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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.

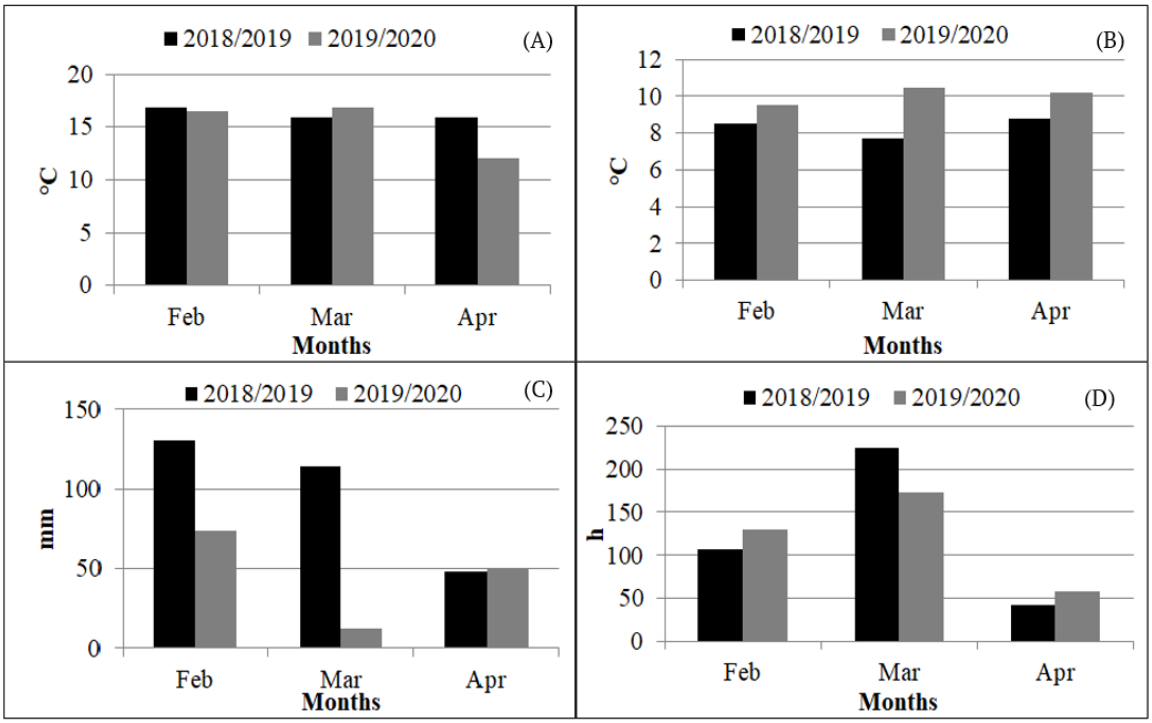

Figure 1. Weather data on average temperature (A), temperature range (B), precipitation (C) and hours of insolation (D) of the São Joaquim weather station for the 2018/2019 and 2019/2020 growing season (Company of Agriculture Research and Rural Extension of Santa Catarina- EPAGRI, 2020).

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.

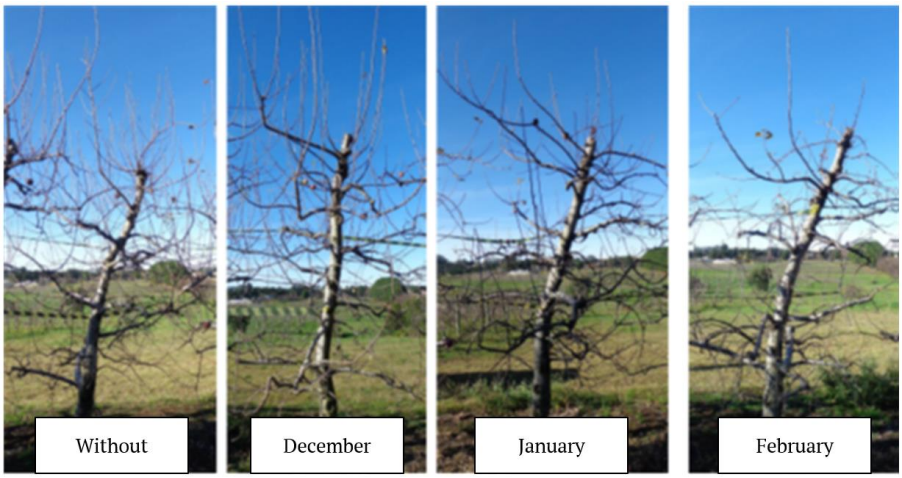

Figure 2. Architecture of plants subjected or not to summer pruning at different times during the winter rest period.

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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