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RESEARCH ARTICLE

Influence of preharvest environmental conditions and postharvest relative humidity on the appearance of orange peel disorder in sweet cherry during fruit development and storage

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ABSTRACT

Orange peel, a physiological disorder, has been reported in Chilean sweet cherries (*Prunus avium* (L.) L.) after 35-45 d under modified atmosphere packaging (MAP). The ontogeny of the disorder is unknown, and preharvest factors have not been thoroughly investigated. This study involved two experiments to elucidate the association between environmental factors and the incidence of the orange peel disorder. In the first experiment, plant water potential, fruit position on the tree, relative humidity (RH), and radiation of sweet cherry trees were studied in 'Regina', 'Kordia', and 'Lapins'. The second experiment focused on the interaction effect of heat during fruit development and the RH during storage in 'Lapins'. The first experiment showed no clear response of orange peel incidence to natural variations in air temperature, vapor pressure deficit, and transmitted solar radiation (PPFD) within trees. However, significant differences in the incidence, severity, and timing of the disorder detection were observed among cultivars. In 'Lapins', orange peel disorder began before harvest in the green stage, 33.7 %, while 'Regina' and 'Kordia' showed negligible incidences of 1.25% and 0%, respectively. At postharvest, no differences were found among tree sections, either in incidence or severity of the disorder. For the second experiment, no differences in incidence were found in heated fruit during preharvest or postharvest RH treatments. However, it was observed that the higher the RH (100%), the lower the severity index (3.6) was for the orange peel disorder. The high incidence of the disorder during postharvest under natural or induced environmental conditions in the three cultivars evaluated demonstrates that RH mainly affects the orange peel disorder.

Key words: Pebbling, physiological disorder, postharvest, *Prunus avium*, quality, relative humidity.

INTRODUCTION

Sweet cherry (*Prunus avium* (L.) L.) has become one of Chile's top ten fruit species and the first crop exported from the Southern Hemisphere. During the 2023-2024 season, Chilean cherry exports reached 413 979 t, and the leading destination was the Asian market (mainly China) with 91% of total shipments (Iqonsulting, 2024), meaning that Chilean sweet cherry is shipped at least 35 d with the concomitant appearance of physiological disorders. During postharvest, Schlegel et al. (2018) identified three disorders that limit the cherry fruit quality: Stem cracking and browning, pitting, and a skin disorder that develops during storage referred to as orange peel, pebbling, alligator peel or lizard peel. Among the symptoms of the orange peel disorder, the most predominant is rough skin, which is very similar to the skin of citrus fruit and has a refined and sculpted appearance, which Schlegel et al. (2018) described as regions below (valleys) and above (peaks) the virtual mid-surface. In addition, rough skin causes a loss of brightness, affecting the cosmetic quality standard of the cherry and its color. The orange peel disorder is usually located in the shoulder area, equatorial, and distal zone of the fruit, and among others, the most susceptible cultivars are Lapins, Regina, Santina, and Sweetheart (Zoffoli et

al., 2017; Schlegel et al., 2018). The orange peel disorder affects the sweet cherry's appearance and its commercial value. This physiological disorder has not been thoroughly studied; however, skin damage induced by osmotic dehydration through water movement and loss is the leading hypothesis behind this disorder (Schlegel et al., 2018). On the other hand, orange peel disorder is associated with cherries stored over 45 d at 0 °C; however, it has also been documented during harvest (Zoffoli et al., 2017). In some cases, the disorder has been detected as early as the seventh day of storage, with severity increasing over time, even in fruit under 100% RH without transpiration (Schlegel et al., 2018). Although this disorder is associated with long-term storage at low temperatures (0 °C), Ross et al. (2020) have suggested it is not exclusive to postharvest and can be observed in cherries during harvest.

Many reported postharvest disorders in fruit species are directly related to preharvest factors, which are dominated by fruit position on the tree, fruiting site characteristics, crop load, mineral nutrition of the developing fruit, water relations, and response to environmental temperatures (Ferguson et al., 1999). Another hypothesis for the orange peel disorder is the position of the fruit on the tree based on the fact that sugar, fructose, sucrose, and sorbitol contents of sweet cherries were evaluated after pruning, resulting in significant differences influenced by the position of the fruit on the tree (Vošnjak et al., 2021), in addition to the different light intersection associated to the tree architecture (Willaume et al., 2004). However, the influence of the fruit position on the tree on the appearance of orange peel disorder is yet to be proven.

This research aimed to determine the phenological time of appearance of the disorder orange peel as a function of the fruit position on the tree, natural microclimatic conditions of the plants, induced heating effect on the trees, and interaction with RH during storage that would affect the incidence of the disorder. We intend to elucidate the ontogeny of the orange peel disorder from preharvest and how the disorder evolves throughout postharvest storage in 'Lapins', 'Kordia' and 'Regina' cherry cultivars in the first experiment, and in the most sensitive cultivar, 'Lapins', in the second experiment.

MATERIALS AND METHODS

Experiment 1

Plant material. The study was conducted on sweet cherry (*Prunus avium* (L.) L.) 'Lapins', 'Kordia' and 'Regina', all of them planted in 2017 and growing in a commercial orchard located in Lago Ranco (40°17'10.0" S, 72°33'26.4" W) (southern Chile) in Los Ríos Region, during the 2021-2022 season. 'Lapins' on 'Colt' rootstock was planted at 5×3 m, whereas 'Kordia' and 'Regina' on 'Gisela 6' rootstock were at 4×1.8 m. Sweet cherry trees were trained in a central axis system (VCL). The trees were under a transparent plastic cover from September to March. The plastic cover consisted of a gable roof using a high-density polyethylene film of 150 µm thickness (Delsantek S.A., Santiago, Chile). A stratified sampling was conducted, four trees were randomly chosen for each cultivar. Each tree was divided into four sections of the canopy: Top east, bottom east, top west, and bottom west. Ten fruits were harvested from each section at each developmental stage, collecting 40 cherries per experimental unit, i.e., for each tree of each cultivar (four trees per cultivar). Sampling was conducted during the following developmental fruit stages: At green or pre-*veraison*, yellow or *veraison*, red, and mahogany red (commercial harvest) according to the cherry phenological stages and color chart used by Villavicencio et al. (2021).

