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Summer Pruning, an Eco-Friendly Approach to Controlling Bitter Pit and Preserving Sensory Quality in Highly Vigorous Apple cv. ‘Reinette du Canada’

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Abstract: Summer pruning reduces vegetative growth in apple trees, but it could have an impact on fruit quality. This study analyzed the effects of summer pruning as an eco-friendly pre-harvest alternative to chemical growth regulation inputs on instrumental and sensory quality of highly vigorous apple cv. ‘Reinette du Canada’, which has been awarded with a Protected Designation of Origin label in two environments. The results showed that summer pruning affected the mineral content of the fruit. Summer pruning reduced bitter pit, but it did not negatively affect fruit weight nor any other instrumental characteristic during storage. Moreover, sensory quality or degree of liking were not affected by summer pruning. Thus, summer pruning could be an eco-friendly pre-harvest alternative to chemical treatments to improve quality in global terms of ‘Reinette du Canada’ apple cultivar, regardless of the location. This technique contributed to the decrease of bitter pit incidence, but did not decrease sensory quality nor degree of liking.

Keywords: calcium; degree of liking; environment; protected designation of origin; storage; sustainable management

1. Introduction

Reduction of fresh produce waste is an essential prerequisite of sustainable horticulture [1]. A great proportion of losses in apple production can be attributed to fruit physiological disorders, usually revealed during cold storage [2]. High acidity apple cultivar ‘Reinette du Canada’ (*Malus × domestica* Borkh.) is awarded with a Protected Designation of Origin (PDO) within the European Community (Commission Regulation (EC) No 2601/2001) due to its characteristic flattened shape, high firmness, high degree of skin russet and high level of acidity [3]. The consumption of this apple cultivar, not only as fresh fruit but also as dessert fruit (roasted apple), is well-known in Spanish homes, and modified atmosphere packaging in refrigeration conditions has been effective to extend the sensory acceptability of roasted apple cv. ‘Reinette du Canada’ [4]. However, this cultivar is severely affected by bitter pit (BP) during storage [5]. BP, is described as one of the most important physiological disorders of apple fruit whose symptoms appear late in the season and during storage, and can cause significant loss of profitability [6]. The incidence of BP in apple is related to the calcium content of the fruit [7]. In addition to Ca levels, the ratio of this nutrient to others such as magnesium, potassium and nitrogen can play a more important role in cell and tissue metabolism than the role of each nutrient alone [8].

‘Reinette du Canada’ is a highly vigorous cultivar, and excessive vegetative vigor has been linked to low levels of Ca in the fruit and the occurrence of BP, because during early fruit development vigorous shoots are strong competitors for available Ca and during this period much of the Ca deposition occurs in fruitlets, and excessive vegetative growth during this period may result in less Ca deposition in fruitlets [9].

Shoot length can be manipulated through horticultural management by using plant growth regulators [10]. Plant growth regulators such as Paclobutrazol can restrict vegetative growth and improve productivity and fruit quality in apples [11]. However, there is a need to reduce the use of chemical fertilizers and pesticides in order to reduce risks to health and the environment [12], since nowadays society demands more environmentally-conscious practices in order to replace chemical pre-harvest treatments. In fact, chemical growth regulation is not allowed within the guidelines for integrated fruit production (IFP) so preference should be given to natural means of vegetative growth control [13]. This is especially important in high quality products such as PDO apple cultivar ‘Reinette du Canada’, in order to satisfy customers who are willing to pay for more expensive apples without chemical postharvest treatments [14]. Summer pruning, which involves removing unproductive shoots called water sprouts [15,16], is performed with the main aim of controlling tree vigor and reducing the need for winter pruning [17]. Summer pruning reduces vegetative growth and, together with paclobutrazol, it is one of the most effective strategies for reducing shoot length in pears [18], as it improves canopy light penetration, re-exposes spur leaves and fruits in the interior canopy of apple trees, enhances fruit quality, concentrates fruit ripeness, and increases the number of flower buds [19–21]. However, summer pruning might limit the carbohydrate availability due to a decrease in the photosynthetic rate as a result of the leaves being removed, thus affecting fruit growth and sugar levels [17]. Summer pruning is considered a sustainable management strategy in dryland apple orchards where it promotes water use efficiency [22], or as an ecological alternative to fertilizers in order to decrease BP incidence during apple storage [14]. Nevertheless, maintaining high-quality standards for sensory quality is one of the priorities for horticultural products [23], and it is not well known how summer pruning performed on apple trees could affect sensory quality of apple fruits.

