higher, however, there was no statistical difference between the cultivars. Vegetative development is dependent on the ability of plants to absorb and process the essential elements for their growth, such as water, CO_2, energy and soil nutrients, and their mobilization is directly linked to climatic events, mainly moisture and temperature, influencing linearly growth (CELEDONIO et al., 2013).

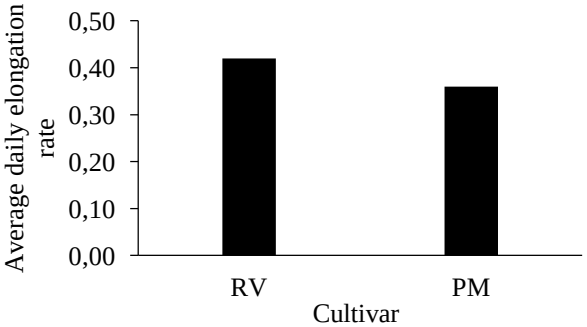

FIGURE 2 - Average daily elongation rate of fig tree branches of the cultivars Roxo de Valinhos (RV) and Pingo de Mel (PM). ns = not significant by the Tukey test, at 5% probability. CV(%) = 22.69.

The final branches length (Figure 3) did not influence by the branches number conducted in the plant, regardless of the cultivar. These data differ from those observed by Dalastra et al. (2011), which evaluates the number of branches in the production of green figs, reports that branch length tends to decrease with increasing number of branches per plant. Caetano et al. (2005) did not observe significant differences for the length of branches when conducted with different number of branches per plant. The results of the present study corroborate the results found by Nava et al. (2015), where the authors mention that the vegetative growth is not affected by the number of branches of the fig tree.

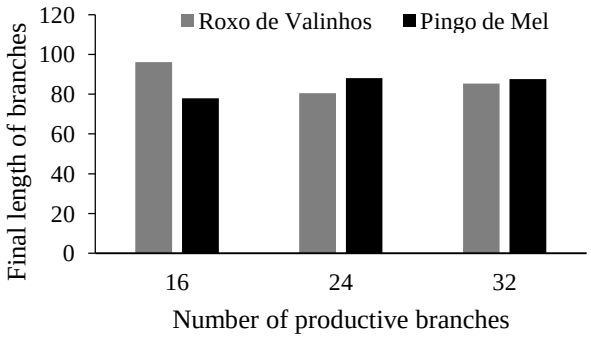

FIGURE 3 - Final length of branches for the cultivars Roxo de Valinhos and Pingo de Mel conducted with different number of branches. ns = not significant by the Tukey test, at 5% probability. CV(%) = 31.82.

The number of fruits per plant (Table 1) was higher in the plants with the highest number of branches, and for the cultivar Roxo de Valinhos there was no statistical difference between 24 and 32 branches and for the cultivar Pingo de Mel the fruit emission was superior when in the conduction of the plants with 32 branches. When comparing the cultivars, no significant difference was found. A lower yield in plants cultivated with conduction systems of the lower number branch can be explained by the low leaf area index of these plants (NAVA et al., 2015).

Corroborating with the results of this work, Nava et al. (2015) evaluating the removal of branches in the productivity and quality of the fig tree, they observed that

when the number of productive branches per plant increased, the number of fruits produced also increased. Dalastra et al. (2011) studying the branches number in the production of green figs, observed that with the greatest number of productive branches in the plant, the number of fruits was also higher.

TABLE 1 - Number of fruits per plant depending on diferents number of branches conducted.

Number of branches	Cultivars	
	Roxo de Valinhos	Pingo de Mel
16	30 bA*	43 bA
24	54 aA	41 bA
32	47 abA	71 aA
CV(%)	32.00	

*Means followed by the same letter do not differ statistically from each other, by the Tukey test, at 5% probability. Lowercase letters represent the columns and uppercase letters represent the rows.

About fruits number per meter of branch, it was observed that it remained satisfactory independently of the treatment. When comparing the cultivars in each number of productive branches, the superiority of the cultivar Pingo de Mel is found in plants submitted to 16 and 32 branches, and cultivar Roxo de Valinhos was only superior in plants with 24 branches (Figure 4). When comparing the different numbers of productive branches, no significant difference was found.

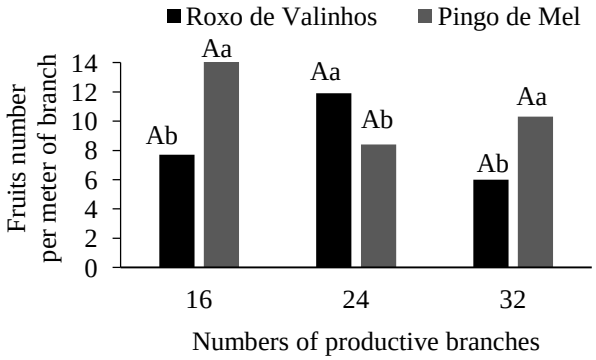

FIGURE 4 - Fruits number per meter of branch for the cultivars Roxo de Valinhos and Pingo de Mel. *Averages with different lowercase letters in each branch length and upper case within the cultivar, differ from each other by the Tukey test, at 5% probability. CV(%) = 42.00.

For the cultivar Roxo de Valinhos, it was observed lower yield of mature fruits when the plants were conducted with 24 productive branches. For driving with 16 and 32 branches there was no statistical difference between them (Figure 5). For the cultivar Pingo de Mel, the three treatments did not present significant differences between them. The same was verified when the different cultivars were compared.

The source:sink relationship can be manipulated in two ways by increasing or decreasing the source strength or the sink strength (DUARTE and PEIL, 2010). This may explain the results obtained in this work, because when we observed the cultivar Roxo de Valinhos, the yield was higher in plants that were submitted to treatment with 24 branches, that is, possibly in this density there was a balance between the source and the sink of photoassimilated. In the 16 branches density, it is possible that a source strength was restricted, not enough to supply the effects of the sinks, and the 32 branches density of the drain strength was much higher, allowing the explanation of the lower observed use in both cases.

The yield of green fruits in the plants with 32 productive branches was superior to the other treatments in both cultivars, however, when compared to each other, the cultivar Roxo de Valinhos showed superiority in all treatments, presenting approximate productivity of 32, 34 and 45% higher than the Pingo de Mel for the plants with 16, 24 and 32 branches, respectively.