'Kordia' was harvested 81 d after full bloom (AFB), which occurred on 21 October 2021, the second cultivar harvested was 'Lapins' 84 d AFB and the last cultivar was 'Regina', harvested 86 d AFB. All fruit was transported to a packinghouse in a refrigerated truck and processed by imposing modified atmosphere packaging.

The commercial harvest was done during the morning between 08:00 and 13:00 h in small plastic crates and then cooled in a hydrocooler equipment at 5 °C. The fruit were later transported in a refrigerated truck to a packinghouse for processing, packaging and storage. At packinghouse, before passing through the hydrocooler once only, the crates with the fruit were covered with a net to avoid damage from the falling water. The fruit were packed in a 1 kg polyethylene terephthalate (PET) container, and a modified atmosphere packaging (5% CO_2 and 15% O_2) was developed by sealing on the top with laser microperforated polypropylene film (View Fresh, Santiago, Chile). A group of 16 PET containers was packed in an 8 kg carton box and placed in forced air tunnel under the commercial system to reach 0 °C pulp temperature. Later, the fruit were stored for 40 d at 0 °C in a commercial chamber, where the atmosphere (CO_2 and O_2) inside each PET container was

evaluated using the Dansensor CheckPoint 3 portable gas analyzer (Ametek Mocon Inc., Minneapolis, Minnesota, USA) at 3, 7, 15, 30 and 40 d at 0 °C. After removal from cold storage, the fruit were kept for three more days at 15 °C, simulating a shelf-life period.

Environmental factors. Daily temperature (maximum, minimum and average) and RH measurements were recorded every 30 min using a U23 Pro v2 sensor (HOBO, Onset, Bourne, Massachusetts, USA), and data were collected throughout the fruit development from green stage until commercial harvest for each cultivar. Solar radiation measurements were made in full sun, at noon (12:00-13:00 h), using a ceptometer LP-80 (Decagon Devices, Pullman, Washington, USA), which measures photosynthetic photon flux density (PPFD). Data were collected at 160 cm from the ground vertically to measure the upper sections and at 80 cm vertically to measure the lower sections, at a 1 m distance for each section, where three subsamples were measured for each section. Transmitted PPFD was calculated as the ratio of PPFD measured within each tree section to PPFD measured above each tree. Radiation readings were measured for each of the cultivars at green stage (December) and at commercial harvest (January), 'Regina' was measured on 9 December and 15 January, 'Kordia' on 9 December and 10 January, and 'Lapins' on 2 December and 13 January. The air temperature and RH data were used to calculate the vapor pressure deficit (VPD, kPa) according to Allen et al. (1998). For comparison purposes, the data recorded by the nearest meteorological station 29.3 km apart (15.1 km in a straight line) (40°09'55.4" S, 72°38'04.7" W) were used.

Plant water status was measured as midday stem water potential (12:00-15:00 h) on three different dates during the sampling period, following the methodology described by McCutchan and Shackel (1992). Two leaves per tree of each cultivar were selected to measure stem water potential using a pressure chamber PMS 615 (PMS Instrument Company, Albany, Oregon, USA). Sampled leaves were placed inside a hermetic aluminum bag for at least 40 min before excision to avoid water loss through transpiration.

Fruit quality assessments during pre and postharvest. All quality assessments were conducted at every phenological stage, at harvest, after 40 d at 0 °C, and 40 d at 0 °C plus 3 d at 15 °C. The incidence of orange peel physiological disorder was determined by the presence or absence of total fruit and expressed as a percentage (%) of affected fruit for each section and sampling stage. The severity of this disorder was determined with a subjective scale of four levels depending on the percentage of the affected fruit surface: Healthy (0%), mild (5%-25%), moderate (25%-50%) and severe (50%-100%). A severity index was also calculated by adding the value given to each severity level (0 healthy or not affected – 1 mild – 2 moderate – 3 severe) of each of the fruits and dividing it by the total number of affected fruits in the section at the corresponding stage.

Experiment 2

Plant material. A second experiment was carried out during 2022-2023 on sweet cherries 'Lapins' planted in 2012-2013 in a commercial orchard located in Tralcao (39°41'35.4" S, 73°07'11.9" W) in Los Ríos Region, 128 km from the orchard studied the first season. The orchard was selected for its known high incidence records of orange peel disorder and its cultivation of a susceptible cultivar such as Lapins (demonstrated during the first-year results). The orchard was 12 yr old, the trees were on 'F12' rootstock in a planting frame of 4 × 3 m, and the rows were 60° to the northeast, trained in a central axis system. The trees were under cover from blooming to harvest (October to January). The structure and the plastic cover type were the same as those used in experiment 1.

Heat treatments (preharvest treatments). Three sweet cherry trees were randomly chosen to impose passive heat treatments (chamber) (Soar et al., 2009), and three trees with no treatments (control). Each of the three chambers enclosed a single tree, comprised of a wooden frame of 2.6 × 2.6 × 2.8 m (L × W × H), and was wrapped with a 100 µm transparent polyethylene film as reported by Rivelli et al. (2024). The imposed passive heat treatment allowed the diurnal temperature to be increased by at least 4 °C. The imposed treatment aimed to simulate a higher background temperature than the usually found in January (21.2 °C) for this geographical zone.

