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Fruit quality of 'Gala' and 'Fuji' apples cultivated under different environmental conditions

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A R T I C L E I N F O

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A B S T R A C T

This study evaluated the effect of growing site environmental conditions on apple fruit quality. Experimental orchards with 'Gala' and 'Fuji' apple strains were established in the subtropical humid climate in Southern Brazil, between 26° and 28°S, in the growing sites of São Joaquim, Caçador and Vacaria, located 1415, 960, and 971 m, respectively, above sea level. Fruit quality was assessed at harvest and after storage from the 5th through 8th year after planting. At each growing site, 'Gala' apples were harvested at one maturity stage and stored in a controlled atmosphere (CA). 'Fuji' apples were harvested at two maturity stages, with early harvested fruit stored in air and late harvested fruit stored in CA. For the São Joaquim site compared with those of Caçador and Vacaria, average temperature during the growing season was lower whereas the number of winter chilling hours and the number of days from bloom to harvest were higher. Fruit weight and red skin area were higher and russeting skin index lower in fruit from the coldest site (São Joaquim) for both cultivars. At harvest, flesh firmness and starch index of fruit from the coldest site were higher or the same as those of the warmest sites (Caçador and Vacaria), depending on cultivar and harvest date. The rate of flesh firmness loss during storage was greater in fruit from the coldest growing site for both cultivars and harvest maturities. Titratable acidity (TA) and soluble solids content (SSC) in fruit from the warmest site were higher or the same as the fruit from the coldest site, depending on when fruit analysis were performed. Although 'Gala' apples produced in all three growing sites were equally affected by external fungal decay, fruit from the warmest site had the highest incidence of *Glomerella cingulata* spot. 'Fuji' apples produced in the coldest site had the highest length/diameter ratio and watercore index at harvest and developed more CO_2 injury and diffuse flesh browning. External fungal decay index for 'Fuji' was the same for the three growing sites for fruit harvested at early maturity, but the decay index was higher for fruit from the warmest site when harvested at an advanced maturity. The study shows the influence of environmental conditions on apple fruit growth and development and the need to adjust production and storage practices to achieve high fruit quality based on pre-harvest environment.

1. Introduction

Apple, one of the most important fruits consumed worldwide, is produced in a wide range of environmental conditions within a latitude range of 25° to 52° (Faust, 2000; Palmer et al., 2003). In Brazil, apples are produced in the humid, subtropical region between the latitudes 26° and 28° S. This region has mild winter conditions that typically is sufficient to trigger tree defoliation and dormancy, however, in some years, the amount of winter cold may not satisfy the requirement necessary for breaking bud dormancy. In this region, fruit have been produced in growing sites with different elevations, from 960 to 1400 m above sea level, in the municipalities of São Joaquim and Caçador in Santa Catarina State, as well as in Vacaria in Rio Grande do Sul State. Although these apple growing sites have the same climate, they have different environmental conditions that can potentially affect final fruit quality.

Fruit appearance is the primary cue that influences consumers'

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initial purchase decision (Jaeger et al., 2018) and this criterion is used to objectively grade apples (OECD, 2010; USDA, 2002). Apple fruit with suboptimal appearance are marketed at a lower price, which reduces producer profitability (Carew and Smith, 2004). Size, color, shape, and absence of defects are some of main fruit quality attributes assessed by consumers at the marketplace (Musacchi and Serra, 2018). External defects include those that develop prior to harvest, such as russet, diseases, insect, and hail injuries, as well as those developing during and after harvest such as skin and flesh discoloration, pitting, bruising and fungal decay (Jaeger et al., 2018; Musacchi and Serra, 2018). Repeat purchase can be reduced when consumers' expectations for internal quality traits including taste, texture, aroma, and absence of disorders are not met (Harker et al., 2003, 2008).

Genotype, environment, and orchard management factors determine the quality of apple fruit (Musacchi and Serra, 2018). Size, color, shape, sugar content, acidity and susceptibility to physiological disorders are apple fruit traits affected by environmental conditions (Karagiannis et al., 2020; Lachapelle et al., 2013; Reginato et al., 2019; Stanley et al., 2000; Yuri et al., 2019). Therefore, considering the climate changes associated with global warming, understanding the responses of different apple genotypes to changing environment conditions may enable selecting appropriate genotypes and orchard management practices to achieve high fruit production and quality (Palmer et al., 2003). In Brazil, apple production has been shifting from warmer to colder (higher altitude) regions, which favors flower bud formation, control of tree growth and fruit yield (Dennis, 2000; Faust, 2000), as well as reduces the incidence of leaf spot caused by *Colletotrichum* spp. (Moreira et al., 2019). However, the role of warmer and colder regions on the quality of different apple genotypes is unclear. Studies in various production regions may also help determining the effects of global warming on apple production and quality.

The objective of this study was to evaluate the effect of growing sites with different environmental conditions on 'Gala' and 'Fuji' apple quality at harvest and after storage.

2. Material and methods

2.1. Growing sites, plants, and experimental design

The experimental orchards were established in 2007 in São Joaquim (28°16′28.32″S, 49°55′59.04″W) and Caçador (26°50′8.42″S, 50°58′26.79″W) in Santa Catarina State, as well as further south in Vacaria (28°30′44″S, 50°56′02″W) in Rio Grande do Sul State. São Joaquim, Caçador and Vacaria are 1415 m, 960 m, and 971 m above sea level, and 92, 229 and 150 km from the sea, respectively. All three locations have a subtropical climate with warm summers and rainfall spread evenly throughout the year (Cfb, Köppen-Geiger classification system). In São Joaquim, Vacaria and Caçador, the soils are typic hapludox, humic Haplumbrept and typic oxisols, respectively. At each site, two sets of three rows were established with six 'Gala' strains ('Royal Gala', 'Imperial Gala', 'Gala Real', 'Maxi Gala', 'Galaxy' and 'Baigent') and three 'Fuji' strains ('Fuji Suprema', 'Mishima' and 'Fuji Select'). In one set of rows, the 'Gala' and 'Fuji' strains were on M.9 rootstock at 1.0 × 3.5 m spacing while the other set of rows had both cultivars on Marubakaido rootstock with M.9 interstem at 1.4 × 4 m spacing.

Trees were trained to a central leader and standard orchard management practices followed the technical recommendations for these growing sites (EPAGRI, 2018). The only exception was the use of bioregulators (mineral oil 3% v/v plus hydrogen cyanamide 0.5% v/v) for dormancy release in Caçador and Vacaria for both cultivars. The soil of the three growing sites was limed to pH 6.0 and fertilized with P and K, according to technical recommendations for these areas (EPAGRI, 2018).

2.2. Fruit harvest, sampling, and storage

Fruit were harvested over four 'Gala' and three 'Fuji' growing seasons (from year 5 to 8 after planting) within the commercial harvest window that was predicted based on fruit starch index. 'Gala' were harvested at one maturity stage, while 'Fuji' were harvested at two maturity stages in each growing site. The intended starch index (1–9 scale) for harvest was 4 to 6 for 'Gala', and 3 to 4.5 for the first harvest and 6.5 to 8 for the second harvest of 'Fuji'. 'Gala' and 'Fuji' grown in São Joaquim have been reported to reach a similar maturity to those grown in Caçador seven days later (Argenta et al., 1995). Therefore, for this study, fruit from São Joaquim were picked seven and five days later than in Caçador and Vacaria, respectively. This harvest scheduling was also necessary due to time constraints for fruit transportation, handling, packing and analyses.