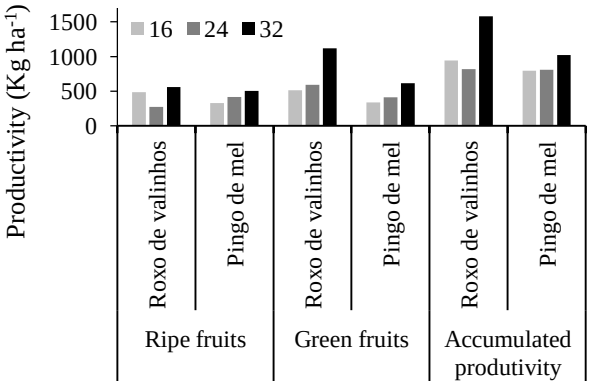

FIGURE 5 - Productivity of ripe fruits (kg ha^{-1}), green fruits (kg ha^{-1}) and accumulated productivity (kg ha^{-1}) for Roxo de Valinhos and Pingo de Mel with different numbers of branches. Averages followed by the same letter do not differ statistically from each other by the Tukey test, at 5% probability. Equal lowercase letters in the columns within each cultivar in the group evaluated and upper case among the cultivars in the same numbers of branches in the evaluated group did not differ significantly. Mature fruits CV (%) = 43.50, green fruits CV(%) = 21.60, cumulative productivity CV(%) = 26.10.

For the accumulated productivity the plants conducted with 32 branches were approximately 40% more productive than the other treatments in the cultivar Roxo de Valinhos, while the cultivar Pingo de Mel did not differ between the treatments. Among the cultivars was verified difference only for the plants conducted with 32

productive branches and Pingo de Mel was about 35% less productive.

Nava et al. (2015) studying the thinning of branches for the quality and productivity of fig Roxo de Valinhos, also observed an increase in productivity per plant with an increase in the number of productive branches. Nienow et al. (2006) evaluating different pruning seasons and number of branches in fig, for the variable yield of mature fruits also observed that in the plants with the highest number of branches there was an increase in productivity. The number of productive branches has a great influence on productivity (CAETANO et al., 2005), and with the highest number of productive branches in the plant the tendency is that there is consequently higher productivity, however, the plant spacing and the climatic conditions of the also become decisive for this increase to take place.

When evaluating the average weight of the fruits (Table 2), it was observed that the cultivars did not present differences in relation to the number of branches studied, however, when compared to each other the cultivar Roxo de Valinhos stood out, producing heavier fruits, independently of branches number with which the plants were conducted. Nienow et al. (2006) evaluated the average weight of figs grown in protected environment and submitted to different periods of pruning and branches number, observed fruits of the cultivar Roxo de Valinhos with 57.50 g. Chalfun (2012) cites that the fruits of the cultivar Pingo de Mel present weight around 35 g, resembling the observed results, being that the average weight of the fruits for this cultivar was of 35.80 g.

TABLE 2 - Effect of the different number of fig tree branches Roxo de Valinhos and Pingo de Mel on average fruit weight, fruit size, soluble solids and dry matter.

Cultivars	16	24	32
	Average weight of the fruit (g)		
Roxo de Valinhos	46.72 aA*	41.69 aA	46.33 aA
Pingo de Mel	37.23 bA	34.56 bA	35.66 bA
CV(%)		18.50	
	Fruit size (cm^3)		
Roxo de Valinhos	20.05 aA	21.42 aA	23.05 aA
Pingo de Mel	15.72 bA	14.86 bA	15.77 bA
CV(%)		14.00	
	Soluble solids (°Brix)		
Roxo de Valinhos	11.65 aA	12.18 aA	12.04 aA
Pingo de Mel	11.85 aA	12.43 aA	12.00 aA
CV(%)		8.10	
	Dry matter (%)		
Roxo de Valinhos	16.46 aA	18.00 aA	17.22 aA
Pingo de Mel	17.00 aB	19.50 aA	18.34 aAB
CV(%)		13.10	

*Averages followed by the same letter do not differ statistically from each other by the Tukey test, at 5% probability. Lowercase letters represent the columns and uppercase letters represent the rows.

The fruit size variable (Table 2), for both cultivars there was no difference between the number of branches. However, when comparing the cultivars, the cultivar Roxo de Valinhos maintained the superiority, in agreeing with the data observed by Rodrigues et al. (2009).

The evaluation of soluble solids (Table 2) did not present significant difference for the different treatments. Rodrigues et al. (2009) evaluating mutant selections of Roxo de Valinhos fig tree showed minimal variation among the treatments studied. Turk (1989), characterized the Brazilian fig as having soluble solids around 11.08ºBrix, similar to the values observed in this work.

About the dry matter contents (Table 2), the cultivars did not differ statistically among themselves. In relation to the number of branches $plant^{-1}$, the Pingo de Mel cultivar presented superiority of dry mass in its fruits when the plants were conducted with 24 productive branches, but did not differ from the treatment with 32 branches.

It is possible to observe that the cultivar Roxo de Valinhos presented larger fruit size and higher fruit weight, but this did not reflect the higher content of dry matter and soluble solids. In general, there is an order for the destination of the photoassimilates for their sinks, firstly they are destined for the formation of roots, later for the fleshy fruit parts and shoot apices and leaves, followed by the cambium, roots and finally the storage (MINCHIN et al., 1997). The cultivar Roxo de Valinhos translocates more carbon to the meristematic parts, followed by the fruit destination (SILVA et al., 2011).

Despite the good indications that we obtained in the first productive year of the plants, to obtain more consistent results it is necessary to evaluate these practices for more productive cycles. In addition, another important aspect is to assess the quality of the fruits more thoroughly, by determining the influence of each management on the nutritional and nutraceutical composition of the fruits.

CONCLUSIONS

The different numbers of branches did not influence the quality of the fruits.

The cultivar Roxo de Valinhos has larger fruits.

Both for the production of fruits for fresh consumption and for the industrialization it is recommended the conduction with greater number of productive branches being between 24 and 32, for the greater productive yield.

