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Radiation and temperature conditions during crop determine tomato fruit susceptibility to postharvest chilling injury

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ABSTRACT

The aim of this work was to improve the understanding about the relationship between solar radiation and temperature exposure during crop and tomato fruit susceptibility to postharvest chilling injury. Two preharvest treatments were applied: direct sunlight exposed plants (Ex) and shaded plants (Sh). After harvest, fruit were stored at 10ºC and 4ºC for ten days. Then, fruit were removed from the chambers and stored at room temperature for four days. Several determinations were carried out: total mineral and calcium content, fruit quality and postharvest parameters, ions leakage and thiobarbituric acid reactive species content and chilling injury index (CII). Ex fruit had higher values of chilling injury index than the Sh fruit, and at the same time, fruit under 4ºC storage treatment presented a more marked damage than those stored at 10ºC, demonstrating that accumulated radiation in the field, as a result of a higher solar radiation exposure, is a critical factor affecting postharvest chilling injury susceptibility. The lower calcium content in the Ex fruit could be one of the causes of their response to chilling.

Keywords: *Solanum lycopersicum*, preharvest, pitting, calcium, fruit storage.

RESUMO

Radiação e condições de temperatura pré colheita determinam a suscetibilidade dos frutos do tomate a danos causados pelo frio pós-colheita

O objetivo deste trabalho foi melhorar o entendimento sobre a relação entre a radiação solar e a exposição à temperatura durante a colheita e a suscetibilidade dos frutos de tomate a danos pós-colheita por frio. Foram aplicados dois tratamentos pré-colheita: plantas expostas à luz solar direta (Ex) e plantas sombreadas (Sh). Após a colheita, os frutos foram armazenados a 10ºC e 4ºC por dez dias. Em seguida, os frutos foram retirados das câmaras e armazenados em temperatura ambiente por quatro dias. Foram determinados: teor de minerais totais e cálcio, qualidade do fruto e parâmetros pós-colheita, vazamento de íons e teor de espécies reativas ao ácido tiobarbitúrico e índice de injúria pelo frio (CII). Os frutos Ex apresentaram maiores valores de índice de injúria pelo frio do que os frutos Sh e, ao mesmo tempo, os frutos sob tratamento de armazenamento a 4ºC apresentaram danos mais acentuados do que aqueles armazenados a 10ºC, demonstrando que a radiação acumulada no campo, como resultado de uma maior exposição à radiação solar é um fator crítico que afeta a suscetibilidade a danos causados pelo frio pós-colheita. O menor teor de cálcio nos frutos Ex pode ser uma das causas de sua resposta ao frio.

Palavras-chave: *Solanum lycopersicum*, pré-colheita, dano no fruto, cálcio, armazenamento de frutos.

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Tomato (*Solanum lycopersicum*) is the second most important vegetable, considering global gross production value (FAOSTAT, 2016). This crop can be cultivated under different light intensity conditions and the use of mesh shade system is recommended over sun-exposed systems, in regions with high solar radiation and temperatures.

Commercially, refrigeration has been predominantly used to delay ripening and to extend tomato shelf-life since the produce quality is affected at high temperatures storage (Arah *et al.*, 2016). However, tomato fruit are susceptible to chilling injury after exposure to temperatures below 13ºC for more than two weeks (Biswas *et al.*, 2016), or just 6 days at 5ºC (Suslow & Cantwel, 2009). One of the most common symptoms is superficial pitting (Park *et al.*, 2018), occurring within a few hours after moving the fruit from the refrigerated chamber to room temperature (Liu *et al.*, 2012). To mitigate chilling injury, a known postharvest technique is warming the produce before it enters the cooling chambers (Lu *et al.*, 2010).

In these regards, it could be deduced that a greater energy balance in the crop should result in a greater heat accumulation in the fruit (Arah *et al.*, 2016) and therefore, the susceptibility to postharvest chilling injury would decrease. However, the evidence obtained from field observations on tomato indicates that the tolerance to low temperature decreases in sun exposed fruit, just as it occurs in kiwifruit and persimmon (Woolf & Ferguson, 2000). To our knowledge, there is no scientific information accurately relating preharvest light and temperature conditions of the crop and the postharvest response to chilling injury. We consider this information could be highly relevant to take into account when deciding the right combination of the crop management and the produce storage.