A total of 100 unblemished fruit were harvested in each block. Twenty-five fruit were assessed for maturity and quality at harvest, and the remaining 75 fruit were assessed for quality after storage. Fruit for storage were placed onto fiberboard trays and trays packed into cardboard boxes (18 kg). Fruit were cooled to approximately 0.8 °C within 36 h after harvest.

Fruit were stored for 210 days at 0.8 °C ± 0.6 °C, with relative humidity maintained at approximately 92%, followed by seven days of shelf life at 22 °C ± 1 °C. 'Gala' apples were stored in CA (1.5 kPa O_2 and 2.5 kPa CO_2). 'Fuji' apples from the first harvest (less mature) were cold stored in air to allow the development of superficial scald and calcium related disorders, while 'Fuji' apples from the second harvest (more mature) were stored in CA with high CO_2 (1.5 kPa O_2 and 2.5 kPa CO_2) to induce CO_2 injury.

CA was initiated beginning 4 h after fruit reached 0.8 °C and setpoints were reached 48 h later. Low O_2 and high CO_2 were established and maintained using compressed N_2, CO_2, air, and a CO_2 scrubber. Concentrations of O_2 and CO_2 were monitored and adjusted every 180 min with an automatic CA system (Isolcell, Laives, Italy), using a CO_2 scrubber, and a VPSA N_2 generator (NeuTec, Lana, Italy).

2.3. Fruit maturity and quality analyses

Maturity and quality attributes were assessed for each fruit except for fresh weight, soluble solids content (SSC) and titratable acidity (TA). Fruit weight was determined in four replications of 25 fruit, while SSC and TA were determined in three fresh juice samples using longitudinal slices of fruit cheeks taken from eight fruit per replication. Assessments of flesh firmness, starch index (1–9 scale), TA and SSC were performed as described previously (Argenta et al., 2020).

The percentage of skin area with red color was determined subjectively by estimating the ratio of reddish surface area to overall fruit surface area. Mottled areas or traces of light-green or yellowish-white background between red stripes were not counted as red-colored areas. Physiological disorders and decay were assessed using subjective scales of severity, where a score of 0 (zero) indicated the absence of disorders and decay. Internal disorders were assessed from four transverse slices across the fruit. The severity of disorders was recorded considering the affected area or number of pits per fruit, as previously described (Argenta et al., 2020).

2.4. Fruit disorder analyses

Fruit with russet was rated as 1, 2, 3, or 4 when present on 1–4%, 5–10%, 11–25%, or >25% of the fruit surface, respectively. Fruit with superficial scald was rated as 1, 2, or 3 when symptoms were observed on ≤15%, 16–40%, or >40% of the surface, respectively. Bitter pit was scored considering the number of brown pits on the skin and or corky lesions in the cortex, where 1, 1–4 pits; 2, 5–9 pits; or 3, >9 pits. These scores were also used for the assessment of lenticel breakdown and *Glomerella cingulata* spot. Lenticel breakdown was characterized by dry and brown spots around the lenticels that was restricted to the skin without extending to the flesh tissue. Fruit cracking on the shoulder area to over the cheek, in the skin and underlying flesh was recorded as absent (0) or present (1). Flesh browning was assessed on a single cross section of fruit at the equatorial region (at upper edge of seed cavity). Fruit affected by this disorder was scored as 1, 1–30% of light-brown cortex; 2, 30–60% of light to dark-brown cortex; or 3, >60% of light to dark-brown cortex. Watercore, flesh browning, and core browning disorders were assessed on a single cross-section of 'Fuji' apples, after cutting the fruit at the equatorial region. The severity of watercore was rated as 1, water-soaked spots with diameter <5 mm around core line vascular bundles; 2, water-soaked blotches spread from vascular bundles, some coalesced and reaching up to 25% of flesh; and 3, >25% of flesh with water-soaked appearance. Rates of core (pith) browning are reported considering the darkness of browning, where 1, diffuse light-brown tissue; and 2, dark-brown tissue. CO_2 injury was assessed on four transverse cross-sections of each fruit. Scores for CO_2 injury were 1, ≤30%; 2, 31–60%; or 3, >60% of flesh with well-defined dark brown areas (brown-heart). CO_2 injury scores were also given to the development of light-brown and dry cavities in the affected tissues, where 1, 1 cavity; 2, 2–5 cavities; and 3, >5 cavities. Fruit with external decay symptoms were rated as 1, ≤1 cm^2 of fruit surface affected by one or more spots; or 2, >1 cm^2 of fruit surface affected. Fruit affected by core rot was rated as 1, <50% of core tissue rotted; 2, 50–100% of core tissue rotted; or 3, damage spread to the cortex tissue.

A disorder incidence index (DI) (%), weighted by severity, was calculated for each disorder, as described by Argenta et al. (2020).

2.5. Environmental data

Temperature, relative humidity, and rainfall data were obtained from weather stations of the Santa Catarina Agricultural Research and Extension Corporation (EPAGRI) and the Brazilian Agricultural Research Corporation (EMBRAPA), both located 0.5 km to 4 km from each experimental orchard. Temperature (°C) and relative humidity (%) were monitored every hour with a chart recorder thermo-hygrograph (Wilh. Lambrecht, Einbeck, Germany). Temperatures were also recorded daily at 9 AM, 3 PM, and 9 PM, using a mercury thermometer (Incoterm, Brazil). Maximum and minimum daily temperatures were determined using mercury and alcohol thermometers, respectively (Incoterm, Brazil). Daily average maximum and minimum temperatures were calculated for the 30-day period both after full bloom and prior to harvest. Daily average temperatures were used to calculate the average temperature from bloom to harvest, every year. The number of hours below 7.2 °C and chilling units accumulated, according to the modified North Carolina model (Ebert et al., 1986), were computed for winter (June through September) of each preceding harvest year. The growing degree days with a base temperature of 10 °C (GDD_{10}), as well as the heat accumulation units were calculated for the period between full bloom and harvest as described (Lindsey and Newman, 1956). Rainfall (mm) was recorded daily at 9 AM, 3 PM, and 9 PM, using a *Ville de Paris* rain gage (Hidromec, RJ, Brazil), which was used to calculate the accumulated rainfall from full bloom to harvest for each apple genotype, every year.

2.6. Statistical analysis

This study focused on comparing the three growing sites regardless of apple strains, rootstocks, or season. Therefore, data for 144 'Gala' experimental units (six strains x two rootstocks x three blocks x four growing seasons) and 54 'Fuji' experimental units (three strains x two rootstocks x three blocks x three growing seasons) were initially subjected to non-parametric Kruskal-Wallis test for multiple comparisons using R (Team-R-Core, 2020) and the add-on package 'agricolae' (Mendiburu, 2020). Climate measures and the length of the growing season from the three growing sites were also compared by non-parametric Kruskal-Wallis test using each year's data as replicates.

Growing sites were compared through canonical discriminant analysis (CDA), using the PROC CANDISC procedure of SAS (University Edition 2017, SAS Institute, Cary, NC) for cultivar, harvest date for 'Fuji', and four groups of variables: 1) quality and maturity at harvest; 2) physicochemical attributes after storage; 3) physiological disorders; and 4) diseases. Values for standardized canonical coefficients (SCC) and canonical correlations (*r*) were multiplied to calculate the parallel discriminant ratio coefficient (DRC). Canonical scores were used to determine how the first two canonical discriminant functions (CDF_1 and CDF_2) accounted for separation among growing sites for each group of variables. Mean standardized canonical scores of growing sites were compared by Tukey's test ($P \leq 0.05$) along canonical discriminant functions (CDFs). Results of CDA were presented only when there was a significant difference among growing sites by Wilk's Lambda multivariate statistical test, while the power of variables to discriminate growing sites are described only for significant CDFs.