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Simultaneous selection of peach rootstocks by mixed models

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Abstract

The term adaptability refers to the ability of a genotype to respond favorably to environmental spur, while stability is the predictability of genotypic behavior. Therefore, the objective was to select *Prunus* rootstock cultivars with greater adaptability and genotypic stability for subtropical environmental conditions using the HMPRVG method. The experiment was conducted in Chapecó, Santa Catarina State, Brazil. Twenty-one rootstock genotypes were evaluated under the 'BRS-Libra' canopy cultivar and one genotype from self-rooted seedlings. The 22 genotypes were evaluated for canopy volume, yield, fruit diameter and fruit set in the growing seasons 2015/16, 2016/17, 2017/18 and 2018/19. Adaptability and stability were measured by means of the harmonic mean relative performance of genotypic values (HMRPGV). In addition, genetic parameters for heritability and ratio test were measured. According to the results, the self-rooted, 'De Guia', 'I-67-52-4', 'Mexico Row 1' and 'Rosaflor' genotypes coincided most frequently in the ranking of the three most adaptable and stable genotypes. On the other hand, the '*P. mandshurica*', 'Rigitano' and 'Santa Rosa' genotypes corresponded to the lowest adaptability and stability values, thus constituting low quality genetic materials for cultivation. It can be concluded that under the tested conditions the HMPRVG method is efficient for the *Prunus* rootstock selection cultivars and the 'BRS-Libra' grafted on 'Mexico Row 1', 'Rosaflor' rootstocks and trees from self-rooted seedlings have greater adaptability and phenotypic stability under the subtropical cultivation conditions.

Keywords: adaptability, stability, *Prunus persica*, harmonic mean

Introduction

The longevity, economic and environmental viability of a peach orchard (*Prunus persica* L.) are directly correlated with the rootstock that is used, as well as the grafting affinity between the cultivar and rootstock (Reig et al., 2019). The recommendation of superior peach genotypes should meet the minimum criteria that allow inferring about the genotypic potential in the cultivation environment (Souza et al., 2017). In this context, the preference for rootstock cultivars with superior characteristics that result in better fruit quality and greater adaptability and production stability is of great interest.

The term adaptability refers to the ability of a genotype to respond supportively to environmental stimulus, while stability is the predictability of genotypic behavior (Mendes & Ramalho, 2018). Several methods have been proposed to evaluate phenotypic adaptability and stability such as AMMI (Gauch, 1992) and GGE biplot (Yan & Rajcan, 2002), however, for the breeder, the major interest is in adaptability and genotypic stability. Thus, the Harmonic Mean Relative Performance of Genotypic values (HMRPGV) method has been expanded in recent years (Rosado et al., 2019; Olivoto et al., 2019; Sousa et al., 2019).

The HMRPGV method is based on mixed models and simultaneously provides, in a single measure on the evaluated character scale, the adaptability, stability and performance of the characteristic (Viana et al., 2010). The use of methods that convert adaptability and stability measures together with productivity into a single value is preferred as it allows simple interpretation, specifically in studies with high genotype numbers (Cruz et al., 2014).

Currently, in Brazil, peach tree breeding has focused mainly on the production of new scion cultivars (Donadio et al., 2019). Having in mind the importance

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and influence of rootstock on the longevity, yield and orchard fruits standardization, studies that seek to evaluate the effect of available rootstock cultivars are essential. In addition, studies in order to evaluate the individual performance of existent rootstock cultivars are of fundamental importance to support the development of new cultivars with superior characteristics to those already available. The main rootstocks used in the production of peach trees in Brazil are 'Okinawa' and 'Capdebosq'. But they usually confer a high vigor to the trees, which prevents the planting in high density (Donadio et al., 2019).

Several studies have applied the HMRPGV methodology in annual crops, such as corn (Mendes et al., 2012), rice (Colombari et al., 2013), beans (Santos et al., 2019) and soybean (Freiria et al., 2018). However, studies focusing on perennial crops such as peach trees, more specifically to evaluate the rootstock, are unpublished and need more development. Therefore, the aim was to select *Prunus* rootstock cultivars with greater adaptability and genotypic stability for subtropical environmental conditions using the HMPRVG method.

Material and Methods

Experimental conditions

The experiment was conducted in Chapecó, Santa Catarina, Brazil (27 ° 07 'S, longitude 52 ° 42' W, 605 m). The local climate is classified as subtropical humid (Cfa - according to Köppen classification). The local soil is clayey and classified as Latossolo Vermelho Distroférrico (Oxisol), with a basic pH, i.e., requiring no liming. The evaluations were carried out in the growing seasons 2015/16, 2016/17, 2017/18 and 2018/19. At the beginning of the evaluations, the trees were three years old. The management of diseases, insects and weeds, pruning and fertilization were carried out according to recommendations for peach (Raseira et al., 2014). No irrigation system and dormancy- breaking chemicals were adopted. The climatic data for the study period are presented in Figure 1.

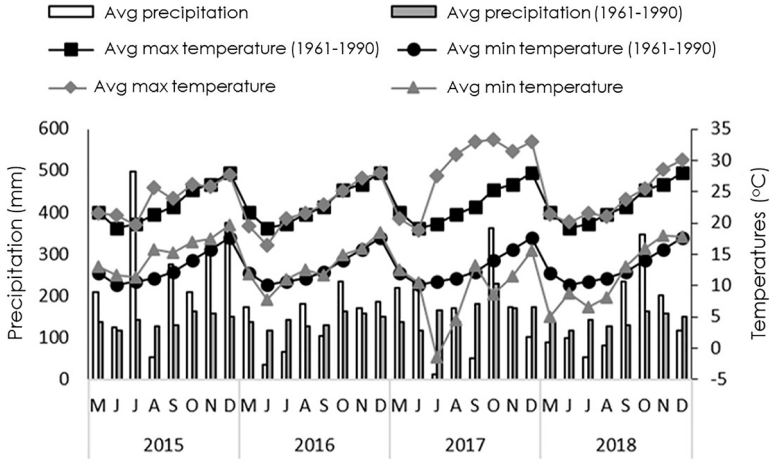

Figure 1. Climate data from May to December from 2015 to 2018 in Chapecó, state of Santa Catarina, Brazil. Source: Chapecó Climatologic station, EPAGRI-CIRAM.

Genotype rootstocks

A total of 21 clonal rootstocks grafted with the 'BRS-Libra' canopy cultivar were studied: 'Barrier' (2), 'Cadaman' (3), 'Capdeboscq' (4), 'Clone' 15 (5), 'De Guia' (6), 'Flordaguard' (7), 'G × N9' (8), 'GF 677' (9), 'I-67-52-4' (10), 'Ishtara' (11), 'Mexico Row 1' (12), 'Nemared' (13), 'Okinawa' (14)), '*Prunus mandshurica*' (15), 'Rigitano' (16), 'Rosaflor' (17), 'Santa Rosa' (18), 'Late 01' (19), 'Tsukuba-1' (20), 'Tsukuba-2' (21) and 'Tsukuba-3' (22) . As a control, we used self-rooted seedlings (without rootstock) of cultivar 'BRS-Libra', totalizing 22 different genotypes evaluated. In the current study, treatment without rootstock, that is, from self-rooted seedlings is called Self-rooted (1).

