



Communication

Effect of Sustainable Preharvest and Postharvest Techniques on Quality and Storability of High-Acidity ‘Reinette du Canada’ Apple

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Abstract: Consumers are more disposed towards paying price premiums for sustainable food products. This fact is especially important for products with a PDO (Protected Designation of Origin) label, such as the high-acidity apple cultivar ‘Reinette du Canada’. However, ‘Reinette du Canada’ apple fruit is affected by two of the main post-harvest disorders in apple cultivars, bitter pit and senescent breakdown. As a means to achieve sustainable production of this high-quality apple cultivar, the effect of a sustainable preharvest technique, summer pruning, in combination with a zero-residue postharvest treatment allowed in organic farming on calcium-related disorders and quality in global terms in this PDO-labelled apple cultivar was assessed during storage. The combination of the two sustainable practices was an effective tool in diminishing senescent breakdown from 43% to 17% and to decrease bitter pit incidence by up to 21% (from 36% to 15%) throughout storage, especially in fruit with high susceptibility to this disorder during storage. Moreover, the overall acceptability was kept high (values of more than 3 on a 5-point scale) when applying this environment-friendly sustainable horticulture practice, which will not only reduce the synthetic pesticide input in the apple agroecosystem, but also contribute to producing fruit with zero residues.

Keywords: bitter pit; fertilisation; fruit quality; senescent breakdown; storage; sustainable agriculture



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1. Introduction

Fruits including apple have gained more popularity recently. They include high content of non-nutritive, nutritive, and bioactive compounds such as flavonoids, anthocyanins, phenolic acids, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals. Apple fruits have a distinct flavour and taste, excellent medicinal value, and health care functions as well [1–3] (Koyuncu 2020; Nybom et al., 2020; Radivojevic et al., 2020). Apple cv. ‘Reinette du Canada’ (*Malus × domestica* Borkh.) is an important cultivar in Spain and other southern European countries. It is valued for its flavour and resistance to bruising [4]. Moreover, this cultivar is characterised by its high level of acidity compared to other apple cultivars, even higher than apple cv. ‘Granny Smith’ [5], which is known for being at the top of the acidic chart. The acidity values of apple cv. ‘Reinette du Canada’ are above 0.80% (malic acid) at harvest and higher than 0.65% after the storage plus ripening period, resulting in high consumer acceptance [6]. These characteristics, together with the appropriate microclimate conditions for the cultivation of apple trees in El Bierzo Valley (Northwestern Spain), have helped to protect this cultivar throughout the

European Community as a PDO (Protected Designation of Origin) ‘Manzana Reineta del Bierzo’ (Commission Regulation EC 2601/2001) [7].

PDO/PGI (Protected Geographical Indication) agricultural products are exported to numerous countries in Europe, Asia and the Americas [8]. Fruit quality in products under a PDO label should be ensured throughout the storage period, since apples are rarely consumed directly when harvested, but are usually stored for periods of up to 6 months to ensure a steady, year-round supply of high-quality fruit [4,9]. However, ‘Reinette du Canada’ apple is severely affected by bitter pit [4], and also by senescent breakdown during storage (it is known that the ‘Reinette’ apple group suffers from senescence during extended storage [10]). These are two of the main post-harvest disorders in apple fruit. Some authors claim that almost all pre-harvest factors that influence the incidence of bitter pit in apple fruit can be directly or indirectly related to the fruit’s calcium nutrition [11]. Senescent breakdown is also due to a nutritional disorder in the orchard [12], but other key factors contribute to the outbreak of this disorder, such as tree age, pruning, fruit maturity, fruit size, storage temperature and storage period [12–15]; therefore, after long storage periods, and after simulated shelf life, these disorders limit the storage length up to 5–6 months [14,16,17].