3. Results

3.1. Environmental conditions

The experimental orchard in São Joaquim had more winter chilling hours and lower average, maximum and minimum temperatures for the entire growing season, including 30 days after bloom and 30 days before the harvest, compared to the Caçador and Vacaria sites (Table 1). The São Joaquim site also had less growing degree days and more days from bloom to harvest compared to the Caçador and Vacaria sites. Rainfall, relative humidity, and insolation were similar for all three sites.

3.2. 'Gala'

3.2.1. Quality at harvest and after storage

Site affected all 'Gala' quality attributes assessed at harvest as well as flesh firmness, TA and SSC assessed after storage (Table 2). Two canonical discriminant functions (CDF_1 and CDF_2) were significant for fruit quality at harvest. However, CDF_1 explained most (70.94%) of the separation among sites. Starch index had the highest power to discriminate among sites along CDF_1 (highest DRC) followed by weight and flesh firmness. TA had the highest power to discriminate among sites along CDF_2 which explained only 29.06% of separation among sites. 'Gala' produced in São Joaquim had a higher percentage of red skin than fruit from Caçador or Vacaria. However, skin red color had a smaller contribution to discriminate site compared to other quality attributes at harvest as indicated by the DRC values of CDF_1.

Discrimination among growing sites at harvest is shown by the differences in canonical scores (Fig. 1). Fruit from São Joaquim had the highest standardized canonical scores along CDF_1 (Fig. 1), representing fruit with the highest starch index and weight at harvest (Table 2), while fruit from Caçador (lower elevation site) had the highest canonical scores along CDF_2, corresponding to fruit with the highest TA at harvest. Fruit produced in Caçador and Vacaria had equal and the lowest canonical scores along CDF_1.

Only CDF_1 was significant for physicochemical attributes assessed after storage. The site discrimination for 'Gala' along CDF_1 was explained mostly by SSC. According to CDF_1, the canonical scores were highest for fruit from Caçador, intermediate for São Joaquim, and lowest for Vacaria reflecting SSC after storage for Vacaria fruit.

The loss of 'Gala' flesh firmness during storage plus shelf life were 29.8 N, 23.7 N and 27.2 N for São Joaquim, Caçador and Vacaria fruit, respectively (Table 2). Similarly, the TA loss during storage was slightly greater in São Joaquim fruit compared with fruit from Caçador or Vacaria.

3.2.2. Physiological disorders

Site had a significant effect on incidence index for 'Gala' russeting,

Table 1
Average environmental conditions in São Joaquim, Caçador and Vacaria experimental orchards, during four and three growing seasons of 'Gala' and 'Fuji' apples, respectively.

Environmental parameter	'Gala'			'Fuji'		
	São Joaquim	Caçador	Vacaria	São Joaquim	Caçador	Vacaria
1. Bloom to harvest						
Mean temperature	16.1 b	19.6 a	18.8 a	16.1 c	19.4 a	18.6 b
Maximum temperature [(2)]	21.7 b	25.9 a	25.4 a	21.6 b	25.8 a	25.1 a
Minimum temperature [(2)]	12.0 b	15.0 a	13.6 a	12.0 b	14.9 a	13.6 a
Growing degree-days [(3)]	1002 b	1295 a	1224 a	1176 b	1636 a	1543 a
Rain precipitation (mm)	514.2	585.2	471.3	912.3	862.3	756.1
Relative humidity (%)	79.2	80.1	77.5	78.2	79.0	76.5
Insolation (hours)	790.6	797.6	–	1136.1	1158.2	–
2. 30 days after full bloom						
Mean temperature	14.1 b	17.5 a	16.1 a	13.7 b	17.1 a	15.7 a
Maximum temperature [(2)]	19.6 b	23.8 a	22.5 a	19.1 c	23.2 a	22.0 b
Minimum temperature [(2)]	10.1 b	13.0 a	11.1 ab	9.8 b	12.8 a	10.7 ab
3. 30 days before harvest						
Mean temperature	17.2 b	20.5 a	20.0 a	16.0 b	18.6 a	17.6 a
Maximum temperature [(2)]	22.8 b	26.9 a	26.4 a	21.0 b	25.1 a	23.9 ab
Minimum temperature [(2)]	13.2 b	16.0 a	15.2 a	11.6 b	14.3 a	13.2 a
4. Winter environment						
Hours below 7.2 °C	914.8 a	555.0 b	701.5 ab	882 a	545 b	777 a
Chilling units [(1)]	2192.3 a	959.0 c	1447.5 b	2198 a	1046 c	1562 b
Days from full bloom to harvest	145 a	126 b	127 b	163 a	157 b	156 b

Means followed by different letters in each row and cultivar are significantly different according to Kruskal-Wallis test (α = 0.05).
[(1)] Determined during the winter (June through September) based on the North Carolina model (Ebert et al., 1986).
[(2)] Mean of daily maximum or minimum temperature.
[(3)] Heat accumulation unit.

Table 2
Quality of 'Gala' apples in São Joaquim, Caçador, and Vacaria, Brazil. Quality attributes were assessed at harvest and after 210 days of cold (0.8 °C) storage in a controlled atmosphere (1.5 kPa O_2, 2.5 kPa CO_2), plus seven days of shelf life (22 °C). Values are means of six 'Gala' strains, two rootstocks, four years and three blocks (n = 144). Values highlighted in gray for multivariate analysis, showing parallel discriminant ratio coefficients (DRC) for canonical discriminant functions 1 (CDF_1) and 2 (CDF_2).

Attribute	Sites			DRC	
	São Joaquim	Caçador	Vacaria		
		At harvest		CDF_1	CDF_2
Red skin area (%)	75.1 a	68.7 b	50.6 c	0.0659	−0.0163
Weight (g)	151.0 a	133.7 b	125.5 c	**0.2933**	0.0539
Starch index (1–9)	6.6 a	4.1 c	5.8 b	**0.4898**	0.4096
Flesh firmness (N)	76.6 a	77.3 a	72.4 b	**0.1110**	−0.2195
Soluble solids (%)	13.5 a	13.5 a	12.5 b	−0.0175	0.0033
Titratable acidity (%)	0.399 a	0.412 a	0.308 b	0.0575	0.7691
Canonical correlation[(1)]				0.9363***	0.8628***
Proportion (%)[(2)]				70.94	29.06
	After storage			CDF_1	CDF_2
Flesh firmness (N)	46.8 b	53.6 a	45.2 b	−0.2894	1.3451
Soluble solids content (%)	13.6 b	14.4 a	12.8 c	**1.2002**	−0.1319
Titratable acidity (%)	0.307 b	0.346 a	0.272 b	0.0892	−0.2132
Canonical correlation[(1)]				0.7659***	0.1394^{ns}
Proportion (%)[(2)]				98.62	1.38

Means followed by different letters in each row are significantly different according to Kruskal-Wallis test (α = 0.05).
[(1)] Canonical correlations between growing sites and quality and maturity attributes. ***: $P < 0.0001$; ns: not significant ($P > 0.05$).
[(2)] Proportion of total variance explained by CDFs.

cracking, and flesh browning but not for lenticel breakdown (Table 3). Only CDF_1 was significant for physiological disorders. Bitter pit and russeting had the highest DRCs to discriminate among the sites with a lower contribution from flesh browning. DRCs and canonical scores for CDF_1 indicate 'Gala' from São Joaquim had less russeting and more bitter pit than Caçador or Vacaria fruit.