Experimental design

The experiment was conducted in randomized block design with four replications. Each replication was consisted by one plant. The experimental plot was implanted in a spacing of 5m × 2m conducted in "Y" form (epsilon), as proposed by Grossman & Dejong (1998). No irrigation system was adopted.

Evaluated Characters

The influence of different genotypes on canopy volume, expressed in m^3, was evaluated by collecting data regarding the width, thickness and height of the canopy with a tape measure assistance. Two mixed branches located in the middle part of the plant, one in each main branch, were selected during the flowering

period. In each secondary branch, the number of flowers and, subsequently, fruit set before thinning were counted to verify the proportion between flowers and fruit set, expressed in %. Genotypes were also evaluated for yield per tree (kg tree^{-1}), fruit diameter (mm) with the aid of a digital caliper and soluble solids (°Brix) using bench refractometer.

Statistical analysis

Repeatability and interaction model genotype x measurement

Matrix mode

$$(1) \quad y = Xm + Zg + Wp + Ti + e,$$

Where y is the phenotype vector, m is the fixed effects vector of the measurement-repeat combinations (the vector m contemplates all measurements at all repetitions and adjusts simultaneously for the effects of repetitions, measurement, and repetition x measurement interaction) summed to the overall average; g is the random vector of genotypic effects; p is the random vector of the permanent environment effects (plots), i is the random vector of the effects of the genotype x measurement interaction, and e is the random vector of residuals. Capital letters represent the incidence matrices for these purposes, and are, respectively, the variances of genotypic effects, permanent environment, genotype x measurement and residual interaction.

Algebraic model

$$y_{ijk} = u + r_j + m_k + rm_{jk} + g_i + gr_{ij} + gm_{ik} + e_{ijk},$$

Wich:

u is the effect of the average (fixed);

r_j is the effect of repetition j (fixed);

m_k is the effect of measure k (fixed);

rm_{jk} is the effect of the interaction repetition x measurement (fixed);

g_i is the genotype effect *i* (random) $g_i N(0, \sigma_g^2)$

gr_{ij} is the permanent environment effect (random) $gr_{ij} N(0, \sigma_p^2)$

gm_{ik} is the effect of genotype x measurement interaction (random), $gm_{ik} N(0, \sigma_{gm}^2)$

e_{ijk} is the residue (random), $e_{ijk} N(0, \sigma_e^2)$

Genetic parameters

Inheritance of individual plots in the broad sense (h_g^2)

$$(2) \quad h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_p^2 + \sigma_{gm}^2 + \sigma_e^2}$$

Average heritability of genotypes in the broad sense (h_{gm}^2)

$$(3) \quad h_{gm}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_p^2}{j} + \frac{\sigma_{gm}^2}{k} + \frac{\sigma_e^2}{jk}}$$

Genotypic correlation through measurements (r_{gmed})

$$(4) \quad r_{gmed} = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_i^2}$$

Harmonic Mean of Relative Performance of Genotypic Values (HMRPGV)

A method for classifying genotypes considering yield and stability simultaneously is the harmonic mean of genotypic values (MHVG), and considering yield and adaptability simultaneously is the relative performance of genotypic values (PRGV) on crops or measurements. In order to contemplate productivity, adaptability and stability, the harmonic mean of relative performance of genotypic values - HMRPGV (Resende, 2007) can be used.

The harmonic means of genotypic values ($\boldsymbol{MHVG_i}$) of each genotype were obtained by:

$$(5) \quad MHVG_i = \frac{n}{\sum_{I,K=1}^{i,k} \frac{1}{VG_{ik}}},$$

where: n is the number of harvests (measures or harvests - quarter in the case); *i* is the number of genotypes (22 genotypes); $\boldsymbol{VG_{ik}}$ is the genotypic value of genotype i in crop (measure) *k*, that is, are the capitalized genotypic values for the genotype x measurement interaction.

The relative performance of genotypic values ($\boldsymbol{PRVG_i}$) of each genotype was obtained by:

$$(6) \quad PRVG_i = \frac{1}{n} \sum_{K=1}^{k} \frac{GV_{ik}}{m_k},$$

where: m_k is the phenotypic mean of each measurement (harvest).

Simultaneous selection for productivity, stability and adaptability is given by the harmonic mean of the relative performance of genotypic values ($\boldsymbol{MHPRVG_i}$) obtained by:

$$(7) \quad MHPRVG_i = \frac{n}{\sum_{K=1}^{k} \frac{1}{PRVG_{ik}}},$$

Software and statistical tests

The random effects of the models were tested using the likelihood ratio test (LRT) considering the chi-square statistic with a degree of freedom and 5% of probability. The Selegen software (Resende, 2016) was used in all statistical procedures.

Results and Discussion

Likelihood Ratio Test

Through the likelihood ratio test (Figure 2), only the fruit diameter character showed no significant genotypic effect (p> 0.05). Therefore, for the other characters evaluated there is significant effect of genotypes (p <0.05), this indicates that there is genetic variability and that it is possible to select superior genotypes. For permanent environment effects, significance was observed (p <0.05) only for fruit set. This is because, this trait is largely affected by internal and external environmental factors such as competition for nutrients and climate conditions (Morimoto et al., 2019). Regarding the effects of the interaction Genotypes × Environments (G × E), significance was observed for all analyzed variables (Figure 2).

GxE interaction can be statistically explained as genotype classification in different orders in different environments, or genetically as differences in gene expression under different environmental conditions (Lynch & Walsh 1998; White et al., 2007). One of the main ways to overcome the adverse effects of GxE interaction is to identify cultivars with greater genotypic stability and adaptability (Resende, 2007) and across selection strategy that capitalize on GxE interaction (Rocha et al., 2019).