Some studies have shown that foliar sprays or postharvest treatments with solutions of calcium salts are effective in reducing senescence breakdown disorders [18] or bitter pit [19,20]. Different calcium sources to control bitter pit have been tested recently. Foliar treatments of CaCO_3 were effective in decreasing bitter pit incidence in ‘Reinette’ apple cultivars after 3 months of storage [21]. However, calcium chloride (CaCl_2) remains the most effective salt due to its lower point of deliquescence in comparison to other calcium salts [22], and it is the cheapest form of calcium available to growers [23,24]. On the other hand, in recent years, attention has been focused on the need to develop apple cultivars that can be stored for long periods without the need for such chemical treatments. This is because of the movement towards more sustainable agricultural activities, which has grown recently to improve the ecological impact of agriculture [25], and the increasing public concern over the presence of agrochemical residues in foods and on the environmental impact of agriculture. Therefore, there has been an increase in demand for research to increase nutrient-use efficiency, improve environmental sustainability, and maximise quality [9,26] of high-quality fruit. Thus, good agricultural practice programs that increase efficiency input usage, optimise the use of biological and chemical/physical resources in the agroecosystem, and that reduce the negative impact of agriculture on the environment have become more common [27,28]. Moreover, the promotion of low pesticide input strategies is particularly important in apple agroecosystems, which are considered to be high pesticide input environments [29]. It is not surprising that the superiority of organic food is based on low or zero pesticide residues and on preserving environmental and economic sustainability [30]. In the long term, the production of fruit with minimal residues is a major concern to the public for fresh perishable fruit. The reduction of fungicide residues on fruit may encourage people to consume fruit and contribute to sustainable fruit production [31]. In recent years, consumer concern for quality, nutraceutical characteristics and food security, together with an increasing awareness of the environmental impact of many chemicals, has grown [32,33], and consumers are more likely to pay price premiums for sustainable food products [7,34]. This is even more important for PDO-labelled products like ‘Reinette du Canada’, because consumers consider that PDO/PGI labels guarantee their authenticity, safety, and high quality [35,36].

Taking the aforementioned reasons into account, it is important in the production of high-quality fruits not only to carry out fertilisation using external inputs such as CaCl_2 , which has been proven to be effective in controlling calcium related disorders, but also to make use of zero-residue inputs, in order to test their effectiveness on the postharvest quality of sustainable fruit products. However, Val et al. [22] stated that the high number of treatments that are needed to increase the calcium content in the fruit skin, have phytotoxic effects on the apple tree, and suggested that lower concentrations should

be sprayed onto the trees. Thus, the control of apple disorders such as bitter pit should be supplemented with other sustainable approaches in order to reduce their negative incidence on fruit quality.

Summer pruning of extended and vigorous shoots has been an effective tool to improve light penetration and distribution within the tree canopy [37,38], which can enhance fruit quality in apple trees [39], and is regarded as an ecological alternative to decrease bitter pit incidence during the storage of high quality apple cv. 'Reinette du Canada' [7].

The effect of preharvest and postharvest factors on the quality of the fruit has been studied before. However, there is a lack of information about the combined effect of sustainable techniques involving both preharvest and postharvest factors on fruit quality. Therefore, the objective of this work was to investigate the effect of a sustainable preharvest technique, summer pruning, in combination with a zero-pesticide-residue postharvest treatment that is allowed in organic farming on postharvest disorders and fruit quality of 'Reinette du Canada' under the PDO label to achieve sustainable fruit production.

2. Materials and Methods

2.1. Plant Material and Treatments

Apple trees were selected for uniformity based on tree size, trunk cross-sectional area 20 cm above the graft (25–35 cm in central leader and 75–85 cm in open vase), and tree foliage density.

Samples of apple cv. 'Reinette du Canada' were harvested in September 2016 during the commercial harvesting period according to days after full bloom and SSC (Soluble Solid Content): TA (Titratable Acidity) ratio to ensure a sufficient quality of 'Reinette du Canada' apple that would allow the fruit to be marketed with the 'PDO' designation after storage [4]. The fruit, selected for uniformity of size and freedom of defects, was picked from two commercial apple orchards in two municipalities in El Bierzo Valley, Cabañas Raras and Congosto: Orchard 1, with a history of bitter pit, was characterised by 14-year-old trees, grafted on M9 rootstock, grown at a density of 1905 trees per ha and trained to central leader system; Orchard 2, consisting of 23-year-old trees, grafted on MM106 rootstock, grown at a density of 400 trees per ha and trained to open vase. The two orchards had similar characteristics in terms of soils, loam texture, pH 6.9 ± 2 ; height above sea level, 570 ± 5 m; average annual temperature, 11.6 °C; and average annual rainfall, 623 mm. Nutritional and orchard management practices, including preharvest calcium applied after flowering, were comparable in both orchards. Dormant pruning was performed during the previous winter season following Guerra and Guerra's [40] recommendation for this cultivar. The total fruit load per tree was approximately 25 and 100 kg in central leader and open vase training systems, respectively.

The treatments consisted of two sustainable practices performed both before and after harvest, summer pruning (preharvest) and postharvest calcium treatment (postharvest). Thus, the summer hand pruning treatment was performed in early July, thinning out long vigorous shoots more than 30 cm in length. Regarding the postharvest calcium treatment, the fruit was treated immediately after harvest and before storage by dipping the fruit for 120 s with the coating Nutropit® (Biagro S.L., Valencia, Spain), a source of calcium chloride regarded as a zero pesticide residue product that is currently allowed in organic farming, which was prepared by diluting Nutropit (14% calcium) with water to 0.3%. This source of calcium was chosen on the basis of its low toxicity and its potential use in organic horticulture.