Taking into account tomato can be cultivated under different kinds of light conditions, depending on the cultivation system, and the potential impact it could have on its postharvest life, the aim of this work was to improve the understanding about the relationship between the grade of exposure to solar radiation, as one of the main preharvest environmental factors on tomato crop, and the fruit's susceptibility to postharvest chilling injury.

MATERIAL AND METHODS

Plant material

The experiments were carried out in the Facultad de Ciencias Agrarias of the Universidad Nacional del Litoral (FCA UNL) experimental field (31º23'59"S, 60º53'58"W) in 2019. The tomato cultivar used was 'Superman' (F1 Seminis, Missouri, USES). The crop was conducted under typical cultural work. Seeds were planted in polystyrene trays with 228 cells (20 cm^3 per cell) using a mixture of peat and perlite substratum (80%/20% proportion), respectively. The transplant was carried out when the plants had four green leaves. The plants disposal was in lines separated at 1.4 m, obtaining a final density of 24,000 plants/ha, leading to a single stem. The soil corresponded to the typical Argiudol group, characterized by its silt-loam texture. Surface drip irrigation was used with drip lines installed on every furrow. The irrigation was the same for all treatments.

Preharvest treatments and measurements

The treatments were: direct sunlight exposed plants (Ex) and shaded plants (Sh). Black raffia meshes (35% shade, 36 g/m^2) were used to cover Sh. The covering was applied when 98% of the plants was at the anthesis of the first bunch (around 45 days after sowing). In both treatments, Ex and Sh, the accumulated daily solar radiation, the air temperature, and the vapor pressure deficit at 3:00 pm, were determined using an iMetos 3.3 station (IMT 300, Pessl Instruments, Austria). The temperature of 60 fruit from 20 plants was measured (three fruit per plant) at 3:00 pm, using a laser infrared thermometer (Sinometer Mod. GM-320, China) during a ten-day period before harvest.

Postharvest treatments

The harvest was carried out 67 days after transplantation (97 days after sowing). At that moment, all the plants (Ex and Sh) had similar development status and presented several fruit in light red stage of ripeness (grade 5, according to the UC Postharvest Technology Center), which was the state at which harvest was performed. A set of 160 fruit was taken from Ex and 160 fruit, taken from Sh. The sampling was carried out randomly. Immediately after harvest, 80 fruit per each treatment were stored at 10±0.5ºC in an electric cooler cabinet and 80 fruit per each treatment were stored at 4±0.5ºC in a laboratory fridge (both treatments under RH ≥90%, reached by humidifiers and controlled via dataloggers). The storage lasted ten days under the descripted conditions. Then, the fruit were removed from the chambers and stored at 22±0.5ºC, for 4 days.

Measurements

From each combination of preharvest and postharvest treatment (Ex 10ºC; Ex 5ºC; Sh 10ºC; Sh 5ºC) 40 fruit were intended for non-destructive measurements, and 40 fruit, for destructive measurements. Immediately after harvest, and before temperature storage, the following non-destructive measurements were carried out: weight (with analytical scale), firmness (with TR Turoni BC-TR53215 durometer, Italy) and superficial color (with Minolta CR-400 colorimeter, Japan). The color index was calculated according to HunterLab (1996), following next equation:

$$\frac{1000a}{Lb}$$

Where: ***a*** is relative to the green-red opponent colors, with negative values toward green and positive values toward red. ***L*** is the lightness value, defining black at 0 and white at 100. ***b*** represents the blue-yellow opponents, with negative numbers toward blue and positive toward yellow.

Besides, samples were taken for the following destructive measurements: fruit water content (obtained by the difference between fresh weight and constant dry weight, which were measured with analytical scale and expressed as a percentage), electrolytes leakage percentage (Zhao *et al.*, 2009), thiobarbituric acid reactive substances (TBARS), total soluble solids (TSS) acidity, and total mineral and calcium content (AOAC, 1984). Samples for electrolytes leakage, TSS and acidity measurements were immediately used, and after these determinations, they were frozen at -20ºC until TBARS, total mineral and calcium contents determinations.

For TBARS measurements, an extract was carried out from 250 mg of homogenized tissue, which was precipitated with 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) and then centrifuged at 5000 g for fifteen min, to eliminate precipitated proteins. An aliquot of the supernatant was taken, and the TBARS determination was made according to Rice-Evans *et al.* (1991).