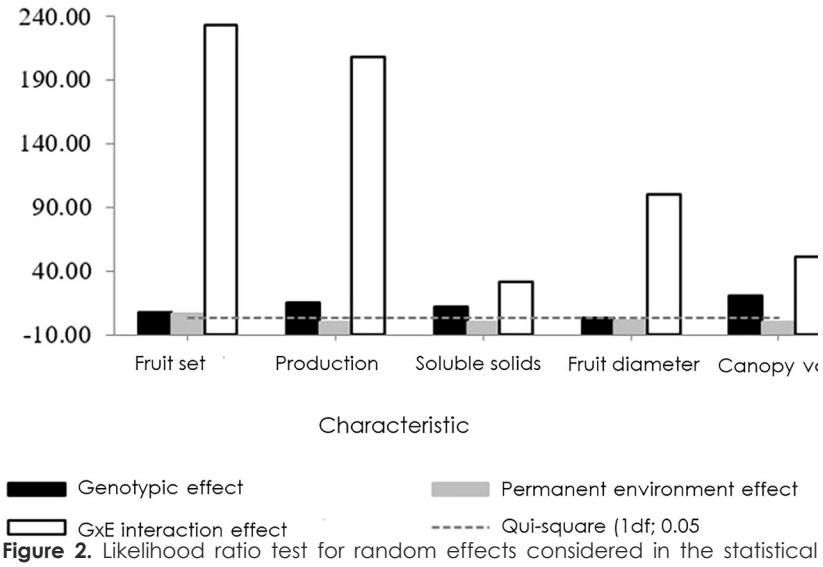

Figure 2. Likelihood ratio test for random effects considered in the statistical model. All bars above the dashed line indicate significant effects by the Chi-square test at 5% probability.

Heritability of individual plots, average genotype heritability, genotypic correlation across measurements and average performance across measurements

The heritability coefficient of individual plots in the broad sense (Table 1) indicates the ratio of genotypic variance to phenotypic variance of individual plots. Based on this, only yield and canopy volume showed medium magnitude heritability. For the other characters (soluble solids, diameter and fruit set), heritability of low magnitude (<30%) was observed indicating that the variance due to the environment has a strong influence.

Soluble solids content, for example, is a character strongly influenced by the environment, associated with temperature variations, rainfall distribution and fruit location in the canopy. Fruits located in shaded portions of the canopy will receive less solar radiation. Consequently, there will be interference in the synthesis of photoassimilates reducing the soluble solids content of the fruit (Alves et al., 2018). Thus, the selection of genotypes based only on the soluble solids content of their fruits is equivalent to a less efficient selection.

For the genotypic correlation through the measurements (Table 1), it was observed the lowest correlation for the fruit diameter character and the largest for canopy volume. The correlation between the measurements represents the proportion of the genotypic variation present between the years of evaluation, how much the heritable fraction represents in the phenotypic variation of the character in question. Thus, fruit diameter has a high phenotypic variation, being of greater proportion of non-heritable scope.

Regarding the phenotypic averages (Table 1), 2017 showed a considerable reduction in fruit set and yield compared to the previous year (2016). This was due to the incidence of severe frost during the full bloom period (Figure 1). As consequence of the low fruit set, there was a reduction in production equivalent to approximately 75% compared to the average of 2016

and 2018. Such environmental adversity is a major factor for the occurrence of GxE interactions and therefore lead to reductions in correlations between measurements. However, the overall yield average observed in the present study resembles that found in southern Brazil (Barreto et al., 2017), northern Tunisia (Yahmed et al., 2016) and northern and southern Italy (Scalisi et al., 2018).

Table 1. Estimation of genetic parameters. Heritability of individual plots in the broad sense (h_g^2), heritability in the broad sense of genotype mean (h_{gm}^2), genotypic correlation through measurements (r_{gmed}), phenotypic mean of each measurement, and overall phenotypic mean through measurements.

Genetic parameters	Fruit set (%)	Production (kg tree^{-1})	Soluble solids (°Brix)	Fruit diameter (mm)	Canopy volume
h_g^2	0.2502	0.3396	0.2146	0.1214	0.3226
h_{gm}^2	0.6132	0.7188	0.6889	0.4480	0.7761
r_{gmed}	0.3064	0.4128	0.4695	0.2026	0.5463
2015 mean	14.18	2.02	8.68	48.91	1.86
2016 mean	20.60	15.54	9.47	56.73	7.12
2017 mean	3.92	4.21	10.28	55.94	12.04
2018 mean	37.45	17.26	8.14	57.59	19.04
Overall average	19.04	9.76	9.14	54.79	10.01

Harmonic mean of relative performance of genotypic values

Selecting genotypes only at the predicted average genotypic value for yield does not guarantee that they will perform better when grown under other conditions (Cruz et al., 2014). Moreover, the main complicating factor in recommending cultivars under this situation is the GxE interaction, as it requires the breeders to adopt different methods to mitigate this interaction and facilitate the selection of superior genotypes (Tiwari, 2019). Therefore, the adaptability and stability analysis of the predicted genotypic values was preceded by the harmonic mean method of relative performance of the genotypic values (HMRPGV).

According to this methodology, 'Tsukuba-3', 'De Guia' and 'Barrier' genotypes formed the group with the highest HMVG values for fruit set (Table 2), indicating that they present the lowest variation in genotypic values. For the variables production and soluble solids, the self-rooted genotypes, 'Mexico Row 1' and 'De Guia' coincided 100% in the ordering of the three best genotypes. The variables fruit diameter and canopy volume refer, respectively, to fruit quality and plant vegetative vigor. 'BRS-Libra' grafted on 'Barrier', 'Clone 15' and 'Santa Rosa' genotypes presented greater stability for fruit diameter while 'De Guia', 'Flordaguard' and 'I-67-52-4' genotypes promoted more stable for canopy volume.

It is noteworthy that, by this method, the lower the standard deviation of genotypic behavior between years of cultivation, the higher the genotypic values of the harmonic mean (Resende, 2007). Therefore, selection by the highest HMVG values implies the simultaneous selection of stability, or even invariance of genotypic values.

As for the genotypes of less stability, '*Prunus mandshurica*' and 'Rigitano' stand out in relation to the others. More specifically, the '*P. mandshurica*' rootstock coincided 100% in the ranking of the three worst genotypes for all evaluated characters. This reveals instability of quality and fruit production as well as vegetative growth of 'BRS-Libra' when grafted on '*P. mandshurica*'. Similarly, the 'Rigitano' genotype was among the three worst genotypes, however, with relatively minor coincidence (60%).

The PRVG values for the self-rooted genotype show that it contributed to the increase of the average of each evaluation year in greater proportion, under the conditions in which it was evaluated. This genotype stands out as of great genotypic adaptability, since it has the highest contribution values to increase the average of each growing season. For traits fruit set, yield and soluble solids, the genotype presented an average 1.37, 1.60 and 1.17 times the average of the year of cultivation in which it was evaluated. However, trees from self-rooted seedlings responded to a smaller degree for fruit diameter and canopy size, since their PRVG were lower.

The '*P. mandshurica*' genotype presented the lowest adaptation, since it coincided 80% in the ranking of the three worst genotypes regarding the PRVG values. In this sense, although there is a worldwide trend for the use of interspecific rootstock × scion combinations observed in both peach (Zarrouk et al., 2006; Neves et al., 2017) and in pear (Melo et al., 2017), in order to introduce useful characteristics such as vigor control, pest resistance and edaphoclimatic adaptation, such a combination does not always result in success.