2.2. Experimental Design

The research method involved a one-factor experiment with 4 treatment levels and three replicates. Moreover, the experiment was conducted at two locations as a means of replicating the experiment over space [41]. The treatments were the control, i.e., fruit coming from trees that were not summer pruned and not subjected to postharvest calcium treatment (1), a sustainable preharvest treatment in the orchard (summer pruning) (2), a sustainable

postharvest calcium treatment that is allowed in organic farming (3), and the combined effect of both the preharvest and postharvest sustainable practices (4). A completely randomised block design with three replicates per treatment was used. The experimental units consisted of two trees per replicate and treatment in each of the orchards, 8 trees were selected per replicate and orchard, making a total of 24 trees per orchard.

2.3. Mineral Concentration and Fruit Quality Assessment

Mineral concentration of apple fruit was determined one month after harvest. For analysis, aliquots of 1 g weight of flesh from 12 fruits per sample were used. This made for a total of 288 fruits for the mineral concentration analyses (12 fruits \times 4 treatments \times 3 replicates \times 2 orchards). Ca and Mg concentrations were determined by atomic absorption spectroscopy and K by flame emission. All nutrient concentrations were expressed as $\text{mg} \times 100 \text{ g}^{-1}$ fresh weight.

At harvest and following storage (60 and 180 days) plus ripening period (a 7-day shelf-life period at 20 °C), fruit quality was determined. The initial 30-fruit samples per treatment, storage time, replicate and orchard were divided into subsamples of 10 fruit for sensory analyses and subsamples of 20 fruit for physiological disorders. This made for a total of 1440 fruits for quality analyses (30 fruits \times 4 treatments \times 2 storage periods \times 3 replicates \times 2 orchards). These samples were stored immediately after harvest in cold-storage chambers at 1.5 °C and 92% relative humidity, conditions that, according to Guerra et al. [6], are suitable for this cultivar to withstand from 10 to 30 weeks of storage depending on the storage conditions.

The number of fruits affected by senescent breakdown and bitter pit was evaluated for 20 fruits per lot and recorded as percentage of incidence regardless of the degree of severity.

Bitter pit was evaluated depending on the presence of visible symptoms. Senescent breakdown was defined externally as a green or yellow skin which becomes brown in the areas of breakdown, and then the fruit was cut longitudinally and transversely to assess senescent breakdown internally as a brown and watery discoloration in the core area [18] (Figure 1).

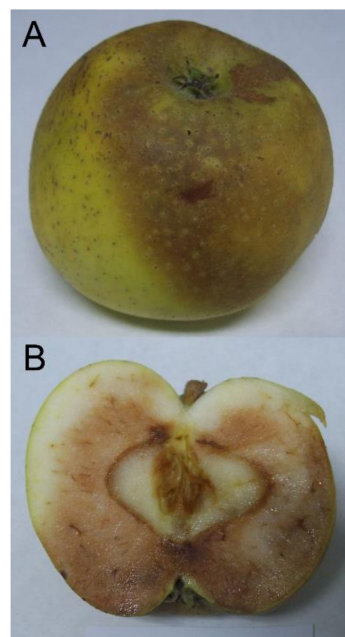


Figure 1. Senescent breakdown symptoms on apple cv. 'Reinette du Canada'. (A) Skin symptom. (B) Flesh symptom.

Overall acceptability was analysed by a panel of 10 judges from the ITACYL (Agriculture Technology Institute of Castilla and León), trained to assess apple fruit attributes.

For fruit of each replicate, overall acceptability was scored on a 5-point scale (1–5), 1 being lowest acceptance and 5, highest acceptance [42].

2.4. Statistical Analysis

Data were analysed with a one-way ANOVA and mean comparisons were performed using the Fisher's least significant difference (LSD) test to examine differences ($p < 0.05$) among treatments. All analyses were performed using IBM SPSS Statistics 26 for Windows.

3. Results

3.1. Bitter Pit and Senescent Breakdown during Storage

The calcium content of the fruit was not influenced by any of the treatments. However, the potassium concentration in the fruit was reduced in fruit coming from pruned trees from both orchards (with and without postharvest treatment) in comparison to the control fruit. Additionally, sustainable practices influenced the magnesium nutrient content in Orchard 1, the environment where bitter pit was a big issue; thus, the combination of summer pruning plus Nutropit[®] provided lower levels of Mg when compared to the control fruit (Table 1).