Twenty fruits were harvested from each tree (chamber and control treatments) at the same fruit phenological stages described in the experiment 1. Then, at commercial harvest, all fruit from each tree were picked (~ 420 cherries per tree). Harvest was carried out between 08:00 and 13:00 h on 27 December 2022, 78 to 81 d after full bloom (AFB), which occurred between 7 and 10 October 2022. Fruits were harvested in small

plastic crates, transported to the postharvest laboratory at Universidad Austral de Chile, and evaluated the same day. The initial quality evaluation consisted of 40 cherries per tree, and the remaining ~ 380 were stored at 0 °C for 35 d, plus 3 d at 20 °C, after which they were evaluated for fruit quality and the appearance of orange peel disorder (incidence and severity).

Relative humidity treatments (postharvest treatments). After commercial harvest, 10 cherries in three replicates (i.e., 30 cherries per tree) were placed in a 1 L PVC box with a transparent lid with a 2 mm hole for gas exchange. Thus, the RH treatments comprised 18 boxes: 2 Heating treatments (chamber and control) × 3 replicates × 3 RH treatments. The RH treatments were as follows: i) 100% RH Treatment, a small plastic cup was placed with 10 mL distilled water; ii) 65% RH treatment, a Petri dish was placed with 60 g calcium chloride and 8 mL distilled water, and iii) 45% RH treatment, 60 g lithium chloride and 8 mL distilled water were used. Relative humidity was monitored through HOBO Temperature/RH Data Logger sensors placed inside each RH treatment.

Environmental factors. Daily air temperature (maximum, minimum and average), RH, solar radiation, and plant water status were measured under the same procedures used in the first experiment. Solar radiation and plant water status data were collected at the green stage or pre-*veraison* (3 December 2022), yellow or *veraison* (10 December 2022), red (18 December 2022), and commercial harvest (27 December 2022). For comparison purposes, air humidity and temperature data recorded by the nearest weather station 33.5 km (14.5 km in a straight line) (39°47'21.6" S, 73°14'06.2" W) was used.

Fruit quality assessments during pre and postharvest. The same quality evaluation described for the first experiment was carried out in each phenological stage for the heating treatments (preharvest treatments), for the RH treatments (postharvest treatments), and after cold storage removal after 35 d at 0 °C plus 3 d at 20 °C.

Statistical analysis

Experiments 1 and 2 were carried out with a completely randomized design. Experiment 1 was performed with four replicates per cultivar. The experimental unit was a single tree. Experiment 2 was performed with three replicates (three trees). A factorial design was performed with two factors: i) Heating treatment with two levels (heated and non-heated fruit), and ii) RH treatments with three levels (45%, 65% and 100% RH), with three replicates each. The results were subjected to ANOVA comparing the differences in fruit quality and orange peel disorder associated with tree sections and phenological stages for season 2021-2022. The interaction between the tree section and the phenological stage was also studied. The same quality parameters were assessed in the 2022-2023 season as a function of the heating treatment and phenological stages, and the RH treatments during postharvest in interaction with the heating treatment. Significant differences were determined using a Tukey test $P \leq 0.05$. The statistical program used for these analyses was InfoStat software version 2020 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina) for both seasons.

RESULTS

Experiment 1

Environmental factors. In general, transmitted PPFD within trees was less than 40% of the PPFD transmitted in December and January, regardless of the cultivar (Figure 1). The transmitted PPFD at the top was slightly higher than at the bottom of the tree. Yet, the highest variation in transmitted PPFD was measured between the sides of the tree (east and west) rather than between heights of the canopy (top and bottom) in 'Regina' and 'Lapins'. This was clearer in the 'Regina' trees, where the transmitted PPFD measured at the east side of the tree tended to be ~ 20% and 40% higher than at the west side in December and January, respectively (Figure 1A). Whereas the transmitted PPFD at the east side remained relatively stable from December to January, a ~ 20% increase in transmitted PPFD at the west side was registered between both measuring dates. In 'Kordia' plants, differences in transmitted PPFD among sections were not the same in December and January (Figure 1B). In December, the transmitted PPFD was slightly higher at the west side of the plant for the top section (7%), but not for the bottom. In January, regardless of the tree height, the transmitted PPFD at the west side was higher than at the east side.

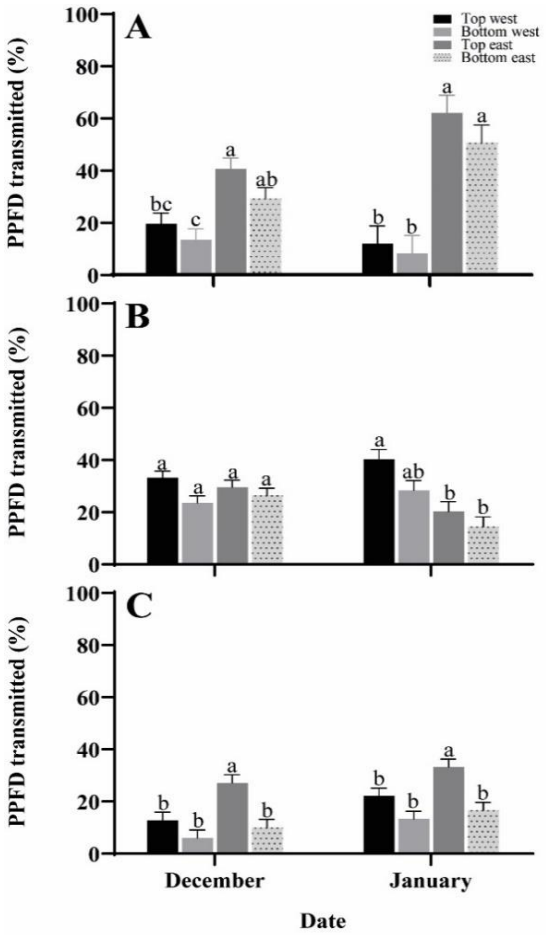

Figure 1. Radiation interception in each of the four sections measured in the canopy for Regina (A), Kordia (B) and Lapins (C) cultivars at stages green or pre-*veraison* and mahogany red (commercial harvest). Letters indicate a significant difference among tree sections. Means are presented ± standard error. PPFD: Photosynthetic photon flux density.