After the postharvest treatments (day fourteen after harvest), all the descripted non-destructive measurements were performed again, on each fruit. Besides, two additional non-destructive measurements were carried out: the natural rot incidence by fungal pathogens (*Botrytis cinerea* and/or *Rhizopus stolonifer*) and the chilling injury index (CII), based on Ding *et al.* (2002) visual scale (Figure 1) and the following equation:

$$CII = \frac{\sum(Cilevel)(totalfruitoftheCilevel)}{Totalfruit}$$

where Ci (chilling injury) 25% to 50% of fruit surface; 3 = pitting covering from 50% to 75% of fruit surface; and 4 = pitting covering >75% of fruit surface.

The destructive measurements/samplings made after harvest, were also carried out after storage (except for the total mineral and calcium contents). Besides, samples for a morphological and anatomical description of the pericarp chilling injury pits were taken.

The percentage of physiological weight loss (PWL) and the percentages of firmness loss were calculated considering initial and final values.

Experimental design and statistics analysis

A completely randomized block design with three repetitions per treatment was followed in the field (preharvest treatments). The fruit was the experimental unit in all determinations. Every measurement was carried out at least making five repetitions each time. An ANOVA was carried out using Infostat software. Significant differences between the means were estimated using LSD test, at a value of $P<0.01$.

RESULTS AND DISCUSSION

Solar radiation, vapor pressure deficit, air temperature and fruit temperature

The daily average solar radiation values were approximately 40% higher in the Ex compared to Sh (Figure 2A). The values of daily temperature and vapor pressure deficit of the air at 3:00 pm, during the experiment, showed significant differences between the treatments. Significant differences were observed between the air temperature, and the vapor pressure deficit at 3:00 pm values; Sh always presented an average of 12% lower values compared to those for the Ex (data not shown).

The average diurnal temperature of the fruit, during the ten days before harvest, was around 20% higher in the Ex fruit (reaching maximum values around 36ºC) compared to Sh fruit (Figure 2B). Field heat is an important factor affecting the fruit's energy intake, and it is not simple to separate the effect of solar radiation from that of the air temperature (Woolf & Ferguson, 2000). However, it is clear that accumulated energy by the fruit is a response to the integrated balance of radiation and heating of the crop. Several factors contribute to this balance, just like albedo, which can reach a temperature between 4 and 10ºC higher in a red state respect to the same fruit in a green state (Woolf & Ferguson, 2000).

The effect of greater sensitivity to chilling on fruit exposed to higher preharvest field heat values, could be considered contradictory since heat shock treatments are used to mitigate this damage during postharvest storage (Biswas *et al.*, 2012). The maximum temperature reached by the fruit in the Ex fruit treatment was around 36ºC, just like the value used by Lurie & Klein (1991) as heat treatment prior to the three weeks storage period at 2ºC, resulting in lower chilling injury compared to untreated fruit. Contrasting these authors observations with our results, we can suggest that accumulated field heat has not the same effect than a postharvest shocking heat treatment. Woolf & Ferguson (2000) affirmed that the response to the chilling injury can be opposite in different fruits, indicating that several produces are more affected by chilling injury when they were directly exposed to sunlight in the field. The mechanisms by which chilling damage is triggered by accumulated solar radiation are not clear, but it could be related to the skin wax thickness and composition, which are affected by light and temperature exposure, determining postharvest susceptibility to cold damage (Purvis, 1984).

Total mineral and calcium content

The lowest values of total mineral and calcium content corresponded to the Ex fruit. The calcium content of the Sh fruit was almost 50% higher compared to the Ex fruit. Water content values in Ex and Sh fruit showed no significant difference (Table 1). Different studies revealed that chilling injury decreases as a function of calcium content increase, just like in banana (Jiao *et al.*, 2018) and pineapple (Hewajulige *et al.*, 2003). Calcium regulates many metabolism paths, including membrane integrity, function and permeability by stabilizing lipid layers, and cell wall structural integrity, rigidity and firmness, among others, delaying softening, weight loss and postharvest decay caused by several molds, under low temperature storage (Demes *et al.*, 2021). In the present