3.2.3. Diseases

Glomerella cingulata spot at harvest and core rot after storage were significantly affected by site whereas no site effect was observed for external decay after storage. Only CDF_1 was significant and represented 74.61% of the separation among sites for the disease indexes. *Glomerella cingulata* spot had the highest DRC along the CDF_1 with a lower contribution from core rot. Fruit grown in Caçador had the most *Glomerella cingulata* spot and the least core rot.

3.3. 'Fuji'

3.3.1. Quality at harvest and after storage

'Fuji' quality assessed at harvest and after storage was influenced by site for both early (except for SSC) and late harvests (Table 4). CDF_1 explained almost the entire separation among sites. At the early harvest, discrimination among sites was primarily due to flesh firmness followed by fruit weight, length/diameter ratio and percentage of red skin. At the late harvest, discrimination among sites was primarily due to percentage of red skin followed by starch index, TA, and fruit weight along CDF_1 as well as due to SSC followed by percentage of red skin along CDF_2.

Early harvest fruit from São Joaquim had the highest standardized canonical score on CDF_1 reflecting the highest flesh firmness, weight, length/diameter ratio and percentage of red skin (Fig. 2). Fruit from Caçador had the lowest standardized canonical score while Vacaria fruit had a standardized canonical score significantly different from and between those of São Joaquim and Caçador. Along CDF_2, Vacaria fruit had the highest standardized canonical scores with lower percentage of red skin and higher starch index than fruit from Caçador and São Joaquim.

Late harvest fruit from São Joaquim had the highest standardized canonical score on CDF_1 reflecting higher skin red color, starch index and weight, and lower TA compared with Caçador and Vacaria fruit.

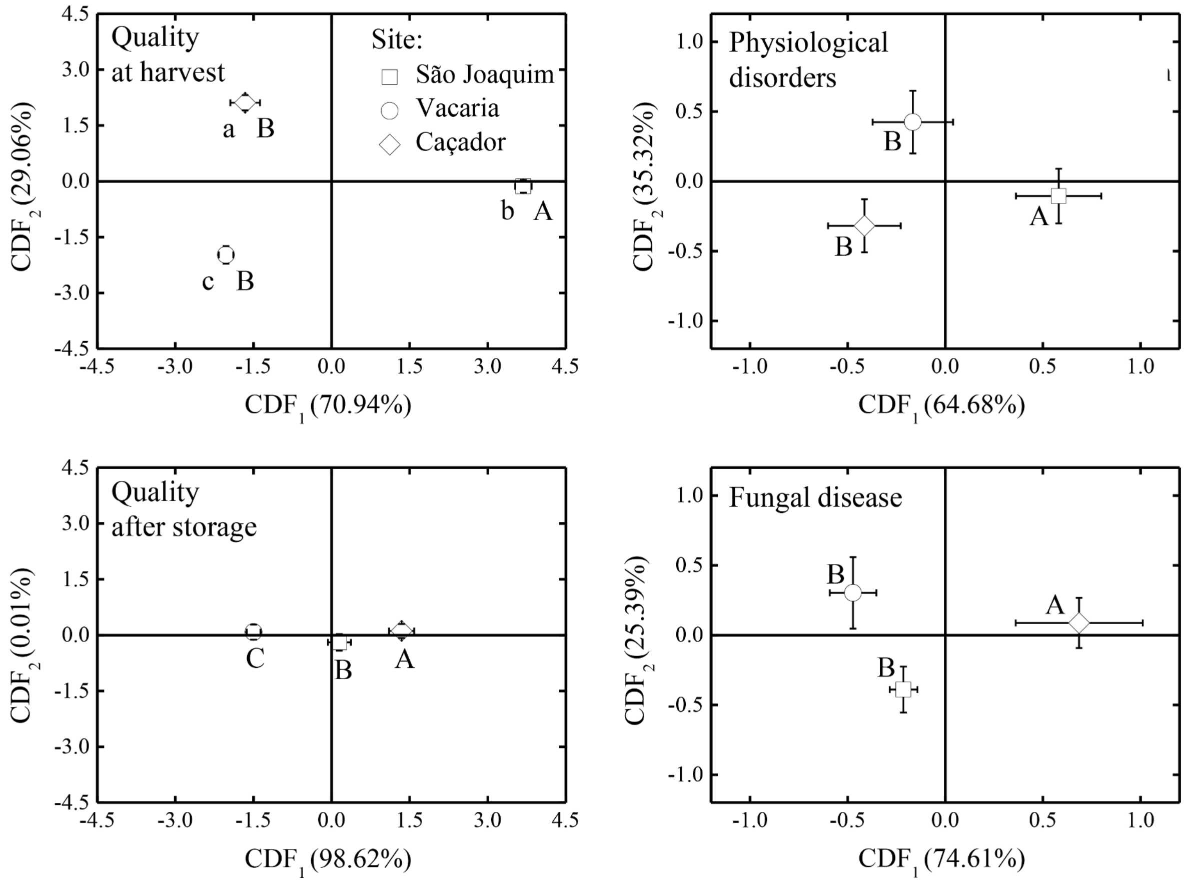

Fig. 1. Canonical scores of growing sites considering the quality attributes for 'Gala' apples assessed at harvest and after storage, described in Table 2 (left graphics), and physiological disorders and fungal disease described in Table 3 (right graphics). Growing sites with mean standardized canonical scores (± SE) followed by different upper-case letter along CDF_1 or lower-case letter along CDF_2 are different by Tukey's test ($P \leq 0.05$). Values in brackets represent the proportion of total variance explained by CDF_1 and CDF_2. Data for six 'Gala' strains, two rootstocks and four years were pooled ($n = 144$).

Table 3
Physiological disorders and decay indexes [incidence (%) weighted by severity] for 'Gala' apples grown in São Joaquim, Caçador, and Vacaria, Brazil. Russeting was assessed at harvest while the other disorders and decay were assessed after 210 days of cold (0.8 °C) storage in a controlled atmosphere (1.5 kPa O_2, 2.5 kPa CO_2), plus seven days of shelf life (22 °C). Values are means of six 'Gala' strains, two rootstocks and four years and three blocks ($n = 144$). Values highlighted in gray for multivariate analysis, showing parallel discriminant ratio coefficients (DRC) for canonical discriminant functions 1 (CDF_1).

Attribute	Sites			DRC
	São Joaquim	Caçador	Vacaria	
	Physiological disorder index			CDF_1
Russeting	17.0 c	21.9 b	24.7 a	**0.3742**
Fruit cracking	4.7 a	1.9 b	3.9 a	−0.0042
Flesh browning	16.6 a	8.4 b	13.8 a	**0.1618**
Bitter pit	0.050	0.005	0.029	**0.4588**
Lenticel breakdown	0.019	0.007	0.031	0.0094
Canonical correlation[(1)]				0.3965*
Proportion (%)[(2)]				64.68
	Disease index			CDF_1
Decay	7.68 a	5.91 a	5.63 a	−0.0010
Core rot	0.023 ab	0.017 b	0.065 a	**0.2419**
Glomerella cingulata spot	0.007 b	1.103 a	0.013 b	**0.7592**
Canonical correlation[(1)]				0.4523**
Proportion (%)[(2)]				74.61

Means followed by different letters in each row are significantly different according to Kruskal-Wallis test (α = 0.05).
[(1)] Canonical correlations between growing sites and attributes (physiological disorders and decay). **: $P < 0.01$.*: $P < 0.05$.
[(2)] Proportion of total variance explained by CDFs.