The RH recorded during the different dates of sampling in 'Regina' was slightly higher than that recorded in the other cultivars and the environmentally recorded, while 'Regina' ranged between 54% and 84% RH (first and last dates), the data recorded outside the canopy (ambient data) and for 'Kordia' and 'Lapins' ranged on average between 48% and 79% (Figure 2A).

With respect to temperature, Figure 2B shows a 1-2 °C average difference between the temperature recorded environmentally and that recorded in the cultivars under cover. While the environmental temperature ranged from 13 to 19 °C on average, the cultivars ranged from 17 to 21 °C.

The VPD was calculated using the corresponding equation for the hours between 13:00 and 15:00 h, when the highest VPD occurred. Figure 2C shows that it is higher on 2 and 9 December in the cultivars (1.2-1.4 kPa) with respect to the environmental (1.0-1.2 kPa), while from 16 December onwards the calculated VPD begins to increase in the environmental record, increasing from 1.0 kPa (16 December) to 1.8 kPa on 10 January with respect to that recorded in the cultivars where for the same dates the VPD oscillates between 0.9 and 1.2 kPa. For the last recording date, it can be observed that the VPD of both environmental and cultivars is between 1.0 and 1.1 kPa.

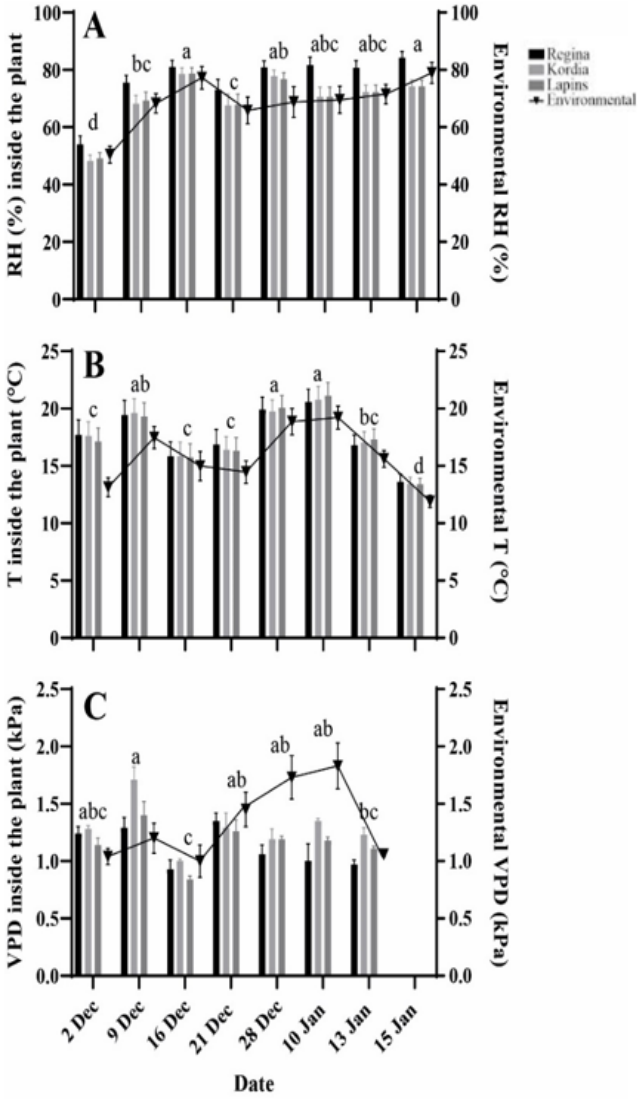

Figure 2. Daily average measurements of relative humidity (A), temperature (B) and vapor pressure deficit (VPD) (C). Calculation performed on one tree per cultivars Regina, Kordia, Lapins (sensor inside the canopy, under cover) and the environment (weather station, without cover) during the sampling period grouped in weeks and for 3 h of the day (between 12:00 and 15:00 h). Letters indicate a significant difference between dates at 95% confidence level. Means are presented ± standard error.

It is worth mentioning that during the sampling period from 2 December 2021 to 15 January 2022 some precipitation occurred close to the sampling dates corresponding to dates 7, 8, 11, 12, 17, 29, 30, 31 December and from 1st to 3 January, which ranged from 0.1 mm for dates 8 and 31 December to 2.77 mm for 29 December.

Stem water potential (SWP) values ranged from -0.7 to -0.5 MPa during the evaluation period (data not shown); in general, SWP in 'Lapins' was 0.2 MPa higher than in 'Kordia', indicating a slightly better plant water status for 'Lapins'. In this study, 'Lapins' and 'Kordia' plants tended to show subtle increases in SWP as the season progressed while a 0.1 MPa increase was observed in 'Regina' at the second measurement.

Fruit quality assessment. 'Regina' showed a low incidence of orange peel disorder at preharvest green stage (1.25%), and did not show any symptoms until harvest, when the incidence increased to 53.1 (Figure 3A). The incidence of the disorder in 'Kordia' was observed at the yellow fruit stage with a 22.5% in one of the tree sections, in contrast to harvest, when the incidence was reduced to 7.5%. On the other hand, 'Lapins' was the most affected cultivar at the green stage, the disorder incidence ranged from 12% to 33.6%, and at harvest the incidence increased between 57.5% and 82.5%. In postharvest, the incidence values in 'Regina' ranged from 67.5% to 52.3% for the 40 d at 0 °C and 40 d + 3 d at 15 °C stages, respectively (Figure 3B). Likewise, in 'Kordia', the values increased to 70% and 98.8% approximately after 40 d at 0 °C and 40 d + 3 d at 15 °C. 'Lapins' showed 98.1% of affected fruits during postharvest, and there were nonsignificant differences in the incidence of the disorder among tree sections ($P \leq 0.05$).