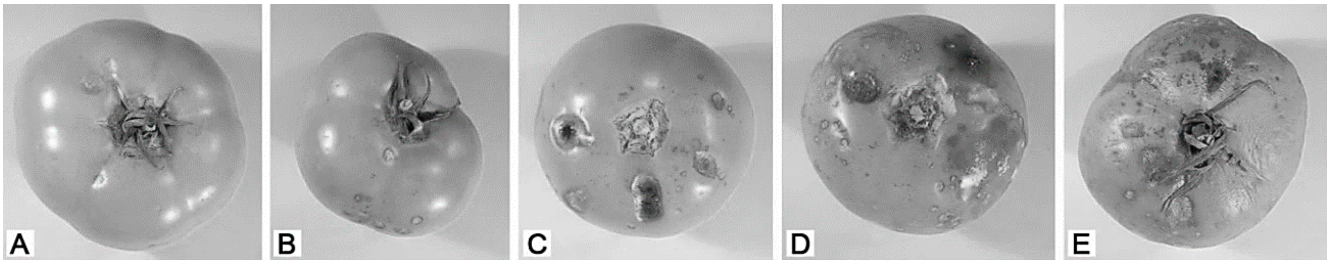

Figure 1. Chilling injury visual scale used for the CII calculation. A) CI = 0; B) CI = 1; C) CI = 2; D) CI = 3; E) CI = 4. Esperanza, Argentina, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA UNL), 2019.

study, lower calcium values were observed in Ex fruit, showing the greatest chilling injury. Among the most important environmental factors contributing to calcium transport to the fruit are high air temperature and vapor pressure deficit which promotes transpiration (Ho & White, 2005). These two factors were precisely higher in the Ex fruit compared to the Sh fruit. It is known that transpiration is a critical process to import calcium to the leaves. Transpiration was reported to progressively decrease as the fruit matures, meanwhile leaves always present high transpiratory activity (Xiloyannis *et al.*, 2001). The reduction of the fruit transpiration reduces calcium intake (Montanaro *et al.*, 2006). The calcium movement through the phloem between leaves and fruit is limited (Xiloyannis *et al.*, 2001). Our results showed that Ex fruit accumulated less calcium compared to fruit coming from Sh plants. This decrease of calcium concentration would have been caused by higher solar radiation and air temperature (Bertin, 2005). Our observations would suggest that calcium could be an intermediary between the effect of the preharvest conditions and postharvest chilling injury.