The separation of sites based on flesh firmness, TA and SSC assessed after storage was mostly explained by CDF_1 for both harvests. TA of early harvest fruit had the highest power to discriminate sites along CDF_1, while flesh firmness of late harvested fruit had the highest power to discriminate sites along CDF_1.

After storage, early harvest fruit from São Joaquim had the highest standardized canonical score on CDF_1 with highest TA, while fruit from Caçador had the highest standardized canonical score on CDF_2 with highest SSC. Late harvest fruit from São Joaquim had the highest standardized canonical score on CDF_1 with lower flesh firmness while Caçador fruit had the highest standardized canonical score on CDF_2 with highest SSC.

Flesh firmness (N) loss during storage plus shelf life were 27.6, 20.9, and 24.4 (early harvest) and 7.6, 1.3, and 2.2 (late harvest) for fruit from São Joaquim, Caçador and Vacaria, respectively. TA losses during storage were lowest for both early and late harvest fruit from São Joaquim compared with Caçador.

3.3.2. *Physiological disorders*

Development of physiological disorders before and after harvest were affected by site (Table 5). Watercore index increased with advanced harvest maturity and was higher in fruit produced in São Joaquim compared to Caçador and Vacaria. CO_2 injury was detected only in late harvest fruit with the highest index for São Joaquim fruit. Core browning only developed in early harvest fruit with the highest index in fruit from São Joaquim and Vacaria. Diffuse flesh browning index was highest in São Joaquim fruit regardless of harvest date. Bitter pit incidence, low in fruit from all three sites, was highest for early

Table 4
Quality of 'Fuji' apples produced in São Joaquim, Caçador, and Vacaria, Brazil. These attributes were assessed at harvest and after 210 days of storage at 0.8 °C, plus seven days of shelf life at 22 °C. Fruit were harvested at two maturity stages. Fruit of early harvest were stored in air and fruit of late harvest were stored in controlled atmosphere (1.5 kPa O_2 and 2.5 kPa CO_2). Averages represent the quality data obtained from three 'Fuji' strains, two rootstocks, three blocks, and three growing seasons (n = 54). Data highlighted in gray for multivariate analysis, showing parallel discriminant ratio coefficients (DRC) for canonical discriminant functions 1 (CDF_1) and 2 (CDF_2).

Attribute	Sites			DRC	
	São Joaquim	Caçador	Vacaria		
	At early harvest			CDF_1	CDF_2
Red skin area (%)	49.3 a	41.4 b	33.3 c	**0.1429**	**0.6598**
Weight (g)	175.0 a	139.6 b	141.8 b	**0.1902**	−0.0445
Length/diameter ratio	0.855 a	0.791 c	0.821 b	**0.1850**	0.1298
Starch index (1–9)	3.5 b	3.0 b	4.4 a	0.0246	**0.2240**
Flesh firmness (N)	78.6 a	73.4 b	73.6 b	**0.3774**	0.0329
Soluble solids (%)	13.9	14.1	14.0	0.0442	−0.0076
Titratable acidity (%)	0.389 b	0.442 a	0.434 a	0.434 a	0.0055
Canonical correlation[(1)]				0.9350***	0.7432***
Proportion (%)[(2)]				84.92	15.08
	At late harvest			CDF_1	CDF_2
Red skin area (%)	72.8 a	61.5 b	48.0 c	**0.3832**	**0.3403**
Weight (g)	190.2 a	162.2 b	152.3 c	**0.1866**	−0.0599
Starch index (1–9)	7.8 a	6.5 b	7.2 ab	**0.2340**	**0.2260**
Flesh firmness (N)	68.6 a	66.8 b	67.6 ab	0.0426	0.0317
Soluble solids (%)	15.9 a	15.9 a	15.1 b	−0.0416	0.4476
Titratable acidity (%)	0.375 b	0.438 a	0.422 a	**0.1952**	0.0143
Canonical correlation[(1)]				0.9271***	0.6276***
Proportion (%)[(2)]				90.40	9.60
	After air storage (early harvest)	CDF_1	CDF_2		
Flesh firmness (N)	51.3 ab	52.7 a	49.4 b	0.0124	0.2292
Soluble solids (%)	13.8 b	14.3 a	13.6 b	0.0227	**0.7723**
Titratable acidity (%)	0.148 a	0.138 b	0.129 c	**0.9649**	−0.0016
Canonical correlation[(1)]				0.6007***	0.4889***
Proportion (%)[(2)]				64.26	35.74
	After CA storage (late harvest)	CDF_1	CDF_2		
Flesh firmness (N)	61.1 b	65.5 a	65.3 a	**0.8534**	0.0997
Soluble solids (%)	15.7 ab	16.0 a	15.4 b	0.0563	**0.9359**
Titratable acidity (%)	0.228	0.230	0.218	0.0903	−0.0356
Canonical correlation[(1)]				0.8127***	0.3957*
Proportion (%)[(2)]				91.28	8.72

Means followed by different letters in each row are significantly different according to Kruskal-Wallis test (α = 0.05).
(1) Canonical correlations between growing sites and physicochemical attributes. ***: $P < 0.001$; *: $P < 0.05$.
(2) Proportion of total variance explained by CDFs.

harvest apples from Caçador and Vacaria.

Only CDF_1 was significant for physiological disorders for early harvest fruit whereas both CDF_1 and CDF_2 were significant for late harvest fruit. Canonical correlation and proportion of total variance were higher for CDF_1 than CDF_2 regardless of harvest date. Core browning in early harvest fruit had the highest power to discriminate sites along CDF_1 with a lower contribution for watercore and diffuse flesh browning. For late harvest fruit stored in CA, watercore and flesh browning had the highest power to discriminate among sites along CDF_1.

After storage, early harvest apples from São Joaquim had the highest standardized canonical score on CDF_1 with more core browning, watercore and diffuse flesh browning compared with Caçador and Vacaria fruit (Fig. 3). Late harvest apples from São Joaquim had the highest standardized canonical score on CDF_1 with more watercore and flesh browning than fruit from Vacaria or Caçador.

3.3.3. *Diseases*

There was no difference among sites for disease index observed in early harvest fruit (Table 6). Late harvest fruit from Caçador had more decay and core rot compared to apples from São Joaquim and Vacaria.

4. Discussion

The results document variability in environmental condition among the three growing sites despite their geographic proximity and similar climate. The highest elevation site (São Joaquim) can be distinguished from the other two sites mainly by lower mean air temperature during winter as well as during fruit growth and development. The average temperature from bloom to harvest were 3.3 °C to 3.5 °C lower at the coldest compared with warmest site.

Establishment and operation of the three experimental orchards were designed to minimize site to site variability. The orchards were established in the same year with the same scion clones on the same rootstocks obtained from the same nursery, planted at the same spacing, trained with same training system, operated with the same orchard management practices. Therefore, it is reasonable to assume that the variability observed in fruit growth, development and quality are likely associated with site to site differences in environmental conditions.

The length of the growing season has been reported to be determined by spring temperatures with a shorter season following relatively warmer springs (Warrington et al., 1999). It has been suggested that for each 1 °C rise in average spring temperature, the time from bloom to harvest maturity is shortened by 3.5 days (Palmer et al., 2003). In our study, the effect of site on the time from bloom to harvest was greater for 'Gala' than 'Fuji' indicating cultivar and/or maturation date can be factors contributing to fruit response to spring temperature. The longer growing season for 'Gala' (19 days) in São Joaquin may be an overestimate, considering that fruit from this site had higher starch index at harvest and could have been harvested one week earlier to meet similar starch index of fruit harvested in Caçador or Vacaria. In New Zealand, the time from bloom to harvest of 'Royal Gala' can vary from 140 to 157 days in the coldest and from 132 to 148 days in the warmest growing sites depending on the production year (Stanley et al., 2000).