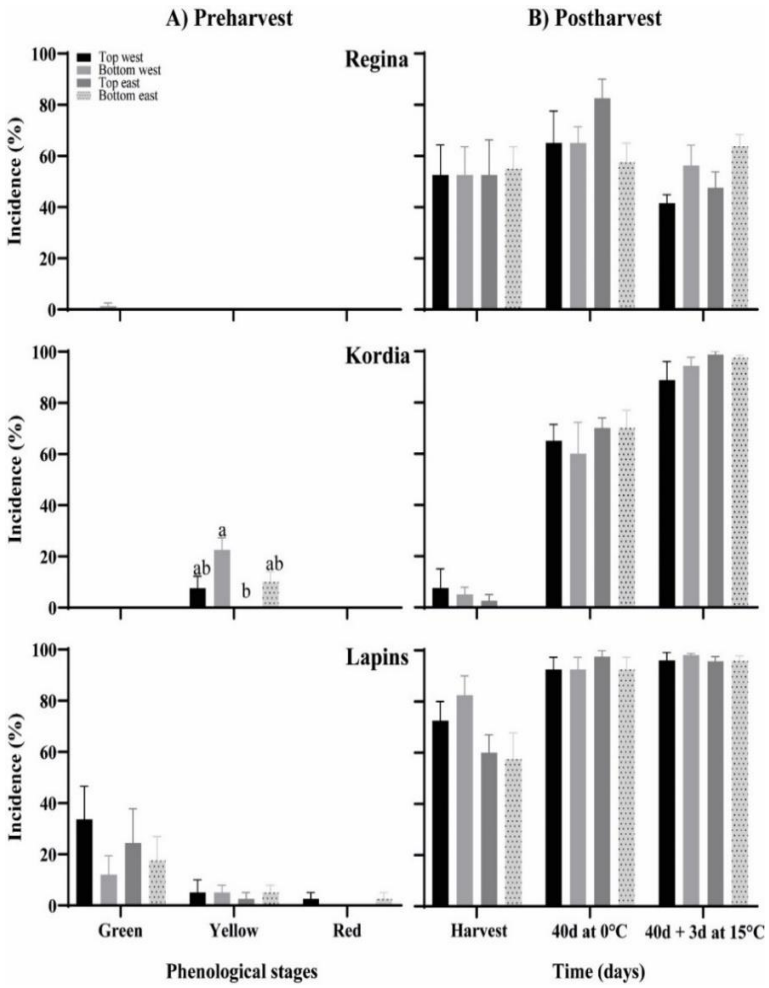

Figure 3. Incidence (%) of orange peel disorder of 'Regina', 'Kordia' and 'Lapins' sweet cherries during pre-harvest (A) and post-harvest (B) for each section and at the stages green or pre-*veraison*, yellow or *veraison*, red, mahogany red (commercial harvest), and after 40 d at 0 °C and 40 d + 3 d at 15 °C. Letters indicate a significant difference between sections at 95% confidence. Means are presented ± standard error.

The severity of the orange peel disorder in 'Regina' was compared to the other cultivars (Table 1). In the first stages, the severity had an index of 0.0 to 1.4 until harvest, corresponding to a level between healthy and mild. Then, the severity increased to an index of 2.3 in 40 d + 3 d at 15 °C, corresponding to a moderately affected fruit. 'Kordia' remained slightly affected (index 1.0) until harvest, to significantly increase its severity index at the last stage (2.6 severe). As for 'Lapins', the fruit remained 'healthy' until the red fruit stage, increasing to 3.0 severely affected fruit at the 40 d + 3 d at 15 °C storage.

Table 1. Severity index of orange peel disorder in sweet cherries fruit for each tree section. Index 0 = healthy, 1 = mild, 2 = moderate and 3 = severe.

		Tree section			
Cultivar	Stage	Top west	Bottom west	Top east	Bottom east
Regina	Green	0.0	0.0	0.0	0.0
	Yellow	0.0	0.0	0.0	0.0
	Red	0.0	0.0	0.0	0.0
	Harvest	1.0	1.4	1.2	1.1
	40 d at 0 °C	1.3	1.5	1.5	1.3
	40 d + 3 d at 15 °C	2.0	2.3	2.1	2.2
Kordia	Green	0.0	0.0	0.0	0.0
	Yellow	0.0	0.0	0.0	0.0
	Red	0.0	0.0	0.0	0.0
	Harvest	1.0	0.0	0.0	0.0
	40 d at 0 °C	1.4	1.3	1.3	1.7
	40 d + 3 d at 15 °C	2.3	2.6	2.6	2.8
Lapins	Green	0.0	0.0	0.0	0.0
	Yellow	0.0	0.0	0.0	0.0
	Red	0.0	0.0	0.0	0.0
	Harvest	1.9	2.0	1.6	1.9
	40 d at 0 °C	2.0	2.2	2.2	2.0
	40 d + 3 d at 15 °C	2.9	3.0	2.9	3.0

Experiment 2

Heating treatment. The fruit under heating treatment in the orchard and the control, showed significant different preharvest data (Table 2). The average temperature difference between the heating and control treatment was 9.2-10.2 °C and 28.6%-37.5% RH higher than the control. The VPD was almost half in the heated treatment than the control.

The orange peel incidence was nearly 100% in the early stages of fruit development and during postharvest. Only the red stage and at harvest showed less than 100% incidence, however, nonsignificant differences were found (Figure 4).