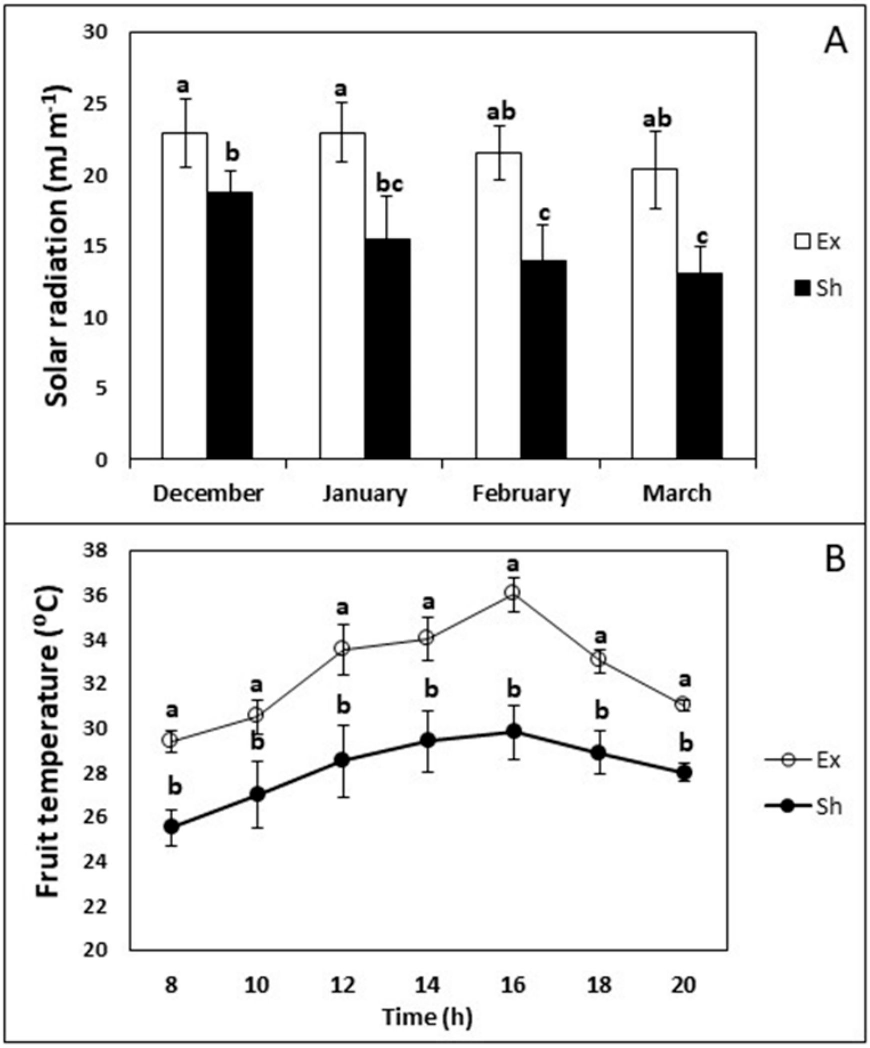

Figure 2. A) Average monthly accumulated daily solar radiation (mJ/m2) during the experiment for tomato from direct sunlight exposed plants (Ex) and shaded plants (Sh). B) Average diurnal temperature of the fruit from sunlight exposed plants (Ex) and shaded plants (Sh), measured during a 10-day period before harvest. Different letters indicate significant differences according to LSD test (P≤0.01). In the case of fruit temperature, the difference was calculated between the two treatments means for each hour. Esperanza, Argentina, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA UNL), 2019.

Physiological weight and firmness loss

Even though no significant difference was detected in PWL between Ex and Sh fruit under the same temperature storage conditions, it was observed that fruit at 10ºC significantly lost more water compared to fruit at 4ºC (Figure 3A). The color shift is a typical process occurring during ripening. It was observed that Ex fruit stored at 10ºC had higher color shift, followed by the Sh fruit stored at the same temperature, and then followed by Ex fruit stored at 4ºC and the Sh fruit stored at 4ºC. The latter combined treatment showed the lower color shift (Figure 3B). Firmness loss is also a process linked to ripening and to fruit quality. The initial average firmness value at harvest was 51.5±7.1 in Ex fruit, and 48.6±4.3 in Sh fruit. It was detected that the Ex fruit stored at 10ºC presented higher firmness loss, followed by Sh fruit stored at 10ºC, Ex fruit stored at 4ºC and the latter followed by the Sh fruit stored at 4ºC (Figure 3C). There were significant differences in natural rot incidence among all treatments (Figure 3D). Higher values were detected for the Ex fruit stored at 4ºC, followed by the Sh fruit stored at 10ºC, then by the Ex fruit stored at 10ºC, and finally, the Sh fruit stored at 4ºC with the lowest incidence values. In regards of these last postharvest quality parameters, the results showed tomatoes color shift and softening responded to a combination of both field heat (Woolf & Ferguson, 2000) and storage temperature, showing greater changes in Ex fruit at 10ºC. Firmness loss could be related to the PWL here also recorded, since firmness is related not only to cell wall integrity but also to cell turgor. As shown, the PWL was mainly influenced by the storage temperature (Tadesse *et al.*, 2015). The main contributing factor for the fruit postharvest pathogen incidence is the softening rate, as well as temperature and environmental