Taking the aforementioned into account, instrumental and sensory attributes as well as physiological disorders are critical for premium quality fruits such as PDO apple cultivar ‘Reinette du Canada’, and it is essential for these products to achieve quality through eco-friendly practices regardless of location where the orchard is located. Thus, the aim of this research was to investigate the effects of summer pruning as an eco-friendly pre-harvest technique, on BP incidence and sensory quality of PDO ‘Manzana Reineta del Bierzo’ apple cv. ‘Reinette du Canada’ during storage.

2. Materials and Methods

2.1. Locations

Two orchards, located in two locations in El Bierzo Valley (León, Castilla y León, Spain), namely Cabañas Raras and Almazcara both locations allowed to grow PDO ‘Manzana Reineta del Bierzo’ apple, were used for sample collection (Table 1). Nutritional and orchard management practices were comparable in both locations. ‘Cabañas’ orchard has 14-year-old trees on M9 rootstock, planted at 3.5 × 1.5 m spacing (high-density planting system) and trained as a central leader. ‘Almazcara’ orchard has 23-year-old trees on MM106 rootstock, planted at 5 × 5 m spacing (low-density planting system) and trained as an open center with three main scaffold branches.

Table 1. Geographical and soil characteristics of the experimental locations where apple cv. ‘Reinette du Canada’ was grown.

Location	Geographical Characteristics				Soil Characteristics						
	Lat.	Long.	Alt. (m)	Texture	pH	N (g kg ⁻¹)	P (mg kg ⁻¹)	Ca ²⁺ (g kg ⁻¹)	Mg ²⁺ (g kg ⁻¹)	K ⁺ (g kg ⁻¹)	
Cabañas	42°37' N	6°36' W	567	Loam	7.1	2.0	99.50	1.42	0.20	0.36	
Almazcara	42°36' N	6°30' W	575	Loam	6.7	1.8	13.91	1.16	0.18	0.11	

2.2. Summer Pruning

Trees were selected for uniformity based on tree size and tree foliage density. All trees had been uniformly pruned during the previous dormant season. Dormant pruning of this cultivar with low susceptibility to alternate bearing was performed with pneumatic pruning shears and involved removing vigorous watersprouts, thinning-out cuts and heading cuts, so the number of fruit-bearing shoots left on the trees after pruning was approximately 150 and 600 for central leader and open center respectively, which according to Guerra and Guerra [24] is equivalent to trees bearing a total fruit load of approximately 135 and 540 fruits in central leader and open center in apple cv. 'Reinette du Canada', respectively, according to its characteristic fruit set percentage (number of fruit divided by the number of flowers pollinated and multiplied by 100) of 14%.

Summer hand pruning was carried out on the first week of July (99 days after full bloom), when the growth of most extension shoots had ended. Thinning cuts involved removing entire water sprouts and long shoots over 30 cm in length (Figure 1). Control trees received only dormant (winter) pruning with thinning-out cuts.



Figure 1. Illustration of summer pruning performed in 'Reinette du Canada' apple trees: (a) Control trees; (b) Pruned trees.

2.3. Experimental Design and Storage Conditions

The research works involved one-factor experiments, which were carried out in randomized block designs, with two levels (summer pruning and no summer pruning as the control), in order to analyze the effects of summer pruning on apple trees. The experiment was conducted in two orchards ('Cabañas' and 'Almázcara'). Each orchard had three blocks, each consisting of eight individual trees per orchard. Pruning was applied to four trees of each replication, leaving the other four trees as control (a total of 24 trees per orchard was considered). Blocking was based on the location following the steepest slope of the orchards.

Apple cv. 'Reinette du Canada' (*Malus × domestica* Borkh.) was harvested during the commercial harvesting period on September 2016 from the two orchards according to days after full bloom and TSS (Total Soluble Solids): TA (Titratable Acidity) ratio (≥ 11) to ensure a sufficient quality of 'Reinette du Canada' apple which would allow fruit to be marketed with the 'PDO' designation after storage [5].

Fruit from each replicate, were collected for instrumental, sensory and BP analyses, and then stored immediately after harvest in cold-storage chambers at 1.5 °C and 92% relative humidity. After 10, 20 and 30 weeks, samples of 20 fruit per analysis period were transferred to 20 °C to be analyzed after a 7-day shelf-life period.

