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# Climatic and physiological parameters related to the progress and prediction of apple sunburn damage in a neotropical climate

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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**Abstract: Apple production in neotropical climate is affected by sunburn and the high interannual variability in meteorological conditions makes prediction and management of damage difficult. Non-destructive methods associated with physiological variables are keys to monitoring but their development is still incipient. In our study occurrence of sunburn, meteorological conditions and physiological parameters was monitored throughout four crop cycles. Fruit visual assessment and reflectance measures in field, as well as, pigments, proline and hydric potential in laboratory, were accomplished. The results show that the availability of water in the soil was more related to the evolution of sunburn than air temperature. Plant Senescence Reflectance Index (non-destructive predictor) discriminated between healthy and damaged fruits and fruit hydric potential and proline content were good indicators of sunburn, although such variables are determined when damage has already occurred. Our results suggest focusing future research on the water balance of the system and on the physiological indicators of osmotic stress as a way to predict damage.**

## 1. Introduction

Fruit sunburn has been reported since 1870, and although several studies have touched upon the matter since the early 20th century (Racsko and Schrader, 2012), it is still a cause of significant economic loss in apple production (Reig *et al.*, 2019). Although some expressions of damage may be easily perceived in the field, on occasions the symptoms of sunscald are imperceptible and only appear after months of cold storage (usually after three months), which makes the damage difficult to control (Yuri *et al.*, 2000). Symptoms appear as brown stains on the fruits' exterior, being Granny Smith the most susceptible reported cultivar (Felicetti and Schrader 2008; Hernandez *et al*., 2014).

There is consensus that sunburn is related to the combination of high temperature and irradiance (UV-B range is thought to be essential) during the fruit growth period (Yuri *et al.*, 2000, 2010; Racsko and Schrader, 2012; Torres *et al*., 2013; Darbyshire *et al*., 2015; Torres *et al*., 2016 a, b), however, there is a limited understanding of the physiological aspects of the changes in the fruit's internal quality (Racsko and Schrader, 2012) and generation of sun-related physiological disorders in fruit. This problem requires further research into the environmental and physiological processes that occur prior to and during sun injury development and more importantly, biochemical changes that may contribute to resistance to environmental conditions that cause sun-related disorders in fruit (Morales-Quintana *et al*., 2020).

Stress adaptation mechanisms of plants, such as chlorophyll reduction (Ballester *et al*., 2017), dissipation of excitation energy, increase of solutes of low molecular weight (Wen and Moriguchi, 2015) and changes in pigmentation (Merzlyak *et al*., 2003) have been previously studied in relation to sunburn in apple fruit. Based on these results, in the last decade, work has been done on the development of non-destructive methods to predict and detect sun damage based on the composition and location of skin pigments as well as the optical properties of the underlying fruit tissue (Solovchenko *et al*., 2010; Torres *et al*., 2016 a, b). In this direction, sunburn has been related to: fruit reflectance values in the visible and near-infrared (NIR) spectra (Solovchenko *et al.*, 2010; Torres *et al*., 2016 a), crop water stress index and chlorophyll fluorescence (Torres *et al*., 2013, 2016 b).

About temperature effect, it has been reported that increases in fruit temperature above a certain limit may cause enzymes denaturation and protein coagulation, leading to tissue damage (Yuri *et al*., 2010). Studies performed in cv. Fuji fruits showed a highly susceptible caused by excessive heat and did not sustain damage when exposed to UV radiation only (Yuri *et al*., 2000). Air temperatures of 38-42°C increases the heat-shock proteins induction (Woolf and Ferguson, 2000) and sunburn symptomatology appear with fruit temperature of 46°C and higher (Racsko and Schrader, 2012).

The water status of the plant and fruit has also been related to sunburn, although fewer studies have focused attention on this aspect. Recent work in Chile analyses the association of acclimation events with fruit water relations and osmoregulation occurring in sun-exposed fruit tissue (Torres *et al.*, 2013). Studies in Japan and South Africa discuss the effect of foliar ABA on antioxidant levels and the incidence of sunburn with variable results (Mupambi *et al*., 2018). Antioxidant system plays a crucial part in the elimination of free radicals under stress conditions (Chen and Murata, 2002). Compatible solutes such as proline, betaine and polyols are accumulated in response to abiotic stress (Suzuki, 2015). These solutes affect the osmotic balance and the membrane stability and have been proven to maintain turgor pressure, cellular volume and electrolyte concentration (Roberts, 2005). Proline is known to be a stabilizer of sub-cellular structures (Kautz *et al*., 2015) and although many studies have established a connection between proline and antioxidant activity in apple plant leaves and xylem under abiotic stress (Šircelj *et al*., 2005; Nemeskéri *et al*., 2015; Afonso *et al*., 2017) no relationship between proline and sunburn has yet been reported.