An example is that presented in the present work, which revealed that the combination *P. mandshurica* ×

P. persica resulted in graft incompatibility. According to Darikova et al. (2011) incompatibility in interspecies combinations occurs mainly due to anatomical, morphological and physiological differences between grafting components.

Table 2. Ranking of genotypes considering productivity and stability by the MHVG method, productivity and adaptability by the PRVG method and productivity, stability and adaptability by the HMRPGV method. Values in parentheses are rootstock genotypes coded according to the following relationship: 'Self-rooted' (1), 'Barrier' (2), 'Cadaman' (3), 'Capdeboscq' (4), 'Clone 15' (5), 'De Guia' (6), 'Flordaguard' (7), 'G × N9' (8), 'GF 677' (9), 'I-67-52-4' (10), 'Ishtara' (11), 'Mexico Row 1' (12), 'Nemared' (13), 'Okinawa' (14), '*Prunus mandshurica*' (15), 'Rigitano' (16), 'Rosaflor' (17), 'Santa Rosa' (18), 'Late 01' (19), 'Tsukuba-1' (20), 'Tsukuba-2' (21) and 'Tsukuba-3' (22).

Order	Fruit set			Production			Soluble solids		
	MHVG	PRVG	HMRPGV	MHVG	PRVG	HMRPGV	MHVG	PRVG	HMRPGV
1	16.09 (22)	1.40 (22)	1.35 (1)	7.37 (1)	1.60 (1)	1.59 (1)	10.65 (1)	1.17 (1)	1.17 (1)
2	15.18 (6)	1.37 (1)	1.30 (10)	6.76 (12)	1.40 (17)	1.39 (17)	9.86 (12)	1.09 (17)	1.09 (17)
3	14.77 (2)	1.33 (9)	1.24 (6)	6.38 (6)	1.33 (12)	1.32 (12)	9.78 (6)	1.08 (12)	1.07 (12)
4	13.06 (4)	1.31 (6)	1.24 (9)	6.37 (14)	1.32 (6)	1.30 (6)	9.51 (14)	1.05 (6)	1.05 (6)
5	13.02 (10)	1.30 (10)	1.22 (2)	6.27 (10)	1.28 (10)	1.26 (10)	9.45 (10)	1.04 (10)	1.04 (10)
6	12.06 (1)	1.28 (2)	1.18 (11)	6.06 (17)	1.20 (14)	1.14 (14)	9.31 (17)	1.03 (14)	1.03 (14)
7	10.96 (11)	1.19 (11)	1.11 (22)	5.9 (2)	1.16 (2)	1.09 (2)	9.30 (2)	1.03 (2)	1.03 (2)
8	10.49 (9)	1.16 (12)	1.10 (8)	5.40 (4)	1.11 (13)	1.09 (13)	9.22 (4)	1.02 (13)	1.02 (13)
9	10.01 (14)	1.14 (8)	1.00 (12)	5.21 (13)	1.06 (3)	1.03 (3)	9.10 (13)	1.01 (3)	1.00 (3)
10	9.86 (3)	1.09 (4)	0.95 (4)	5.01 (3)	1.06 (7)	1.01 (22)	9.09 (3)	1.00 (7)	1.00 (22)
11	9.75 (8)	0.98 (14)	0.95 (17)	4.33 (22)	1.05 (4)	1.00 (21)	9.09 (22)	1.00 (4)	1.00 (21)
12	9.56 (20)	0.98 (17)	0.94 (3)	4.21 (20)	1.05 (21)	0.98 (4)	9.03 (20)	1.00 (21)	1.00 (4)
13	8.74 (19)	0.95 (3)	0.87 (14)	4.2 (11)	1.03 (8)	0.98 (7)	8.89 (11)	0.98 (8)	0.98 (7)
14	8.22 (17)	0.88 (20)	0.87 (20)	4.08 (21)	1.02 (22)	0.93 (20)	8.84 (21)	0.98 (22)	0.97 (20)
15	7.66 (18)	0.86 (19)	0.86 (19)	4.05 (7)	0.94 (9)	0.92 (8)	8.82 (7)	0.97 (9)	0.97 (8)
16	7.13 (12)	0.78 (13)	0.67 (13)	3.99 (19)	0.93 (20)	0.90 (19)	8.79 (19)	0.97 (20)	0.97 (19)
17	4.99 (21)	0.74 (7)	0.63 (21)	3.67 (9)	0.91 (19)	0.86 (9)	8.77 (9)	0.97 (19)	0.97 (9)
18	4.98 (13)	0.74 (18)	0.58 (7)	3.44 (8)	0.86 (5)	0.80 (5)	8.59 (8)	0.95 (5)	0.95 (5)
19	4.10 (7)	0.72 (16)	0.58 (16)	3.26 (5)	0.78 (11)	0.64 (11)	8.41 (5)	0.93 (11)	0.93 (11)
20	4.08 (16)	0.7 (21)	0.56 (18)	1.81 (16)	0.52 (16)	0.48 (16)	8.40 (16)	0.93 (16)	0.93 (16)
21	3.13 (15)	0.57 (5)	0.45 (15)	0.62 (18)	0.23 (18)	0.19 (18)	8.39 (18)	0.92 (18)	0.92 (18)
22	0.52 (5)	0.54 (15)	0.12 (5)	-0.06 (15)	0.15 (15)	-0.03 (15)	7.94 (15)	0.88 (15)	0.87 (15)