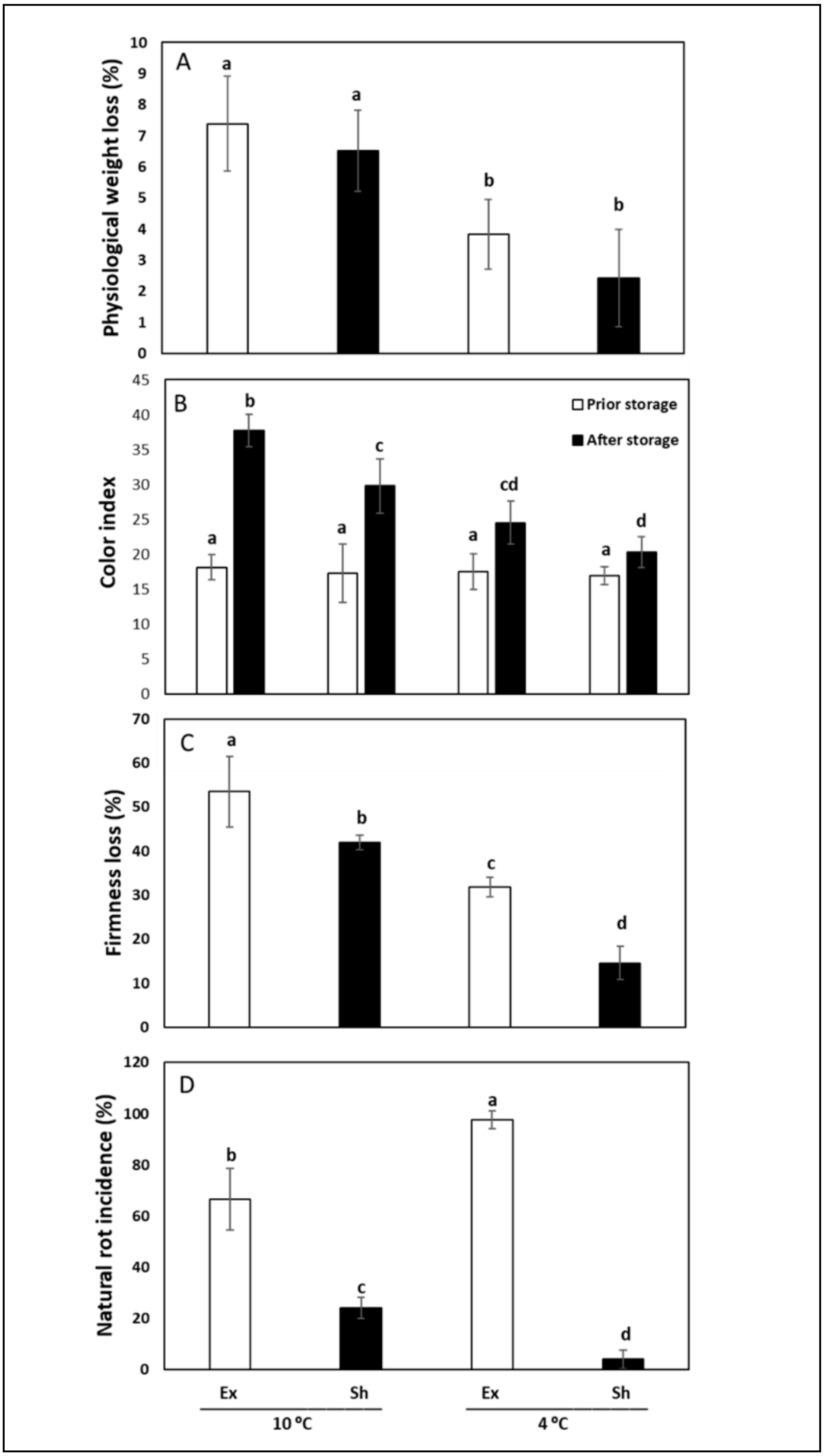

Figure 3. A) Physiological weight loss (PWL) percentage of fruit from sunlight exposed plants (Ex) and shaded plants (Sh). **B)** Color index of fruit from sunlight exposed plants (Ex) and shaded plants (Sh), prior and after storage. **C)** Firmness loss of fruit from sunlight exposed plants (Ex) and shaded plants (Sh). **D)** Postharvest rot natural incidence of *Botrytis cinerea* and/or *Rhizopus stolonifer* (%) of fruit from sunlight exposed plants (Ex) and shaded plants (Sh) after 14 days of storage. After harvest, fruit were stored at 10ºC or 4ºC for 10 days and then kept at 22ºC for 4 days. Different letters indicate significant differences according to LSD test (*P<0.01*). Esperanza, Argentina, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA UNL), 2019.

moisture, which limits fruit shelf-life, increasing postharvest losses (Brummell & Harpster, 2001).

Total soluble solids and acidity

The TSS percentage and the acidity experienced no significant changes between the harvest and after storage, and there were no significant differences among treatments (data not shown).