Although growing site influenced many maturity and quality

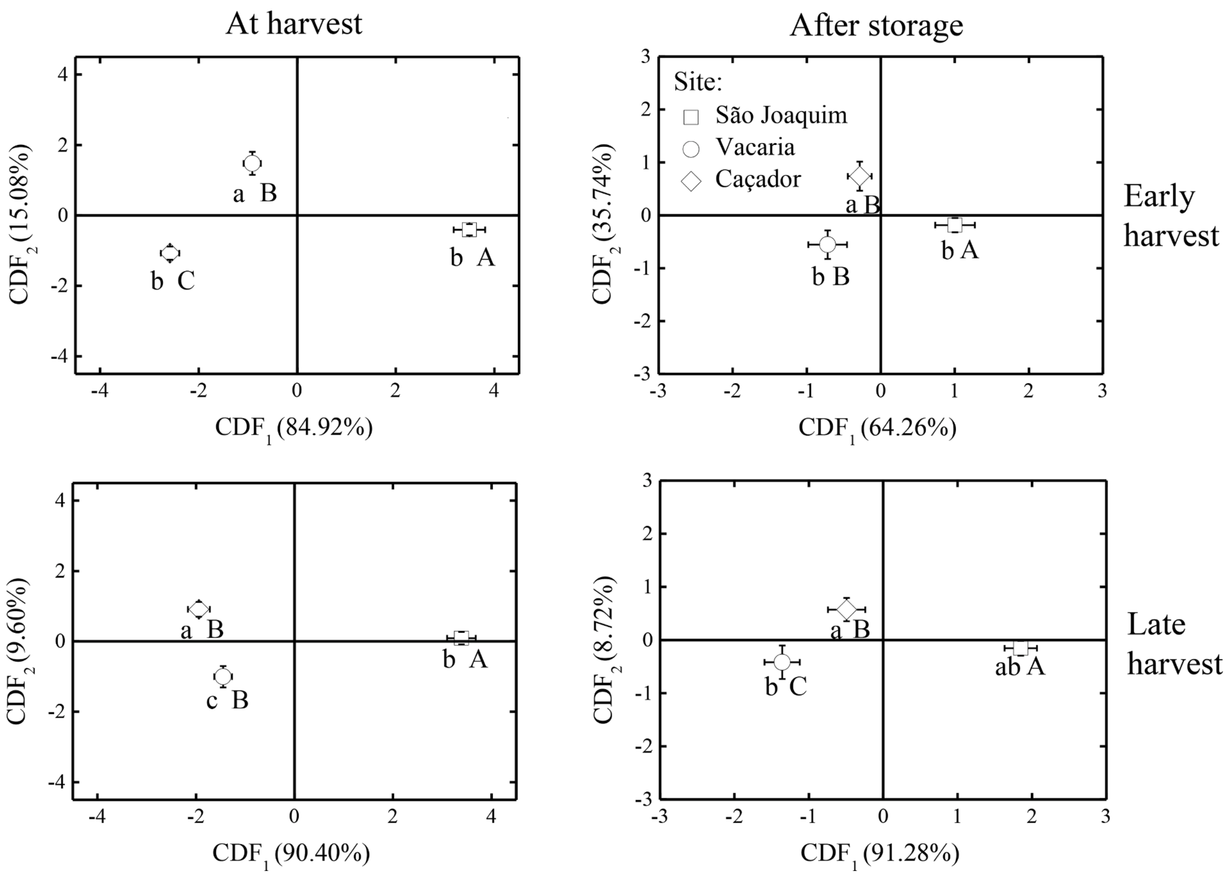

Fig. 2. Canonical scores of growing sites considering the physicochemical attributes for 'Fuji' apples assessed at harvest (left graphics) and after storage (right graphics) described in Table 4. Fruit were harvested at early (top graphics) and late (bottom graphics) maturity stages. Early harvest fruit were stored in air and late harvest fruit in a controlled atmosphere (CA: 1.5 kPa O_2, 2.5 kPa CO_2). Growing sites with mean standardized canonical scores (± SE) followed by different upper-case letter along CDF_1 or lower-case letter along CDF_2 are different by Tukey's test ($P \leq 0.05$). Values in brackets represent the proportion of total variance explained by CDF_1 and CDF_2. Data obtained from three 'Fuji' strains, two rootstocks, three blocks, and three growing seasons ($n = 54$).

Table 5
Physiological disorders index [incidence (%) weighted by severity] for 'Fuji' apples produced in São Joaquim, Caçador, and Vacaria, Brazil. Watercore and russeting were assessed at harvest while the other disorders were assessed after 210 days of cold (0.8 °C) storage plus seven days of shelf life (22 °C). Early harvest fruit were stored in air and late harvest fruit in a controlled atmosphere (CA: 1.5 kPa O_2 and 2.5 kPa CO_2). Data obtained from three 'Fuji' strains, two rootstocks, three blocks, and three growing seasons ($n = 54$). Results highlighted in gray for multivariate analysis indicate parallel discriminant ratio coefficients (DRC) for canonical discriminant functions 1 (CDF_1) and 2 (CDF_2).

Attribute	Sites			DRC	
	São Joaquim	Caçador	Vacaria		
	After air storage (early harvest)			CDF_1	CDF_2
Watercore	3.569 a	1.050 b	0.131 b	**0.2959**	0.3391
Russeting	9.6 b	15.1 a	12.7 ab	−0.0577	0.1953
Core browning	5.299 a	0.049 b	2.038 a	**0.5420**	0.0876
Bitter pit	0.088 b	0.444 a	0.199 a	0.0330	0.0075
Flesh browning	0.484 a	0.000 b	0.008 b	**0.2120**	−0.0018
Superficial scald	38.2	32.4	34.3	−0.0251	0.3722
Canonical correlation[1]				0.8262***	0.3197^{ns}
Proportion (%)[2]				94.97	5.03
	After CA storage (late harvest)			CDF_1	CDF_2
Watercore	45.163 a	1.708 c	6.778 b	**0.7172**	0.0521
Russeting	9.8 b	15.3 a	13.7 ab	0.0103	0.0095
CO_2 injury (browning)	8.25 a	4.03 b	5.14 b	0.0510	**0.1110**
CO_2 injury (cavities)	4.280 a	2.844 b	3.096 ab	−0.0066	0.0226
Bitter pit	0.077	0.078	0.041	0.0105	0.0349
Flesh browning	8.069 a	0.000 b	0.052 b	**0.2171**	0.0516
Superficial scald	0.03	0.13	0.66	0.0004	**0.7184**
Canonical correlation[1]				0.9785***	0.5459***
Proportion (%)[2]				98.15	1.85

Means followed by different letters in each row are significantly different according to Kruskal-Wallis test (α = 0.05).
[1] Canonical correlations between growing sites and physiological disorders. ***: $P < 0.001$. ns: $P \geq 0.05$.
[2] Proportion of total variance explained by CDFs.