Most of the existing research has been carried out in latitudes similar to that of the present study but in more arid climates such as, Chile, Australia, and South Africa (southern hemisphere) or Spain, Turkey, and Washington State (north hemisphere), however, few studies have addressed sunburn in humid growth-season conditions like in Eastern New York State (Reig *et al*., 2019). The region where the study was conducted, defined as neo-tropical (Bernardi *et al*., 2016), has been considered restrictive for apple quality in relation to sunburn aspects (FAO-MGAP, 2013) due to the occurrence of high temperatures during the fruit growth period. Changes in El Niño evolution after 1976 may have played a role in altering the relationship between temperature extreme events in Uruguay and the atmospheric circulation (Renom *et al.*, 2011). The average maximum temperatures of the summer period show a high iner-annual variability (71-86%) and lower variability in the medium (10 years) or long-term (>30 years) components, 23% and 6% respectively (Tiscornia *et al*., 2016), so it is expected that the climate in the region will continue to be favorable to the occurrence of burning. The aims of this work were to study apple sunburn progress and its relation to meteorological variables in a neo-tropical climate, and to establish correlations between fruit physiological parameters and reflectance index.

## 2. Materials and Methods

*Plant material*

The experiment was conducted during the 2012/2013 to 2015/2016 crop cycles (hereinafter, cycles 1 to 4), on a Granny Smith/M7 plantation established in 2003. The crop is located in Uruguay (southeastern of South America) with the coordinates of 34°38’18’’ S and 56°40’06’’ W and 45 meters above sea level.The climate of this regional ecotone is classified by Bernardi *et al*. (2016) as neo-tropical. Crop had planting distance of 4x1.5 m, rows arranged from N to S and trained in central leader system. The soil types are mainly Argiudolls and Hapluderts and a drip irrigation system with a maximum daily watering capacity of 4.5 mm is installed.

Three fruits per tree from ten trees per row, in a total of five rows were selected between 40 and 50 days after full bloom (DAFB) in the four evaluated cycles. Trees and rows were randomly marked, and 150 fruits exposed to radiation were classified by visual assessment of different external initial conditions: A) 50 fruits with no visible sunburn (HF=healthy fruits); B) 50 fruits with red color (RF=red fruits); C) 50 fruits with an early degree of sunburn (SBF=sunburn fruits) [sunburn browning, according to the classification of Racsko and Schrader (2012)] as indicated in figure 1. The flowering dates for cycles 1 to 4 were, September 27 (cycle 1), October 28, 3 and 14, to cycles 2, 3 and 4 respectively.

The exposed side of each fruit was defined as the one directly exposed to sunlight, facing the space between rows, and the internal side as the one facing the trunk, with no direct exposition to solar radiation.