2.4. Instrumental Quality

At harvest and following storage and the ripening period, the fruit was analyzed for weight, color, firmness, iodine–starch index (harvest only), TSS, TA and physiological disorders.

External fruit color was measured using a Minolta, CR-200 colorimeter (Ahrensburg, Germany) and results were expressed in the CIE L*, a*, b* system. The target color was calculated as hue angle = $\tan^{-1} b^*/a^*$. Flesh firmness (N) was calculated using an Effegi penetrometer (Effegi TR Turoni and C.; Forlì, Italy) mounted on a hand-operated press and fitted with a 11.1 mm diameter plunger. Iodine–starch index (1–10 scale) was evaluated by dipping an equatorial slice of the fruit into iodine–iodize solution (20 g IK + 10 g I2 + 1 L H₂O) for 1 min and comparing the color pattern with a reference chart, Ctifl-Eurofru Code. TSS (%) and TA (% malic acid) were determined in 5 fruit samples, with a digital refractometer (Atago, DR-A1, Tokyo, Japan), and by titrating 10 mL of juice with NaOH 0.1 N up to pH 8.2, respectively. The TSS:TA ratio was then calculated.

Physiological disorders BP and shriveling were determined. The number of fruits affected by disorders was recorded, so that the incidence of fruit with each of the disorders could be calculated. BP was evaluated on a 0–1 scale depending on the presence of visible symptoms, where: 0, no visible symptoms; 1, fruit having more than 1 pit on the surface.

2.5. Sensory Analysis

At harvest and following storage plus the ripening period, sensory analyses were carried out by a panel of 10 judges from the ITACYL (Agriculture Technology Institute of Castilla and León), trained to assess apple fruit attributes following the methodology described by Guerra et al. [3]. Degree of liking was scored on a 5-point scale (1–5), 1 being lowest acceptance and 5, highest acceptance [25].

2.6. Mineral Concentration Analysis

Mineral concentration of apple fruit was determined at harvest. Twelve fruits per experimental plot were used for the analysis. For analysis, aliquots of 0.5 g of the skin of the fruit of the different samples were used. Ca and Mg concentrations were determined by atomic absorption spectroscopy and K by flame emission. Nutrient concentrations were expressed as mg 100 g⁻¹ fresh weight.

2.7. Soil Analysis

Soil samples were randomly taken using a soil probe to a depth of 50 cm in each orchard. These soil cores were mixed to make a composite sample. Total N was analyzed using the Kjeldahl method. K, Ca and Mg were estimated using the inductively coupled plasma-atomic emission spectroscopy (ICP-AES) technique [26]. Phosphorus (Olsen) was determined using the extraction method [27].

2.8. Statistical Analysis

The results from each orchard were subjected to a one-factor analysis of variance (ANOVA) in a system of randomized blocks with three repetitions. Mean comparisons were performed using the Fisher's least significant difference (LSD) test to examine differences ($p < 0.05$) between treatments. All analyses were performed using the SAS software version 9.1.2 (SAS Institute Inc., Cary, NC, USA, 2004).

3. Results

3.1. Fruit Mineral Concentration

Summer pruning affected the mineral content of the fruit. Although Ca content of the fruit was not significantly affected by summer pruning in neither of the locations, significant differences between treatments were found for K content, so fruit coming from pruned trees had a lower level of K than control fruit. This made that K/Ca ratio in control fruit from 'Cabañas' was higher than fruit from pruned trees in this location (Table 2).

Table 2. Effect of pruning on nutrient concentration (mg per 100 g fresh weight) in apple cv. 'Reinette du Canada' at harvest.

Location	Factor	Ca	K	Mg	K/Ca
Cabañas	Control	12.644 ¹	167.022 a	33.172	13.357 a
	Treatment	13.819	138.257 b	28.992	10.020 b
Almázcara	Control	11.459	159.190 a	27.786	13.913 a
	Treatment	11.722	136.742 b	28.220	11.899 b

¹ Different letters within nutrient and location indicate significant differences according to LSD test ($p < 0.05$).

3.2. Physiological Disorders during Storage

Summer pruning reduced BP during storage in comparison to control, so BP incidence in fruit coming from pruned trees was lower than BP in fruit from control trees (Table 3). This trend was only significant in 'Cabañas' location. BP during storage was lower for fruit coming from the 'Almázcara' location in comparison to fruit from the 'Cabañas' location.