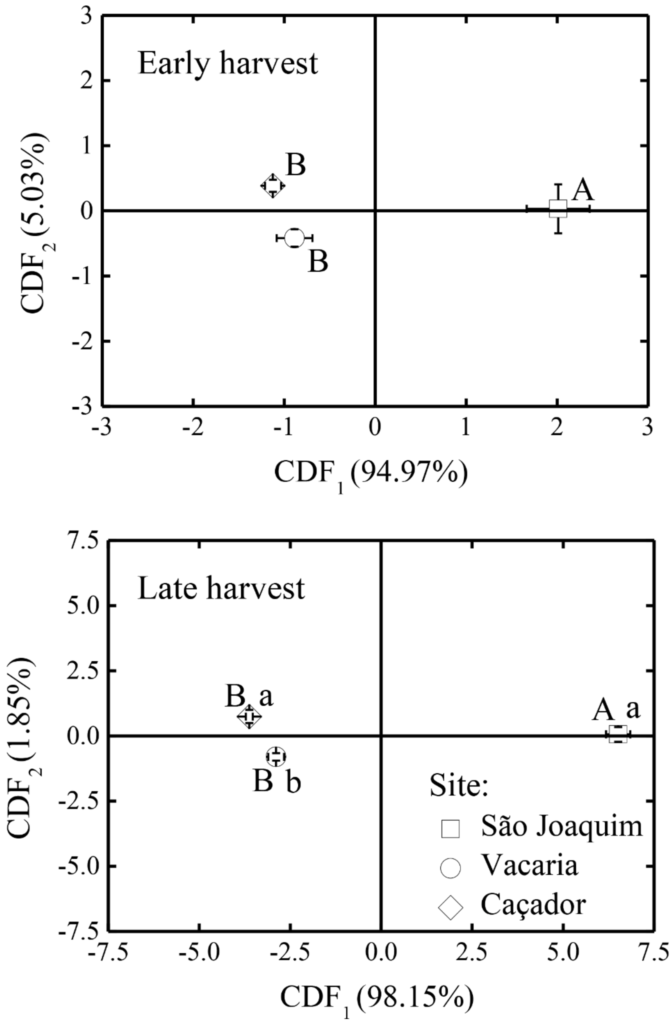

Fig. 3. Canonical scores of growing sites considering the physiological disorders for 'Fuji' apple fruit assessed at harvest and after cold (0.8 °C) storage, described in Table 5. Fruit were harvest at early and late maturity stages. Early harvest fruit were stored in air and late harvest fruit in a controlled atmosphere (CA: 1.5 kPa O_2, 2.5 kPa CO_2). Growing sites with mean standardized canonical scores (± SE) followed by different upper-case letter along CDF_1 or lower-case letter along CDF_2 are different by Tukey's test ($P \leq 0.05$). Values in brackets represent the proportion of total variance explained by CDF_1 and CDF_2. Data obtained from three 'Fuji' strains, two rootstocks, three blocks, and three growing seasons ($n = 54$).

Table 6
'Fuji' disease index for fruit produced in São Joaquim, Caçador, and Vacaria, Brazil. Apples were stored for 210 days (0.8 °C) plus seven days of shelf life (22 °C). Early harvest fruit were stored in air and late harvest fruit in a controlled atmosphere (CA: 1.5 kPa O_2, 2.5 kPa CO_2). Data obtained from three 'Fuji' strains, two rootstocks, three blocks, and three growing seasons ($n = 54$).

Disease	Site		
	São Joaquim	Caçador	Vacaria
	early harvest		
Decay	16.8	20.1	16.3
Core rot	1.01	0.66	1.01
	late harvest		
Decay	10.8 b	16.7 a	8.6 b
Core rot	1.32 ab	1.63 a	0.78 a

Means followed by different letters in each row are significantly different according to Kruskal-Wallis test (α=0.05).

attributes, the multivariate statistical analysis (CDA) showed that some attributes have greater power than others to discriminate the fruit from each site depending on cultivar. Fruit size was one of the quality attributes with the highest power to discriminate fruit among the three sites for both cultivars. Variation in fruit size within a cultivar has been reported to be primarily related to fruit cell number (Coombe, 1976). Apple trees growing in low temperatures within the 40–50 days after pollination produce smaller fruit (Stanley et al., 2000; Warrington et al., 1999). These authors postulated that lower spring temperatures suppress cell-division leading to smaller fruit at harvest, despite the longer cell-division phase induced by lower spring temperatures. It is interesting to point out that heat accumulation from full bloom to harvest maturity was lower at the cooler growing site, despite a longer period from full bloom to harvest. The same was reported by Stanley et al. (2000), who compared growing locations with different mean temperatures in New Zealand.

Cell division before anthesis has also been considered a major determinant of cell number and fresh weight of fleshy fruit (Coombe, 1976). Additionally, fruit from flowers opening early are larger compared to fruit from late bloom due to a greater cell number at anthesis (Coombe, 1976). A somatic mutation that results in larger size has also been associated with enhanced floral organ size with higher cell number and larger cell size (Malladi and Hirst, 2010). Studies have demonstrated that for 'Gala' and 'Fuji' apples, female floral organs on trees cultivated in the temperate climate in Ohio, USA, are larger than those cultivated in the subtropical climate in southern Brazil (Francescatto et al., 2016). Based on field trails at different growing locations (between 34 and 38°S), Reginato et al. (2019) suggested fruit size is primarily dependent on the maximum temperature during winter with colder areas yielding larger fruit. Therefore, the highest fruit weight from the coldest site in São Joaquim may be associated with a temperature effect on floral development and as well as greater cell expansion after anthesis. Floral bud development may be negatively affected by high temperatures in the winter as well as in the summer (Palmer *et al.*, 2003). Sub-optimal fruit growth leading to small fruit size associated with high temperatures, particularly at night, may be related to excessive respiration and consumption of carbohydrate reserves (Faust, 2000).

'Fuji' apples from the warmest site were more oblate and more rounded at the coldest site. Variation in fruit shape has been reported for other cultivars and is related to cool temperatures during the first few weeks after bloom that stimulate growth of the apical portion of the fruit leading to a high length/diameter (L/D) ratio (Dennis, 2003). 'Golden Delicious' fruitlets exhibit increased gibberellic acid activity with increased orchard elevation and a higher L/D ratio at harvest from high elevation orchards (Eccher, 1986). 'Gala' L/D ratio was not assessed because a preliminary visual assessment indicated no site effect on this parameter.

Flesh firmness for many apple cultivars at harvest is determined by fruit maturity and size (Johnston et al., 2002a). In the present study, fruit from the coldest site had similar ('Gala') or higher ('Fuji') firmness at harvest compared with fruit from the warmer sites despite being larger and having a higher ('Gala') or similar ('Fuji') starch index. However, fruit from the coldest site had similar or lower firmness than fruit form the warmest site after storage indicating that softening rate can be higher for fruit produced under colder conditions. This result is consistent with studies showing small fruit softened more slowly after harvest compared with large fruit, especially when harvested at advanced maturity (Harker et al., 1997; Johnston et al., 2002b). Small fruit have a higher cell wall density resulting in stronger flesh tissue compared with that of large fruit (Harker et al., 1997). Our results are also similar to those of a study conducted in Chile where softening of 'Gala' apples from cold sites was greater compared with softening of fruit from warm sites even when fruit from all sites had similar firmness at harvest (Yuri et al., 2019). However, for late season cultivars 'Fuji', 'Granny Smith' and 'Cripp's Pink', the softening rate during storage was not affected by growing site environmental condition (Yuri et al., 2019). Other studies have suggested low air temperatures from 31 to 60 days after full bloom (DAFB), followed by high temperature and precipitation from 61 to 90 DAFB and high temperatures from 91 DAFB until harvest negatively impact 'McIntosh' apple firmness at harvest (Lachapelle et al., 2013). Collectively this information indicates average temperature as well as temperature extremes can affect fruit firmness prior to and after harvest.