The severity index of orange peel disorder for heating and control treatments at the different phenological stages showed nonsignificant differences (Table 3). The control fruit showed a moderate to severe damage at harvest (index = 2.6), whereas the heated fruit presented severe damage after 35 d at 0 °C (index = 3.0). Both treatments presented the same severity level at the end of storage (index = 3.0).

Relative humidity treatments. The fruits were affected in a 100% incidence by the orange peel disorder after 35 d at 0 °C plus 3 d at 20 °C. No differences were found between the heated and control fruit and among the RH treatments (data not shown). On the other hand, the severity index showed a significant difference among the RH treatments; hence, the higher the RH (100%), the lower the severity index (3.6) was (Table 4). Significant differences were found between the heated and control fruit at the interaction level.

Table 2. Preharvest factors of phenological stages green or pre-*veraison*, yellow or *veraison*, red, and mahogany red (commercial harvest) of sweet cherries 'Lapins' with heating and control treatments. Lowercase letters represent differences between treatments according to the Tukey's multiple comparisons test (P ≤ 0.05). Uppercase letters represent differences between the top and bottom sections of the sweet cherry tree. PPFD: Photosynthetic photon flux density; VPD: vapor pressure deficit.

Preharvest factors	Stage		Treatment	
			Heat	Control
PPFD available, µmol m^2 s^{-1}	Green		1916.8^{b}	2309.8^{a}
	Yellow		1746.6^{b}	2175.8^{a}
	Red		1797.9^{b}	2004.8^{a}
	Harvest		1830.7^{b}	2158.1^{a}
PPFD transmitted, %	Green	Top	8.8^{aA}	6.5^{aA}
		Bottom	3.3^{aB}	2.3^{aB}
	Yellow	Top	8.8^{aA}	8.0^{aA}
		Bottom	2.9^{aB}	2.5^{aB}
	Red	Top	8.5^{aA}	13.6^{aA}
		Bottom	3.9^{aB}	3.8^{aB}
	Harvest	Top	7.1^{aA}	4.5^{bA}
		Bottom	3.2^{aB}	2.7^{aB}
Temperature, °C	Green		24.6^{a}	23.3^{b}
	Yellow		35.0^{a}	24.8^{b}
	Red		33.6^{a}	23.9^{b}
	Harvest		32.3^{a}	22.9^{b}
Relative humidity, %	Green		56.8^{a}	55.0^{a}
	Yellow		86.7^{a}	50.0^{b}
	Red		88.3^{a}	59.7^{b}
	Harvest		86.2^{a}	48.8^{b}

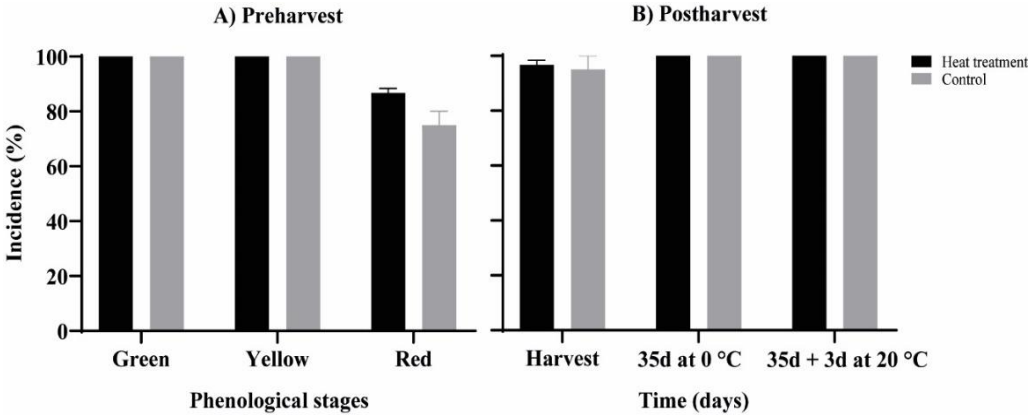

Figure 4. Incidence of orange peel disorder of 'Lapins' sweet cherry during pre-harvest (A) and post-harvest (B) for the heating and control treatments at the stages: Green or pre-*veraison*, yellow or *veraison*, red, mahogany red (commercial harvest), and after 35 d at 0 °C and 35 d + 3 d at 20 °C. Letters indicate a significant difference between stages at 95% confidence. Means are presented ± standard error.

Table 3. Orange peel disorder severity index of sweet cherries 'Lapins' with heating and the control treatments at different phenological stages. Index 0 = healthy, 1 = mild, 2 = moderate and 3 = severe. Means followed by the same letter within each row are not statistically different according to the Tukey's multiple comparisons test (P ≤ 0.05).

Phenological stage	Treatment	
	Heat	Control
Green	1.3[a]	1.3[a]
Yellow	1.9[a]	1.8[a]
Red	1.4[a]	1.4[a]
Harvest	2.0[b]	2.6[a]
35d at 0 °C	3.0[a]	2.8[b]
35d + 3d at 20 °C	3.0[a]	3.0[a]

Table 4. Severity index of orange peel disorder in sweet cherry fruit 'Lapins' under heating (chamber) and RH treatments. Index 0 = healthy, 1 = mild, 2 = moderate, 3 =severe and 4 = extremely severe. Means followed by the same letter within each column (lowercase) and within each row (uppercase) are not significantly different according to the Tukey's multiple comparisons test (P ≤ 0.05).