Table 3. Effect of pruning on bitter pit and shriveling incidence (%) in apple cv. 'Reinette du Canada' at harvest and during storage.

Location	Factor	Bitter-Pit (Weeks after Harvest)				Shrivelling
		0	10	20	30	30
Cabañas	Control	0.0 ¹	27.0 a	30.9 a	36.4 a	9.3
	Treatment	0.0	10.9 b	19.7 b	22.7 b	16.7
Almázcara	Control	0.0	13.7	15.5 a	18.1 a	0.0
	Treatment	0.0	13.9	11.9 b	10.7 b	0.0

¹ Different letters within the same column and location indicate significant differences according to LSD test ($p < 0.05$).

Significant differences regarding shriveling were not found at the end of storage between treatments in either of the locations (Table 3).

3.3. Instrumental Quality Attributes

At harvest and, in general, after storage, differences between treatments regarding quality parameters were not evident in either of the locations (Tables 4 and 5). The only quality attribute affected by summer pruning was firmness, so fruit from summer pruned trees was softer than control fruit at harvest (differences only significant in 'Cabañas' location). However, these differences were reduced during storage (Table 5). Fruit weight, color, TSS, TA or TSS:TA were not significantly affected by summer pruning in either of the locations.

Table 4. Effect of pruning on instrumental quality attributes of apple cv. ‘Reinette du Canada’ at harvest.

Location	Factor	Fruit Weight (g)	Hue	Starch Index (1–10)	Firmness (N)	TSS (%)	TA (%)	TSS:TA
Cabañas	Control	190.05 ¹	110.98	3.6	98.39 a	11.31	0.88	12.94
	Treatment	183.24	111.31	4.4	90.31 b	10.25	0.84	12.36
Almázcara	Control	184.14	109.20	3.3	105.86	11.80	0.90	13.18
	Treatment	181.83	111.03	3.1	101.70	11.27	0.83	13.67

¹ Different letters within the same attribute and location indicate significant differences according to LSD test ($p < 0.05$).

Table 5. Effect of pruning on instrumental quality attributes of apple cv. ‘Reinette du Canada’ during storage.

Storage Time (Weeks)	Location	Treatment	Hue	Firmness (N)	TA (%)
10	Cabañas	Control	104.37 ¹	41.64	0.79
		Treatment	105.34	37.54	0.83
10	Almázcara	Control	104.47	46.85	0.80
		Treatment	105.02	44.32	0.82
20	Cabañas	Control	102.60	41.59	0.72
		Treatment	103.35	37.66	0.67
20	Almázcara	Control	101.28	50.37	0.74
		Treatment	102.44	44.51	0.72
30	Cabañas	Control	98.70	35.71	0.67
		Treatment	97.86	31.99	0.66
30	Almázcara	Control	95.04	44.92	0.53
		Treatment	98.08	42.79	0.55

¹ Different letters within the same attribute, storage time and location indicate significant differences according to LSD test ($p < 0.05$).

3.4. Sensory Quality and Degree of Liking

In general, summer pruning had no effect on either of the sensory properties nor on degree of liking (Table 6; Figure 2).

Table 6. Effect of pruning on sensory quality attributes of apple cv. ‘Reinette du Canada’ at harvest and during storage.