Electrolyte leakage and TBARS content

The electrolyte leakage value was higher in Ex than in Sh fruit after harvest (Figure 4A). The values increased during storage. The highest electrolyte leakage values were detected in Ex treated fruit stored at 4ºC, followed by Ex treated fruit stored at 10ºC. Fruit under Sh treatment and stored at both 4ºC and 10ºC had the lowest electrolytes leakage values. The last two treatments do not present significant differences between them. TBARS content (Figure 4B) showed a similar pattern to that detected in the electrolyte leakage measurements. The initial values, after harvest showed no difference between Ex and Sh fruit. After storage, all levels increased, with the higher values in Ex fruit at 4ºC, followed by Ex fruit at 10ºC and then the Sh fruit, with no significant differences between the storage conditions. One of the main effects of storing vegetables at low temperatures is the change in fatty acids of phospholipids composition, since it generates membrane damage, cell membrane permeability changes and a cascade of events leading to disruption of cell structure. Membrane damage can be measured by ion leakage and by TBARS levels, as an oxidative stress parameter. Our results indicate accumulated field solar exposition affected the membranes stability at low storage temperature, which consequently resulted in much higher chilling injury susceptibility and natural rot incidence (Santos *et al.*, 2017). The development of the diseases was affected by the presence of pits on the fruit epicarp since the collapse of the cells could represent a suitable area for the entry of pathogens. The results of our study showed a higher TBARS (a byproduct of the ROS action) content and ion leakage levels in Ex fruit, compared to Sh fruit, indicating oxidative stress and a decrease in the membrane stability. Several researches support the relationship between calcium and chilling injury, since it is known that the application of calcium solutions decreased the damage by low temperature in fruit (Bagnazari *et al.*, 2018).