Effect	Days of storage			
	35 d at 0 °C		35 d + 3 d at 20 °C	
Heat treatment				
Heat	3.8[a]		3.8[a]	
Control	3.8[a]		3.7[a]	
RH treatment				
45%	3.9[a]		3.7[a]	
65%	3.9[a]		3.7[a]	
100%	3.6[b]		3.7[a]	
Heat × RH	Heat	Control	Heat	Control
45%	3.8[Aa]	4.0[Aa]	3.7[Ba]	3.8[Aa]
65%	3.7[Bab]	4.0[Aa]	3.7[Ba]	3.8[Aa]
100%	3.7[Bab]	3.4[Bb]	3.9[Aa]	3.5[Bb]

DISCUSSION

Experiment 1

Environmental factors. Differences in transmitted photosynthetic photon flux density (PPFD) among the tree sections indicated differences in canopy density that were not the same for each cultivar throughout fruit development. In 'Regina' (Figure 1A), the differences between east and west are even larger than between the other tree heights, which is not similar for 'Kordia' or 'Lapins' (Figures 1B and 1C). The large increase in transmitted PPFD (~ 20%) on the east side of the 'Regina' trees in January suggests that the harvest may have caused the most severe defoliation in this cultivar (Figure 1A), which would be expected for a cultivar with a higher crop load. These results suggest that 'Regina' trees had the lowest intra-canopy uniformity of light conditions among the cultivars. On the other hand, 'Lapins' tended to have the lowest values of transmitted PPFD at each tree section, but the highest uniformity of light conditions (Figure 1C). In grape berries, Calderon-Orellana et al. (2014) suggested that changes in microclimate uniformity during fruit growth and development may induce the occurrence of some physiological disorders, such as berry shrinkage, at harvest. Plants under the plastic covering exhibited a nearly 20% reduction in the PPFD, similar to that reported by Blanco et al. (2021) for plastic-covered sweet cherry trees, furthermore, it has

been reported that depending on climatic conditions, reductions can range from 15 % to 40 % (Salvadores and Bastías, 2023). However, the reduction in environmental PPFD in covered plants may increase the growth of shoots and leaves at the expense of fruit development due to the induction of shade avoidance syndrome (SAS) (Roig-Villanova and Martínez-García, 2016).

The study site was less than 5 km from the Ranco lake, the third largest lake in Chile (lake area of 442 km^2). Therefore, the RH and vapor pressure deficit (VPD) values recorded in the orchard (RH > 50% and VPD > 2.0 kPa) were not representative of an area with high evaporative demand (Figures 2A and 2C). Blanco et al. (2019) reported that under plastic-covered tunnels, RH increased by 6% to 20% and air temperature by 3 to 15 °C, which may negatively affect fruit growth and quality in sweet cherry orchards. In this study, RH values inside the plastic shelter were generally close to those outside, with the exception of 'Regina' (Figure 2A). The RH values in the 'Regina' plot were between 5% and 10% higher under shelter (Figure 2A). In the present study, 'Regina' plants also showed the higher transmission of PPFD radiation within the tree (Figure 1A), which may have increased transpiration of the inner leaves, leading to an increase in RH from December to mid-January. It has been reported that summer pruning of fruit trees not only increases light penetration within the tree, but also increases plant transpiration of interior leaves (Marini and Burden, 1987). The air temperature under the plastic covering was often similar or higher than outside the covering for all varieties (Figure 2B). However, the effect of the higher air temperature inside the plastic covering was not sufficient to increase the VPD inside the covering above that recorded in the open field (Figure 2C). The low atmospheric water demand under the plastic cover was associated with high midday stem water potential values (between -0.7 and -0.5 MPa). The measured stem water potential (SWP) represented the optimal plant water status for cherry trees under field conditions (Blanco et al., 2018) and under protected cultivation (Blanco et al., 2021; Palma et al., 2023). These results suggest that the physiological disorder of orange peel was not due to pre-harvest water stress. However, changes in fruit water status and movement of water and minerals into the fruit during ripening (Quiroz et al., 2023) may still be potential determinants of orange peel disorder.

Fruit quality assessment. Winkler et al. (2020) determined the variation of Ca and K contents and the fruit size in cherry fruit concerning the position of the fruit on the tree. In their study, they divided the tree into different sections and orientations, finding nonsignificant effects of the position of the fruit in the canopy on the variables mentioned above. Despite the significant differences ($P \leq 0.05$) found among tree sections in our study, no clear pattern could indicate that one orientation induced more or less orange peel disorder, which was present in all sections and orientations of the tree regardless of cultivar, with nonsignificant differences among them (Figure 3). Orange peel disorder symptoms have been observed at harvest (Zoffoli et al., 2017), during and after storage, as early as 7 d of storage (Schlegel et al., 2018). In our work, we found that the disorder may appear as early as the green stage in 'Lapins', when 33.65% of the fruits showed symptoms of the disorder compared to 'Regina' and 'Kordia' in the same stage (1.25% and 0%, respectively) (Figure 3A). Despite 'Regina' being susceptible, according to (Schlegel et al., 2018), in our study it showed lower incidence than 'Kordia' (Figure 3). Thus, the cultivars that showed high to low susceptibility were Lapins > Kordia > Regina.

Schlegel et al. (2018) reported that the severity of orange peel differed among cultivars, and the fruit surfaces were mostly macroscopically smooth and shiny for all the cultivars studied, except for 'Regina'. In their study, 'Regina' presented symptoms from harvest similar to the results shown in this work (Table 1). However, 'Regina' showed a mild severity of the disorder at harvest, whereas Schlegel et al. (2018) described 'Regina' as one of the cultivars with the highest susceptibility.

Overall, the severity of the disorder increased throughout the fruit development and storage (Table 1). For instance, 'Lapins' presented a mild severity at the green fruit stage, which progressively increased to a high severity at 40 d at 0 °C plus 3 d at 15 °C, affecting between 50% and 100% of the fruit surface (Table 1). Similar behavior was reported by Toivonen and Kappel (2012) in a study of the effect of cold storage duration on quality attributes in different cultivars, including 'Lapins'. Hence, the orange peel disorder symptoms increased as the storage period extended, affecting all the cultivars studied except 'Skeena' and 'Sonata'.