*Field tests*

The sunburn progress in each marked fruit was assessed by observation. Its frequency varied between 1 week and 1 month, with weekly observations predominating. In each observation, fruits were reclassified according to the categories mentioned above (HF, RF, SBF). Reflectance measurements of the exposed side of ten fruits of each condition were recorded with an ILT 950 spectroradiometer (International Light Technologies, USA) at 91, 99 and 154 DAFB in cycle 4. Percent reflectance was calculated based on a dark spectrum and a reference spectrum from a white reference standard. The content of Chlorophyll (CHL), Anthocyanins (ANT), Carotenoids (CAR), Flavonoids (FLA) and Senescence indexes were calculated based on the reflectance measurements. Chlorophyll was calculated according to the following indexes: CHL1, CHL2 (Mullan, 2013) RARSa, RARSb, PSSRa, MSR, CL1, CL2 (Solovchenko *et al*., 2010) and Anthocyanins, according to the Anthocyanin Reflectance Index (ARI) (Solovchenko *et al*., 2010). Carotenoids were calculated according to the following indexes: RARSc (Mullan, 2013), CRI1 and CRI2 (Solovchenko *et al*., 2010) and Flavonoids according to the Flavonoid Reflectance Index (FRI) (Solovchenko *et al*., 2010). The Normalized Phaeophytization Index (NPQI), the Pigment Simple Ratio (PSR), the Normalized Difference Pigment Index (NDPI), the Structural Independent Pigment Index (SIPI) (Solovchenko *et al.,* 2010) and the Plant Senescence Reflectance Indexes (PSRI480 PSRI500) (Mullan, 2013) were also calculated (Table 1). An automated meteorology station located 1900 m from the crop recorded the maximum temperature (Tmax) (°C) and rainfall (RF) (mm) variables in the fruit’s growth period. The soil water balance (SWB) for each growth cycle was calculated. Plot characteristics and local and regional meteorology stations were used. Local variables used were irrigation (mm), daily rainfall (mm), root deep (m), phenological stages (days) and soil texture. The ETo (reference evapotranspiration) (mm) was recorded at the meteorology station of INIA Las Brujas using Penman-Monteith (Allen *et al.,* 1998). Crop coefficient (Kc) was adjusted to reflect the wetting frequency of soil surface and local climatic conditions according to Allen *et al.* (2006):


Fig. 1 - Examples of fruit categories. RF=red fruits, HF=healthy fruits, SBF=sunburn fruits.

$$K_{cini} = K_{cini\,(*)} + \frac{(I\text{-}10)}{(40\text{-}10)} \left[k_{cini\,(**)} - K_{cini\,(*)}\right]$$

$$K_{cmid} = K_{cmid}\,(Tab) + \left[0.04\,(u_2 - 2) - 0.004\,(RH_{min} - 45)\right] \left(\frac{h}{3}\right)^{0.3}$$

Where:

$K_{cini(*)}$: value for Kc ini from figure 29 in Allen *et al*. (2006).

$K_{cini(**)}$: value for Kc ini from figure 30 in Allen *et al*.

(2006).

I: average infiltration depth (mm).

$K_{c\ mid}$ (Tab): value for Kc mid taken from Table 12 in Allen *et al*. (2006) apples, cherries and pears crops with active ground cover without frosts.

$u_2$: mean value for daily wind speed at 2 m height over grass during the mid-season growth stage (m $s^{-1}$), for 1 m $s^{-1}$<$u_2$<6 m $s^{-1}$.

RHmin: mean value for daily minimum relative humidity during the mid-season growth stage (%), for 20%<RHmin< 80%.

h: mean plant height during the mid-season stage (m) for 0.1 m < h < 10 m.

*Laboratory*

Five fruits of each damage category were sampled on the same dates on which the reflectance measurements were carried out (91, 99 and 154 DAFB) and in addition to the 112, 142 and 170 DAFB of cycle 4 for laboratory evaluations. Hydric potential of the fruits' tissue (ΨF) was determinate with Wp4c dew-point potentiometer (Decagon Devices, USA) on the exposed side (ΨFE) and the internal side (ΨFI). Each measurement was made on 3 $cm^2$ of epidermis with 1 mm of sub-epidermal tissue. To determine CHL, CAR and Proline content (PRO), 0.5 g of epidermis without sub-epidermal tissue was removed. The sample was macerated with 0.5 ml of a methanol-chloroform-water compound (MCW, 12:5:1), producing two phases. In phase 1, pigments CHLa, CHLb and CAR were determined, recording absorbances at 665.6, 647.6 and 480 nm, respectively (Wellburn, 1994). In phase 2, after reacting to a one-hour immersion at 90°C with acid ninhydrin and the addition of toluene, PRO was determined with spectrophotometry, recording absorbance at 515 nm (Troll and Lindsley, 1955; Charest and Phan, 1990). To determine ANT, 0.2 g tissue was incubated in methanol-acid for 48 h, recording absorbance values at 530 and 657 nm (Wellburn, 1994).