Continuation…

Order	Fruit diameter			Trunk diameter		
	MHVG	PRVG	HMRPGV	MHVG	PRVG	HMRPGV
1	57.93 (2)	1.06 (2)	1.06 (2)	7.47 (6)	1.34 (6)	1.32 (12)
2	57.55 (5)	1.05 (5)	1.05 (5)	7.03 (7)	1.32 (12)	1.30 (6)
3	56.66 (18)	1.04 (18)	1.04 (18)	7.03 (10)	1.31 (7)	1.30 (7)
4	56.00 (4)	1.03 (4)	1.03 (4)	7.00 (1)	1.31 (10)	1.29 (10)
5	55.55 (19)	1.02 (19)	1.02 (19)	6.69 (12)	1.28 (1)	1.24 (1)
6	55.22 (16)	1.01 (7)	1.01 (7)	6.6 (13)	1.24 (13)	1.23 (13)
7	55.17 (12)	1.01 (8)	1.01 (8)	6.24 (8)	1.16 (8)	1.15 (8)
8	55.16 (7)	1.01 (12)	1.01 (12)	5.73 (17)	1.11 (17)	1.11 (17)
9	54.88 (8)	1.01 (16)	1.01 (16)	5.67 (4)	1.09 (2)	1.08 (2)
10	54.86 (21)	1.01 (21)	1.01 (21)	5.61 (2)	1.08 (4)	1.08 (4)
11	54.84 (22)	1.00 (1)	1.00 (1)	5.47 (3)	1.07 (3)	1.07 (14)
12	54.75 (1)	1.00 (10)	1.00 (10)	5.41 (14)	1.07 (14)	1.06 (3)
13	54.73 (10)	1.00 (11)	1.00 (11)	5.35 (9)	1.05 (9)	1.05 (9)
14	54.63 (11)	1.00 (22)	1.00 (22)	3.90 (5)	0.92 (20)	0.88 (20)
15	54.19 (17)	0.99 (6)	0.99 (6)	3.62 (20)	0.86 (19)	0.84 (5)
16	53.86 (6)	0.99 (17)	0.99 (17)	3.50 (19)	0.84 (5)	0.84 (19)
17	53.49 (13)	0.98 (13)	0.98 (13)	3.38 (16)	0.82 (22)	0.78 (16)
18	53.48 (14)	0.98 (14)	0.98 (14)	2.79 (22)	0.81 (21)	0.75 (22)
19	52.80 (20)	0.97 (15)	0.97 (20)	2.56 (21)	0.80 (16)	0.72 (21)
20	52.27 (15)	0.97 (20)	0.96 (15)	2.30 (18)	0.64 (18)	0.59 (18)
21	50.30 (9)	0.94 (9)	0.92 (9)	1.95 (11)	0.53 (11)	0.50 (11)
22	49.88 (3)	0.91 (3)	0.91 (3)	-0.23 (15)	0.35 (15)	-0.15 (15)

Harmonic means of relative performance of genotypic values (HMRPGV) are based on genotypic values predicted through mixed models, which group stability, adaptability and productivity into a single statistical evaluation, facilitating the selection of superior genotypes (Resende, 2007). According to this criterion, the 'Self-rooted', 'De Guia', 'I-67-52-4', 'Mexico Row 1' and 'Rosaflor' genotypes coincided most frequently in the ranking of the five most adaptable and stable genotypes. On the other hand, the '*P. mandshurica*', 'Rigitano' and 'Santa Rosa' genotypes corresponded to the lowest adaptability and stability values, thus constituting low quality genetic materials for cultivation.

The evaluation of a genotype in different agricultural years is extremely important in research. The identification of productive genetic materials with greater stability and adaptability within the evaluated characters may attenuate the G × E interaction. From this it is possible to recommend rootstock cultivars with greater precision. In this sense, the harmonic mean genotypic values (HMRPGV) allows selection based on stability, adaptability and yield in a single statistic.

From the adopted methodology it is possible to identify the best performing genotypes for each evaluated character in the four years of cultivation and, thus, select them for recombination starting a program of genetic improvement of peach rootstocks.

The self-rooted genotype stood out with respect to the traits fruit set, production and soluble solids. The 'Barrier' genotype presented a similar behavior to the self-rooted one. However, the fruit diameter character was added. Two other genotypes that presented yield and stable and adaptable soluble solids content were 'Mexico Row 1' and 'Rosaflor', which differ only in the size of the canopy that is included in the 'Mexico Row 1' genotype.

The genotypes 'Clone 15' and 'Capdeboscq' produced fruits with larger diameter, and the 'Capdeboscq' rootstock presented, in addition, larger canopy volume. From this perspective, recombination between the 'Barrier' and 'Capdeboscq' genotypes is recommended, for example, when the goal is to combine adaptability and stability of all characters evaluated in the present study into a single genotype.

However, it is noteworthy that the self-rooted genotype had advantageous characteristics for quality and fruit production in terms of adaptability and stability. Thus, such genotype presents itself as a viable alternative to the beekeeper, since the removal of the rootstock component will result in lower cost for the formation of the nursery tree, favoring the farmer at the moment of the tree acquisition.

Conclusions

The HMPRVG method was efficient for the selection of *Prunus* rootstock cultivars. It can be concluded that under the tested conditions, 'Mexico Row 1', 'Rosaflor' rootstocks and plants from self-rooted seedlings had greater adaptability and phenotypic stability under subtropical cultivation conditions. '*Prunus mandshurica*', 'Rigitano' and 'Santa Rosa' genotypes showed lower adaptability and phenotypic stability, and were not recommended to peach rootstock breeding program.

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FISIOLOGIA VEGETAL

MANUAL DE AULAS PRÁTICAS

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Este projeto teve o intuito de contribuir na formação acadêmica dos estudantes da UFMA e de outras instituições de ensino superior que vierem a utilizar como referência bibliográfica. Gostaríamos de deixar registrado aqui, nossa enorme gratidão a todos os professores e colaboradores, das diversas instituições de ensino superior que colaboraram de forma direta e indireta, da concepção à concretização, desta obra intitulada "**Fisiologia Vegetal – MANUAL DE AULAS PRÁTICAS**". A todos vocês nossa eterna gratidão. Nossa gratidão também se estende aos monitores não bolsistas da disciplina de Fisiologia Vegetal, ministrada aos cursos de Agronomia, Biologia e Zootecnia do CCAA/UFMA, que se empenharam ao máximo para que o conteúdo aqui disponível fosse desenvolvido. Isto implica enfatizar que todos os discentes absortos neste projeto, ao longo de sua implementação, condução e conclusão, aqui apresentada, desenvolveram não apenas habilidades de busca, seleção e implementação, mas sim, avaliação crítica do processo de ensino-aprendizagem de forma holística e interdisciplinar, buscando com o engajamento individual junto ao grupo tornarem-se agentes ativos no seu processo de construção e transmissão do saber. Desta forma, didaticamente, estes estudantes possibilitaram desenvolver material de fácil compreensão e didático a ser base aos que seguem este desafio de compreender os processos que transcorrem durante o desenvolvimento fisiológico das plantas.