Storage Time (Weeks)	Location	Treatment	Skin Color Uniformity	Skin Whiteness	Skin Russeting	Firmness	Crispness	Juiciness	Acidity	Sweetness	Flesh Mealiness	Sourness
0	Cabañas	Control	3.67 ¹	3.67	2.78	3.56	4.00	4.00	3.67	1.44	1.44	1.33
		Treatment	4.00	3.33	2.33	2.89	3.78	3.89	2.78	1.56	1.44	1.44
0	Almázcara	Control	4.11	3.11	3.00	4.33	4.33	4.56	3.78	1.56	1.33	1.67
		Treatment	3.22	2.67	3.56	4.44	4.22	4.00	4.11	1.33	1.44	1.33
10	Cabañas	Control	2.90 ¹	3.29	2.34	2.11	2.65	3.44	2.96	2.07	1.77	1.49
		Treatment	3.40	2.81	2.57	2.20	2.91	3.56	2.51	1.82	1.76	1.46
10	Almázcara	Control	3.39	2.06	3.56	2.83	3.56	3.83	2.98	2.17	1.44	1.61
		Treatment	3.94	2.78	2.94	2.39	3.28	4.06	2.59	2.78	1.50	1.33
20	Cabañas	Control	3.60 ¹	2.75	2.39	1.88	2.79	3.64	3.06	2.00	1.83	1.35
		Treatment	3.67	2.78	1.78	2.06	2.89	3.44	2.56	2.17	2.00	1.44
20	Almázcara	Control	3.56	3.02	2.69	2.47	2.98	3.73	2.82	1.69	1.91	1.53
		Treatment	3.75	3.03	2.82	2.53	3.28	3.70	2.63	1.98	1.82	1.57
30	Cabañas	Control	4.07 ¹	2.58	1.83	2.28	3.36	4.31	3.14	2.22	1.64	1.56
		Treatment	3.79	3.03	1.75	2.03	3.31	3.44	2.61	2.17	1.97	1.28
30	Almázcara	Control	3.79	2.07	2.71	2.26	3.19	3.21	2.87	2.26	2.42	1.42
		Treatment	3.33	2.83	2.71	1.99	3.06	3.41	2.82	2.28	1.99	1.34

¹ Different letters within the same attribute, storage time and location indicate significant differences according to LSD test ($p < 0.05$).

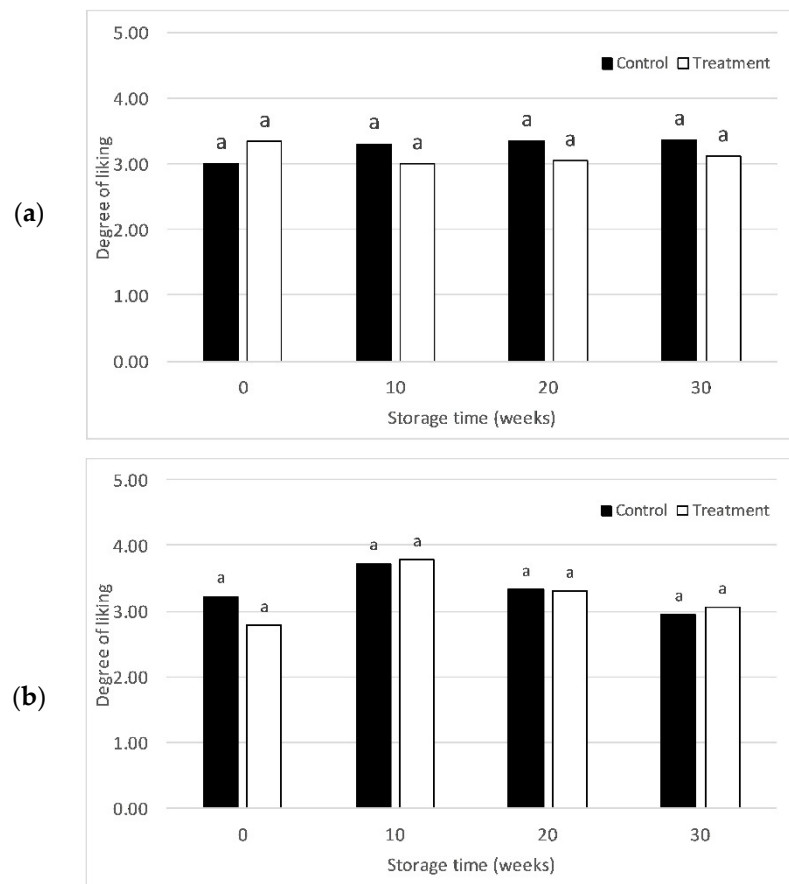


Figure 2. Effect of summer pruning on degree of liking of ‘Reinette du Canada’ apple fruit grown in two locations, ‘Cabañas’ (a) and ‘Almázcara’ (b). Bars represent mean values and different letters above bars indicate significant differences according to LSD test ($p < 0.05$).

With regard to russetting, fruit from the ‘Cabañas’ location always achieved a lower score than that from the ‘Almázcara’ location. On the other hand, in the first stages of storage whiteness was higher for the ‘Cabañas’ location than for the ‘Almázcara’ location.