Table 1. Effect of sustainable techniques on mineral concentration (mg/100 g FW) in apple fruit cv. 'Reinette du Canada'.

Treatment	Orchard 1			Orchard 2		
	Ca	K	Mg	Ca	K	Mg
Control	1.98a	78.77a ¹	4.953a	1.91a	76.18a	5.231a
Nutropit ^{®2}	1.89a	71.88b	4.671ab	1.98a	77.34a	5.267a
Summer pruning	1.74a	66.08b	4.471ab	1.50a	59.83b	5.377a
Summer pruning + Nutropit [®]	1.87a	67.43b	4.283b	1.62a	56.33b	4.936a

¹ Different letters within the same column indicate significant differences according to LSD test ($p < 0.05$). ² Calcium chloride (14% calcium).

The application of Nutropit[®] alone did not decrease the incidence of bitter pit in either of the sites, and the effect of the summer pruning alone was only effective to significantly decrease bitter pit incidence at the beginning of the storage in one of the orchards (after 60 days in Orchard 1). However, the combination of summer pruning plus Nutropit[®] was the most effective tool to decrease bitter pit incidence throughout storage, especially in the fruit from the orchard with high susceptibility to this disorder during storage (Orchard 1); thus, the combined effect of these two sustainable strategies was able to reduce bitter pit by up to 21%, from 36% (control) to 15% (Nutropit[®] plus summer pruning) after 180 days of storage in the high-bitter-pit-incidence environment. In Orchard 2, the combination of the two sustainable practices decreased the bitter pit incidence at the beginning of the storage. However, at the end of storage, differences among treatments were not significant anymore (Table 2).

Table 2. Effect of sustainable techniques on bitter pit incidence (%) in apple cv. 'Reinette du Canada' during storage.

Treatment	Orchard 1		Orchard 2	
	60 Days	180 Days	60 Days	180 Days
Control	33a ¹	36a	14a	15a
Nutropit ^{®2}	27a	24ab	9ab	11a
Summer pruning	13b	23ab	10ab	12a
Summer pruning + Nutropit [®]	13b	15b	2b	11a

¹ Different letters within the same column indicate significant differences according to LSD test ($p < 0.05$). ² Calcium chloride (14% calcium).

As expected, since senescent breakdown is related to overripening, the outbreak of this disorder was only displayed in the long term (after 180 days of storage plus the 7-day

shelf-life period); therefore, at this stage, the fruit from Orchard 2 showed a high incidence of this disorder, which is the orchard with high susceptibility to this disorder. Neither the summer pruning nor Nutropit[®] by themselves were useful for decreasing the incidence of senescent breakdown at the end of storage. Nevertheless, the combined effect of the two sustainable strategies was able to diminish senescent breakdown in fruit growing in the apple orchard where this disorder was more prevalent (Orchard 2); consequently, after 180 days, the incidence of senescent breakdown was significantly reduced (by 26%), from 43% to 17% when combining Nutropit[®] plus summer pruning in comparison to the control fruit (Table 3).

Table 3. Effect of sustainable techniques on senescent breakdown (%) in apple cv. ‘Reinette du Canada’ after 180 days of storage.

Treatment	Orchard 1	Orchard 2
Control	14a ¹	43a
Nutropit ^{®2}	11a	31ab
Summer pruning	9a	43a
Summer pruning + Nutropit [®]	12a	17b

¹ Different letters within the same column indicate significant differences according to LSD test ($p < 0.05$). ² Calcium chloride (14% calcium).

3.2. Overall Acceptability

Fruit quality during storage in terms of fruit weight was unaffected by any of the treatments. Mean fruit weight of apples from Orchard 1 and Orchard 2 were 192.1 g and 187.3 g, respectively. Moreover, overall acceptability of apple fruit during storage was unaffected by any of the treatments in both apple orchards (data not shown).

4. Discussion

The calcium content of the fruit was not affected by the application of Nutropit[®], which is in accordance with the statement of Val et al. [22], in the sense that fruit flesh contents are not affected that much even though high concentrations of input are used to increase the Ca content in the fruit. Although many years of research have shown that bitter pit is induced by low calcium concentrations, many recent studies indicate that the total fruit tissue Ca content may not be the only reason for bitter pit development [43]. Lang et al. [44] reported a significant reduction in the incidence and the severity of the calcium-related bitter pit using mulch as a sustainable alternative to calcium spraying, despite not finding significant effects on fruit mineral concentration. Moreover, Torres et al. [25] pointed out that the risk of a false-positive or false-negative was high when using Ca mineral content as a prediction method for bitter pit. On the other hand, high levels of potassium and magnesium have been found in fruit tissues with calcium deficiency disorder symptoms [7,45]; consequently, the lower levels of potassium and magnesium for fruit subjected to the combination of summer pruning plus Nutropit[®], especially in Orchard 1, the environment with a history of bitter pit, could be related to the lower bitter pit incidence achieved with this treatment.