PREFÁCIO

Compreender e descrever o funcionamento dos vegetais é uma condição *sine qua non* para sobrevivência da sociedade moderna que consome em escala geométrica os alimentos produzidos em ritmo aritmético. Além de alimentos, os consumidores de nossa complexa teia alimentar buscam, também, na Fisiologia Vegetal soluções farmacológicas, climatológicas, dentre outras. As plantas produzem uma multiplicidade de compostos químicos, muitos ainda desconhecidos pela ciência, e que podem conter a solução para a maioria dos problemas de saúde pública. Nesse sentido, a Fisiologia Vegetal ganha relevância e jurisprudência para ser abordada inclusive nas áreas de formação mais distantes das ciências naturais, onde esses temas já têm sido frequentemente estudados. A prerrogativa de tratar os temas fisiológicos em nível aprofundado e apropriado para atender as demandas de nossa sociedade é de: estudantes, pesquisadores e professores da área de ciências naturais e da educação. Nesse manual o aspecto didático foi trabalhado com muita sensibilidade, permitindo que esse material seja facilmente utilizado por alunos da educação básica ao ensino superior. O presente manual permitirá o leitor imergir com muita facilidade nos ambientes e mecanismos de absorção e transporte de água, síntese e transporte de carboidratos, germinação de sementes, dentre outros, pois contém elementos didáticos que permitem ao leitor visualizar as manifestações bioquímicas e físicas desses eventos, assim como os ambientes onde esses eventos ocorrem. As atividades práticas protocolizadas no manual consolidam a percepção destes cenários, são carregadas de instruções que permitem aplicá-las tanto aos alunos da educação básica quanto aos alunos da graduação, sobretudo nos cursos ligados à produção vegetal.

A possibilidade de utilização desse material na educação básica mostra a preocupação dos autores em fomentar uma produção que atende à demanda da graduação, mas que pode suplementar com muita eficiência os temas relacionados à Fisiologia Vegetal ainda no ambiente da educação básica. Sendo assim uma excelente ferramenta de trabalho para os professores de Biologia e de Ciências.

Prof. Edison Fernandes da Silva
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TESTE DE TETRAZÓLIO

INTRODUÇÃO

Os testes rápidos para avaliar a qualidade das sementes são fundamentalmente importantes para agilizar a tomada de decisões referente ao manejo de lotes de sementes durante as etapas de pós-colheita (RODRIGUES et al., 2015). Nesse sentido, o teste de tetrazólio (Tz) tem-se mostrado uma ferramenta muito interessante, uma vez que identifica a viabilidade e o vigor das sementes de forma rápida (de modo geral, obtém-se resultados em períodos inferiores a 24 horas) e eficiente (DIAS; ALVES, 2008).

O Tz é fundamentando na atividade de enzimas desidrogenases (em especial a malato desidronegase) (BULAT, 1961; MOORE, 1973), que catalisam reações bioquímicas nas mitocôndrias durante as etapas da glicólise e ciclo de Krebs do processo respiratório. Mais especificamente, o Tz determina de forma indireta a atividade respiratória dos tecidos que compõe as sementes como, por exemplo, o embrião, a radícula e o escutelo.

As enzimas desidrogenases presentes nos tecidos vivos das sementes quando imersos em solução incolor de tetrazólio reduzem o cloreto de 2,3,5-trifeniltetrazólio (TTC) a trifenilformazan, substância de coloração vermelha, estável e indifusível (PETERS, 2007). Dessa forma, a mudança de cor do tecido do embrião para a vermelha indica que há atividade respiratória e, portanto, considera-se que as sementes estão vivas/viáveis. Além disso, a tonalidade de cor vermelha resultante da reação é indicativo do vigor da semente. Nesse sentido, se o tecido for vigoroso, ele apresentará cor vermelha clara. Se for de baixo vigor, o tecido exibirá cor vermelha intensa decorrente da rápida difusão do TTC por meio das membranas celulares danificadas. Por outro lado, os tecidos de sementes não viáveis, não reagem com o TTC e, portanto, não mudam de cor.

OBJETIVO

Determinar a viabilidade das sementes de milho e de soja por meio do teste de tetrazólio.

MATERIAL

Para realização da prática serão necessários os seguintes materiais:

- Semente de milho (*Zea mays*);
- Semente de soja (*Glycine max*);
- Estilete ou lâmina de barbear;

- Béqueres (ou copos plásticos);
- Solução de TTC (cloreto de 2,3,5-trifeniltetrazólio) a 1%;
- 1 frasco de vidro, cor âmbar, para armazenar a solução de tetrazólio*.

***Observação:** Não armazenar a solução de tetrazólio em frascos transparentes, uma vez que se degrada com a luz ou armazená- la em recipientes de metais, visto que o TTC pode ser reduzido a trifenilformazan pela ação dos íons metálicos (BULAT, 1961).

PROCEDIMENTO

Siga atentamente o passo a passo para obter sucesso na prática.

Passo 1

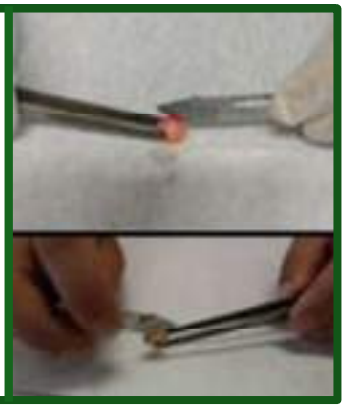
Em 2 béqueres (ou copos plásticos) deixe as sementes de milho e de soja embebidas em água na temperatura ambiente, por 12 a 24 horas.

Passo 2

Para cada espécie, coloque de 10 a 15 sementes previamente embebidas em água em dois béqueres e em um deles adicione água fervente e aguarde por 5 minutos.

Passo 3

Com um estilete ou com uma lâmina, corte as sementes longitudinalmente, expondo o embrião das sementes de milho e da soja.



Passo 4

Coloque as sementes em béqueres com solução de tetrazólio a 1%, fervidas e não fervidas, em béqueres separados. Após alguns minutos, verifique as alterações da cor das sementes.

Passo 5

Observe e compare as sementes que ficaram sob água fervente (imagens à esquerda) com aquelas que não passaram por este procedimento (imagens à direita).

Passo 6

Por fim, elabore um relatório composto com uma breve introdução a respeito do tema, metodologia e resultados, bem como registros fotográficos da prática. Este relatório deve ser entregue na aula seguinte.

APÓS A PRÁTICA VOCÊ SERÁ CAPAZ DE:

1) Descrever a reação que está envolvida no aparecimento da cor vermelha e indicar em que tecidos da semente ela não se desenvolve.
2) Explicar as razões da diferença de coloração observada entre os grãos que foram aquecidos em água quente e os grãos que não foram aquecidos.
3) Indicar a importância prática de se realizar o teste de tetrazólio.

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