Experiment 2

Heat and relative humidity treatments. In the present study, nonsignificant differences were found in the orange peel incidence of the heated sweet cherries (32.3 °C at harvest) compared to the control (22.9 °C at harvest) (Figure 4). However, there were differences in the severity of the orange peel disorder (Tables 3 and 4). Preharvest heating treatment studies in sweet cherries have been mainly carried out on double pistil fruit (Beppu et al., 2001) or blooming (Zhang et al., 2017). On the other hand, postharvest heating treatments evidence a significant effect on reduction of fruit quality condition by promoting softening and fruit darkening (Michailidis et al., 2019; Xin et al., 2021). In a recent postharvest study (Xin et al., 2021), the temperature fluctuation from 5 to 10 °C accelerated the fruit darkening. In contrast with our study, heated cherries during preharvest presented a lower severity index of internal browning, likely associated to the lower VPD compared to the control induced by the high humidity level inside the heating chamber. Beppu et al. (2003) reported that high preharvest temperatures at either low or high irrigation conditions had the same levels of non-structural carbohydrate concentration in sweet cherry trees 'Satohnishiki'. These authors studied the sweet cherry trees under controlled sunlit growth chambers and evaluated starch and sugar content from different tissues such as shoots, trunk, and roots. They found that the effect of temperature was more significant than the soil moisture. It would be interesting to investigate if high temperature and low irrigation conditions result in differential distribution of non-structural carbohydrates in the fruit tissues and, therefore, a range of incidence of orange peel disorder should be expressed. Although temperature was the main factor studied, we do not rule out the possibility that RH, VPD, and radiation may also influence the disorder during preharvest.

Regarding the RH in cherries during postharvest, a recommended RH for storage should be close to 95% at 0 °C (Wani et al., 2014) to minimize water loss and deterioration. Slight variations in RH have a significant impact on fruit quality. For example, fruit stored at 95%-99% RH had less weight loss, firmer and brighter colored fruit with smooth skin, and a greener and more turgid pedicel than 90%-94% RH (Sharkey and Peggie, 1984). Our study showed a lower severity of orange peel disorder for control and heated fruit at 100% RH, while the lower RH treatments, such as 45%, presented a higher severity (Table 4). These results were similar to those reported by Schlegel et al. (2018), where low RH had marked effects on the amount of water transpired by the sweet cherries during storage.

The current hypothesis for the orange peel disorder relates to the cell collapse induced by differential water movement associated with osmotic potential between sweet cherries' flesh and skin, and moisture loss by transpiration (Schlegel et al., 2018). Research has shown that the outer parenchyma has a more negative osmotic potential than the epidermal and hypodermal cell layers of the fruit skin (Grimm et al., 2020). This negative osmotic potential causes water movement and skin dehydration resulting in cell collapse under non-transpiring conditions demonstrated in the orange peel disorder study by Schlegel et al. (2018). The differential rate of carbohydrate accumulation may determine the difference in the osmotic potential between the mesocarp and the epidermal and hypodermal cell layers of the sweet cherry. This suggests that the rate at which the sweet cherry absorbs assimilates is crucial, especially at the ripening stage, when most of the flux of water sap is loaded by the phloem (Grimm et al., 2020). Jia et al. (2020) reported that total soluble solids (probably mannitol) induced a significant decrease in osmotic potential in strawberries.

Likely, osmotic potential and moisture loss occur combined and at different magnitudes throughout the sweet cherry developmental stages. Our data shows that the disorder is expressed from the green stage (in 'Lapins'), possibly due to primarily transpiration (Figure 3). As the fruit matures, the gradient of osmotic potential between the mesocarp and the skin increases, and the water movement promotes the cell collapses characteristic of the disorder. It has been reported that the fruit's osmotic potential increases until the onset of ripening (Jia et al., 2020), after which it decreases (Schlegel et al., 2018). At a ripe stage, there is a significant difference in the osmotic potential of the flesh and the skin of the sweet cherries. During postharvest, these potentials decline slightly, but the difference between them remains constant, and they are not influenced by the transpiration of the cherry (Grimm et al., 2020). Sweet cherries exported under extended periods at low temperatures, even at high humidity, such as the modified atmosphere packaging (MAP), go through a stage of cellular senescence, which leads to compromised membrane integrity and increased pectin depolymerization. This senescent stage may contribute to the osmotic potential gradient between the skin and the flesh cells, especially with the minimal transpiration that still takes place inside the MAP (Grimm et al., 2020).

CONCLUSIONS

This study demonstrated that the development of physiological orange peel disorder begins at the pre-harvest stage since the disorder was found at an early developmental stage in 'Lapins'. However, the position of the fruit on the tree was not shown to be related to the incidence of the disorder, as nonsignificant differences were found between tree sections. In addition, the disorder is unrelated to water stress; however, the development of the disorder could be more critical under more stressed growing conditions promoted by the environment, or when the irrigation cannot counteract the evapotranspiration properly would allow us to rule out or ensure its relationship with the development of orange peel. Finally, the relative humidity affected only the severity of the disorder but not the incidence, whereas nonsignificant effects were found for preharvest heating treatments. Likely, the orange peel disorder depends on the osmotic potential difference between the mesocarp and the peel, and the transpiration process that could express differently from preharvest through storage. Further research is needed as the involvement of the carbohydrates in the appearance of the disorder in relation to the osmotic potential is still unknown.

Author contribution

Conceptualization: A.C., J.P.Z., C.C. Methodology: F.C., A.C., C.C. Formal analysis: F.C. Investigation: F.C., A.C., C.C. Resources: C.C. Data curation: F.C., A.C., C.C. Writing-original draft: F.C., C.C. Writing-review & editing: A.C., J.P.Z., C.C. Funding acquisition: C.C. All co-authors reviewed the final version and approved the manuscript before submission.

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