4. Discussion

The main reason of the postharvest storage loss caused by physiological disorders is the improper proportion of mineral nutrient elements [28]. K/Ca ratio on the peel at harvest could be a good parameter to correlate with BP during storage in ‘Reinette du Canada’ apple cultivar [14]. The fact that K/Ca ratio for fruit from pruned trees was lower in comparison to control fruit may be due to the potential of summer pruning to reduce competition between shoot growth and fruit for available Ca which increased Ca levels in fruits [11]. Therefore, the lower K content and K/Ca ratio of fruit from pruned trees led to a low incidence of BP during storage. This effect of summer pruning is even more promising when taking into account that the conventional alternative to this environmental friendly technique, i.e., growth-inhibiting plant growth regulators that also reduce shoot extension, may not be useful for managing BP incidence in apples [9,29].

BP was lower in fruit from the ‘Almázcara’ location (trees on MM106 rootstock) in comparison to fruit from the ‘Cabañas’ location (trees on M9 rootstock). Donahue et al. [30] pointed out that nutrients (peel Mg/Ca ratio and peel Ca) could be correlated with BP in a stronger or weaker way depending on the rootstock, with M9 clones having the strongest correlations. Ben [31] proved that when stored at 2 °C, the susceptibility of apples to BP depended on the rootstock scion combination. Although it is generally accepted that it is more suitable to grow apples on low vigorous rootstocks [31], fruit from trees grafted

onto rootstocks M9 are mostly affected by physiological disorders such as BP during storage [32,33]. However, trees with a more vigorous rootstock in this experiment (MM106) were trained to an open center system, so the higher energy of this rootstock is diverted into three branches. Therefore, the impact of the rootstock on BP incidence could fade away since the training system and other differences among the locations could also have an impact on the quality of the fruit.

Regarding the differences found in firmness between treatments (pruning) as mentioned before, it is known that summer pruning might limit carbohydrate availability, which could affect fruit growth and sugar levels [17]. Dry matter content is associated with firmer fruit at harvest [34], so fruit firmness from apples from pruned trees, with less carbohydrate availability, obtained lower values of firmness than control fruit. Although this is not shown in Table 4, differences for TSS between treatments were significant at $p < 0.1$. Stover et al. [35] found that summer pruning resulted in lower fruit firmness of apple cv. 'McIntosh' with mid or late harvest dates. Although summer pruning affected firmness at harvest, significant differences tended to diminish during storage, so differences in firmness were not significant at the end of storage. As expected, summer pruning slightly decreased both TSS and TA at harvest, but differences were not significant. Since hue, firmness and TA the instrumental properties that best correlated with consumer acceptance in apple cv. 'Reinette du Canada' [3] were not affected by summer pruning during storage, it could be said that the instrumental quality of 'Reinette du Canada' was not negatively affected by summer pruning.

Firmness of fruit from 'Almázcara' location was higher than fruit from 'Cabañas' at harvest, and this trend was also observed throughout storage. Considering this result, it is clear that location, as a compendium of environmental and orchard management factors such as soil, training system, age or rootstock, had an impact on the firmness of the apple fruit. In spite of these differences, summer pruning would be a useful practice to keep the storability of the apple without losing instrumental fruit quality, regardless of the location used to grow the apple trees.

The depositing of suberized cell layers (periderm) gives rise to the typical brown and corky aspect of the russeted apples [36]. Higher whiteness scores for 'Cabañas' fruit could have something to do with the fact that the skin surface is not as well covered by the dark russeting, and that would lead to a brighter skin. It has been found that although russeting is under genetic control, environmental conditions and orchard management practices can heavily influence russet formation [36].

The fact that neither of the sensory properties nor degree of liking were affected by summer pruning, validates this eco-friendly preharvest alternative to chemical growth regulation input in order not only to decrease the incidence of BP or to keep the instrumental quality high, but also to do this without decreasing the sensory quality of the fruit.

5. Conclusions

Although fruit from pruned trees had a lower rate of firmness than control fruit at harvest, summer pruning did not significantly affect fruit weight nor any other instrumental property at harvest or during storage. Moreover, sensory firmness, any other sensory characteristic or degree of liking were not affected by summer pruning. Thus, it could be said that summer pruning could be a useful way of improving quality in global terms of 'Reinette du Canada' apple cultivar, because this technique contributed to a decrease in BP incidence, but did not decrease sensory quality nor degree of liking. Moreover, summer pruning would be an eco-friendly technique in order to preserve quality of apple fruit regardless of the location of the orchard.

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