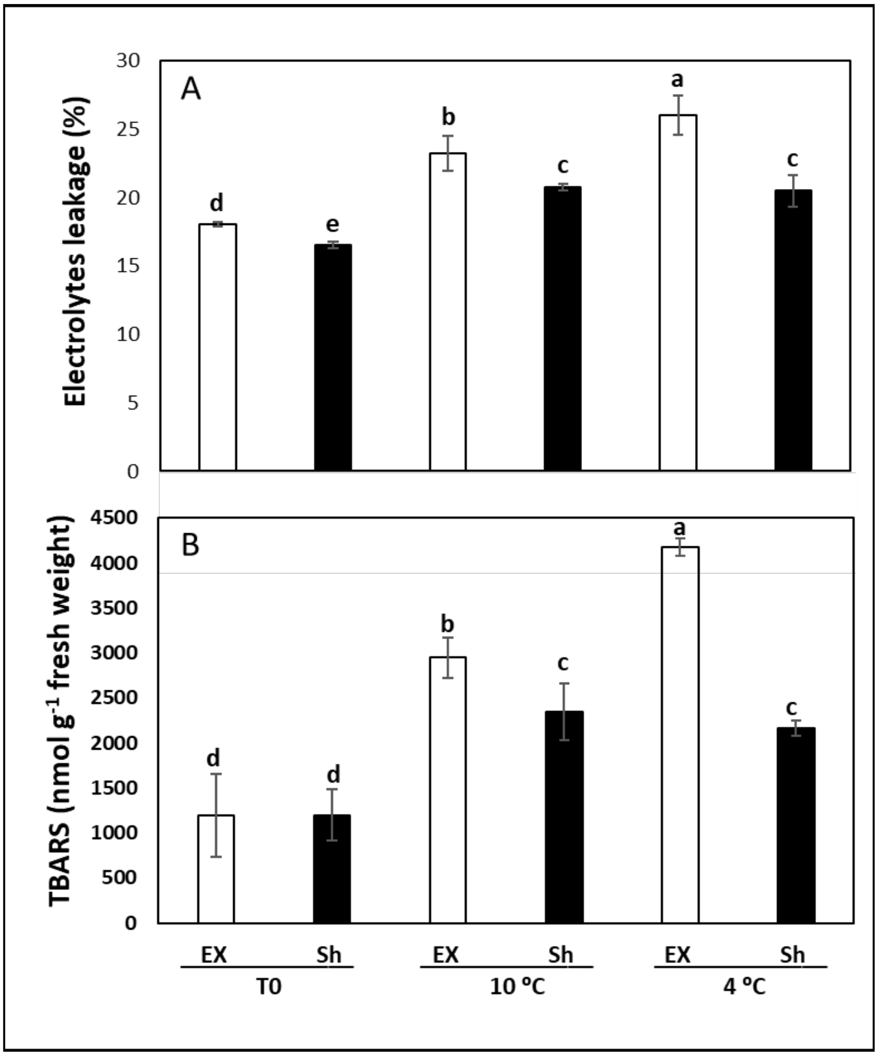

Figure 4. A) Electrolytes leakage percentage (%) of fruit from sunlight exposed plants (Ex) and shaded plants (Sh). **B)** Thiobarbituric acid reactive species (TBARS) content (nmol/g fresh weight) of fruit from sunlight exposed plants (Ex) and shaded plants (Sh). Fruit was immediately harvested (T0) and stored at 10ºC or 4ºC for 10 days and then kept at 22ºC for 4 days. Different letters indicate statistical differences according to LSD test (*P<0.01*). Esperanza, Argentina, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA UNL), 2019.

Table 1. Fruit total mineral and calcium content (mg/g fresh weight) and water content (%), at harvest, from sunlight exposed plants (Ex) and shaded plants (Sh). Different letters indicate statistical differences according to LSD test (*P≤0.01*). Esperanza, Argentina, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA UNL), 2019.

Treatments	Total mineral content (mg/g fresh weight)	Calcium content (mg/g fresh weight)	Fruit water content (%)
Ex	134±12.0 b	170±5.0 b	93.4±3.4 a
Sh	162±9.0 a	250±4.0 a	90.7±4.8 a

Chilling injury

According to the visual scale (Figure 1) Ex fruit at 10ºC presented in general CI=3; Ex fruit at 4ºC presented in general CI=4; Sh fruit at 10ºC presented in general CI=1; Sh fruit at 4ºC presented in general CI=2. The Ex fruit presented a higher CII, compared to the Sh fruit (Table 2). In general, the CII values were also higher in fruit stored at 4ºC than 10ºC. After temperature storage treatment, the Ex fruit showed higher values of CII than the Sh fruit, and at the same time, fruit stored at 4ºC presented a more marked damage than those stored at 10ºC. Considering that tomato is a crop susceptible to chilling injury (Suslow & Cantwell, 2009), the temperatures used in postharvest in this work did result in expression of damage (Biswas *et al.*, 2016). Similar results were reported by other studies. Greater chilling injury can be observed on exposed side versus shaded side of the same fruit (Purvis, 1984). Woolf & Ferguson (2000) reported that kiwifruit is highly susceptible to skin pitting under low temperature storage, when they were exposed to high sunlight. Pits are sunken areas on the epidermis, which are associated with cell collapse, due to the loss of integrity of membranes (Aghdam, 2013).

Based on our observations, it is possible to affirm that accumulated temperature at the field, as a result of higher sun radiation conditions, is a critical factor affecting tomato postharvest chilling injury susceptibility and postharvest pathogen incidence. Fruit calcium contents increased in plants under lower radiation and temperature conditions, therefore, it is possible to be involved in the mechanisms to reduce the postharvest chilling injury. Hence, based on the observations here presented, we ratify that the use of mesh shadow system can attenuate the occurrence of postharvest chilling injury symptoms in tomato. Besides, according to Kittas *et al.* (2012), shading system leads to additional benefits, just like keeping the total fresh tomato yield and reducing losses caused by fruit cracking by 50%.

To our knowledge, this work

Table 2. Chilling injury index (CII) calculated for fruit from sunlight exposed plants (Ex) and shaded plants (Sh) after 14 days of storage. After harvest, fruit were stored at 10ºC or 4ºC for 10 days and then kept at 22ºC for 4 days. Different letters indicate significant differences according to LSD test (*P<0.01*). Esperanza, Argentina, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA UNL), 2019.

Preharvest treatment	**Postharvest treatment**	
	10ºC	**4ºC**
Ex	3.04 ± 0.18 b	3.65 ± 0.29 a
Sh	1.2 ± 0.2 d	1.75 ± 0.21 c

represents the first evidence relating radiation and temperature during preharvest stage with postharvest chilling injury in tomato. These first approaches could help to determine crop management, according to the market target. We consider this study must continue, in order to assess calcium solutions as potential treatments to overcome the limitations imposed by environmental conditions, such as those during the summer. Besides, several crop biometric, yield and water status variables must be determined in future research, to enhance the understanding about the association of integral preharvest conditions and chilling injury susceptibility.

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