The application of Nutropit[®] alone did not decrease the incidence of bitter pit in either of the sites. In the same way, Soppelsa et al. [46] found that the calcium-related disorder known as ‘Jonathan spot’ was not reduced in apple cv. ‘Jonathan’ by calcium chloride spraying. It should be noted that not only the number of products allowed in organic farming is more restricted [47], but organic pest control strategies are typically less effective than synthetic pesticides, and the commercial output of apple production is sensitive to damage, as damaged fruits cannot be sold as high-quality apples for direct consumption on the market [48]. On the other hand, the combination of the two sustainable practices was effective in decreasing bitter pit incidence throughout storage in apple cv. ‘Reinette du Canada’. This trend was more evident in the orchard with higher susceptibility to bitter pit during storage (Orchard 1), than in the orchard with lower levels of bitter pit incidence (Orchard 2), where differences for bitter pit incidence among treatments at the end of

storage were not significant. The fact that Orchard 2 had a lower incidence of bitter pit could be the reason the treatments did not have a significant effect on this disorder. Torres et al. [25], who found a wide range of bitter pit incidence in apple cv. 'Golden Smothee' across orchards classified orchards according to bitter pit risk; thus, orchards with low bitter pit risk would need fewer corrective actions in the form of number of CaCl_2 sprays and dips, since the effect of these would have far less impact on the bitter pit incidence.

The senescent breakdown outbreak occurred at the end of storage plus the 7-day ripening period. In our research, the combined effect of the two sustainable techniques proved to be effective to decrease the incidence of senescent breakdown at the end of storage in fruit coming from the orchard where this disorder was more evident. In agreement with our results, Watkins and Liu [14] also found differences among orchards for this disorder in apple cv. 'Empire' kept in storage. Moreover, Wang and Sugar [45] reported production location to be one of the factors that could have an impact on the incidence of this disorder. The level of senescent breakdown at the end of storage in the control fruit for 'Orchard 2' was similar to the results found by Wang and Sugar [49] for pear cv. 'Bartlett' (38.3%), by Wang and Sugar [50] for 'Doyenne du Comice' after 4 and 6 months, respectively, of cold storage in air plus 5 days of ripening or by Melnyk et al. [17], who found losses of 44.4% in apple cv. 'Reinette Simirenko' after seven months of storage due to senescent breakdown. Mazollier et al. [12] suggested a shortening in the duration of the storage for vulnerable batches in order to minimise the problem that becomes more acute when apples are returned to ambient temperature.

Although it is well known that summer pruning reduces the intensity of photosynthesis towards the end of the vegetation cycle, and, theoretically, it should reduce the deposition of carbonaceous substances which are used at the beginning of growth, inside the tree [51], and could also affect fruit growth and sugar levels [52], neither of the treatments in our experiment had a significant effect on fruit weight nor on overall acceptability of apples; therefore, the combination of summer pruning and postharvest treatment did not diminish the quality of the fruit.

The present work studied the influence of a combination of sustainable preharvest and postharvest techniques on the quality and storability of high-quality 'Reinette du Canada' apple. Further studies can be performed in order to incorporate other measurements related to cell wall degradation enzymes activities such as polygalacturonase, cellulase, xylanase, and pectinase, phenolic compound, and browning enzymes in fruit core. These might be able to provide a better understanding of the mechanisms involved in the outbreak of senescence disorders, fruit softening and, in general, loss of fruit quality.

5. Conclusions

The combined effect of summer pruning, as a sustainable preharvest practice, and Nutropit[®], a postharvest treatment allowed in organic farming, could be considered as alternative sustainable techniques to decrease the incidence of storage disorders in apple cv. 'Reinette du Canada'. Using a combination of these techniques, bitter pit incidence decreased by up to 21%, especially in fruit coming from an orchard with high bitter pit susceptibility, and senescent breakdown was reduced by 26% in an environment with great susceptibility to this disorder. Moreover, the overall acceptability was kept high (values of more than 3 on a 5-point scale) when applying this environment-friendly sustainable horticulture practice, which will not only reduce synthetic pesticide input in the apple agroecosystem but also contribute to produce fruit with zero residues.

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