Although studies have shown earlier apple maturation in response to warm temperatures (Tromp, 1997; Warrington et al., 1999), the effect of growing sites with different temperatures on fruit maturity may not be the same for all cultivars and maturity indices (Yuri et al., 2019). 'Gala' apples reach harvest maturity early in warm sites based on ethylene production and rate of firmness loss (Yuri et al., 2019). However, background color change is faster at colder sites, and starch degradation rate was similar among different sites (Yuri et al., 2019). 'Fuji' apples produced at cold sites had higher firmness, but similar starch index compared with apples produced at warm sites (Yuri et al., 2019). In northern Greece, 'Fuji' maturity based on firmness, soluble solids content and starch index was not impacted by site elevation (20 m vs 750 m) but skin red color development increased with site elevation (Karagiannis et al., 2020).

The higher starch index for 'Gala' grown at the coldest site was unexpected considering a previous long-term study where starch index was similar for apples produced in São Joaquim and Fraiburgo, a relatively warm site near Caçador (Argenta et al., 1995). On the other hand, the results of the present study are consistent with studies showing higher starch accumulation in apples produced under high summer temperatures (Kingston, 1992). Additionally, lower night temperatures during the pre-harvest period may favor conversion of starch into sugars resulting in a higher starch index (Kingston, 1992; Smith et al., 1979; Watkins et al., 1982). In contrast, Toivonen (2019) suggests that in apple orchards exposed to smaller day-night temperature differences, fruit may have lower starch content, possibly due to higher respiratory depletion of carbohydrates in the tree.

For both cultivars, TA and SSC of fruit from the warmest site was similar or higher than in fruit from the coldest site, while TA of fruit from the two warm sites was similar at harvest but different after storage. These results suggest there is no well-defined pattern of TA and SSC responses to these different environmental conditions, consistent with previous studies (Tromp, 1997).

Apple skin red color increases in response to lower temperatures and higher light exposure (Iglesias et al., 2016; Telias et al., 2011; Yuri et al., 2019). Therefore, the greater area of skin red color on fruit of both cultivars produced in the coldest site is likely related to lower temperatures considering that the insolation did not differ between growing sites. The amount of pigment in the fruit is more closely correlated to average night temperature than to the average day temperature (Telias et al., 2011) and a period of cold temperature followed by warm temperature stimulates anthocyanin production (Curry, 1997). However, for this region, the amplitude of maximum and minimum temperatures was the same for the three growing sites and the differences between the coldest and warmest sites was grater for maximum temperature than for the minimum temperature during the 30 days preceding harvest. Fruit from the two warm sites differed in red color development even though these two sites had the same average temperature during the 30 days preceding harvest. These results show that factors in addition to insolation and temperature are determinants of red color development in apples.

Russeting is a periderm formed in hypodermal tissue as a repair process of microcracked cuticle (Khanal et al., 2021). Rainy and humid weather conditions are major factors that favor cuticular microcracking and ultimately russeting (Chen et al., 2020). However, our results show that russeting incidence among growing sites was not related to rain or relative humidity because these factors were similar for all sites. Therefore, other factors are also contributing to russeting development. Studies have suggested an association between russeting and epi- and hypodermal cell morphology (Khanal et al., 2020), and reduced cuticle deposition (Chen *et al.*, 2020). Lower russet incidence at high elevation orchards has been reported to be associated to higher gibberellin (GAs) activity (Eccher, 1986). Greater susceptibility of apple cultivars to this disorder can also be related to higher GA_4/GA_7 ratio in the fruit (Eccher and Hajnajari, 2006). Reduced cuticular microcracking and russet following GA treatment is likely due to its effects on epi- and hypodermal tissues (Knoche et al., 2011). 'Starkrimson' and 'Golden Delicious' apples from cold, high elevation sites have thicker cuticles, longer and thinner epidermal cells, and longer and thinner sub-epidermal cells with more layers (Li et al., 2004). Based on these findings, the reduced development of russet on fruit from the coolest site could be related to the influence of environmental factors, including temperature, on anatomy and morphology of dermal tissue and on cuticle development with reduced risk to cuticular microcracking.

The store of early harvested fruit in air and late harvested fruit in CA allowed comparing 'Fuji' apples from the three growing sites in terms of fruit susceptibility to physiological disorders. The susceptibility of 'Fuji' apples to superficial scald and calcium related disorders have been reported to be higher when fruit are harvested at early maturity and stored in air (Ferguson and Watkins, 1989; Lurie and Watkins, 2012). In addition, the susceptibility of 'Fuji' apples to CO_2 injury increases when fruit are harvested at advanced maturity and stored under CA conditions with high CO_2 levels (Argenta et al., 2002b).

The highest incidence of watercore and CO_2 injury in 'Fuji' grown in the coldest site is consistent with studies reported for other cultivars (Elgar et al., 1999; Harker et al., 1999; Lau, 1998). Additionally, a positive correlation between watercore severity at harvest and susceptibility to CO_2 injury during storage has also been reported (Argenta et al., 2002a).

'Fuji' from the coldest site had the highest incidence of diffuse flesh browning regardless of harvest date. Previous studies have shown diffuse flesh browning development is enhanced in response to cold orchard temperatures as well as by watercore (Meheriuk et al., 1994). The incidence of flesh browning in 'Gala' from the coldest site was similar to the incidence in fruit from one but not both warm sites and may be related, in part, to higher starch index and greater fruit size, as suggested by Lee et al. (2013).

Core browning occurred only in early harvest 'Fuji' and incidence was highest in fruit produced at the coldest site. This result is similar to other studies where early harvest and cool temperatures during the production season increased the risk of core browning (Watkins and Mattheis, 2019).

In summary, the results enhance the understanding of the impact of orchard environment on fruit quality and storability. Fruit grown in warm sites may be smaller with less red color and more russet compared to fruit from cool sites and this lower quality will result in a lower grade based on quality standards. However, cold site fruit can be more susceptible to physiological disorders such as watercore, CO_2 injury, and core browning.

The results from the present study indicate the shorter growing season in warmer compared to cooler growing sites was less for the late season cultivar 'Fuji'. Fruit maturity and quality attributes with the highest power to discriminate among growing sites varied with cultivar and harvest time. In contrast to previous studies, fruit size was more related to winter temperature than spring temperature. Skin red color development was not related to maximum-to-minimum temperature ratio in the 30 days preceding harvest. Differences in russeting development among these growing sites did not appear to be due to rainfall and humidity which was similar among sites. Both cultivars grown at relatively cold site softened at a faster rate during storage compared to warm site fruit suggesting site environment rather than harvest season influences postharvest firmness retention. 'Fuji' superficial scald was not related to growing site temperature in the 30 days preceding harvest as has been suggested previously (Lurie and Watkins, 2012). Fruit from the warmest ‘Gala’ growing site was most affected by *Glomerella cingulata* spot while ‘Fuji’ fruit were more affected by fungal decay and core rot compared to colder site fruit. SSC in warmest site fruit was similar or higher than in coldest site fruit depending on cultivar and analysis time. The findings suggest site and cultivar interactions contribute to apple postharvest characteristics and storage performance.

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CRediT authorship contribution statement

Luiz Carlos Argenta: Conceptualization, Visualization, Writing - original draft, Writing - review & editing. **Cassandro Vidal Talamini do Amarante:** Statistical data analysis, Writing - review & editing. **Sergio Tonetto de Freitas:** Writing - original draft, Writing - review & editing. **Thyana Lays Brancher:** Writing - original draft, Software computing data, Writing - review & editing. **Cristiano Nunes Nesi:** Statistical data analysis, Writing - review & editing. **James P. Mattheis:** Writing - original draft, 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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