*Statistical analysis*

The contrastive analysis of the three fruit conditions regarding pigment quantification and assessment, PRO, ΨF and senescence indexes were done with non-parametric methods, using the Kruskal-Wallis test to compare medians and the Kruskal Nemenyi post-hoc test for the multiple comparisons of pairs, with the R statistical software. Differences were assessed at p≤0.05. Pearson's correlation coefficient was determined for the proportion of sun-damaged fruits and the climatic variables, maximum temperature (Tmax) and soil water balance (SWB). The same analysis was performed for the pigment content tested in the laboratory and those estimated by spectroradiometry in the field, as well as for PRO and ΨF. Best fit regressions for variables with higher correlation coefficients were estimated.

Table 1 - Indexes calculated by reflectance in the field

| Name | Index | Index calculation | Parameter | Source |
|---|---|---|---|---|
| Reflectance Ratio | CHL1 | $R_{750}/R_{550}$ | Chlorophyll | Braun and Payne, 2013 |
| | CHL2 | $R_{750}/R_{700}$ | | Braun and Payne, 2013 |
| Ratio Analysis of Reflectance Spectrum (Chla) | RARSa | $R_{675}/R_{700}$ | Chlorophyll a | Braun and Payne, 2013 |
| Ratio Analysis of Reflectance Spectrum (Chlb) | RARSb | $R_{675}/(R_{650}*R_{700})$ | Chlorophyll b | Braun and Payne, 2013 |
| Ratio Analysis of Reflectance Spectrum (Carotenoids) | RARSc | $R_{760}/R_{500}$ | Carotenoids | Braun and Payne, 2013 |
| Pigment-Specific Simple Ratio | PSSRa | $R_{800}/R_{675}$ | Chlorophyll a | Braun and Payne, 2013 |
| Normalized Phaeophytization Index | NPQI | $(R_{415}-R_{435})/(R_{415}+R_{435})$ | Chlorophyll degradation | Braun and Payne, 2013 |
| Modified Spectral Ratio | MSR | $(R_{750}-R_{445})/(R_{705}-R_{445})$ | Chlorophyll concentration | Braun and Payne, 2013 |
| Pigment Simple Ratio | PSR | $R_{430}/R_{680}$ | Carotenoid-Chlorophyll ratio | Braun and Payne, 2013 |
| Normalized Difference of Pigment Ratio | NDPI | $(R_{680}-R_{430})/(R_{680}+R_{430})$ | Carotenoid-Chlorophyll ratio | Braun and Payne, 2013 |
| Structural Independent Pigment Index | SIPI | $(R_{800}-R_{435})/(R_{415}+R_{435})$ | Carotenoid-Chlorophyll ratio | Braun and Payne, 2013 |
| Chlorophyll Index | CL1 | $(R_{700}^{-1}-R_{800}^{-1})*R_{800}$ | Chlorophyll | (Solovchenko *et al*., 2010) |
| | CL2 | $(R_{640}^{-1}-R_{800}^{-1})*R_{800}$ | Chlorophyll | (Solovchenko *et al*., 2010) |
| Anthocyanin Reflectance Index | ARI | $(R_{550}^{-1}-R_{700}^{-1})*R_{800}$ | Anthocyanin | (Solovchenko *et al*., 2010) |
| Plant Senescence Reflectance Index | $PSRI_{480}$ | PSRI480=(R678-R480)* R-1800 | Carotenoid-Chlorophyll ratio | (Solovchenko *et al*., 2010) |
| | $PSRI_{500}$ | PSRI500=(R678-R500)* R-1800 | Carotenoid-Chlorophyll ratio | (Solovchenko *et al*., 2010) |
| Flavonoid Reflectance Index | FRI | $(R_{410}^{-1}-R_{460}^{-1})*R_{800}$ | Flavonoids | (Solovchenko *et al*., 2010) |
| Carotenoid Reflectance Index | $CRI_1$ | $(R_{520}^{-1}-R_{700}^{-1})*R_{800}$ | Carotenoids | (Solovchenko *et al*., 2010) |
| | $CRI_2$ | $(R_{520}^{-1}-R_{550}^{-1})*R_{800}$ | | (Solovchenko *et al*., 2010) |

## 3. Results

Sunburn progress in the four crop cycles showed a high inter annual variability, with differences in the incidence values (% of damaged fruits) and the moment when the maximum occurs. Maximum values of 70% and 62% of sunburnt fruits were recorded in late December and early January for cycles 2 and 3 respectively (approximately 12 and 14 weeks after full bloom). For cycles 1 and 4, maximum values under 45% were recorded between mid-February and early March, at 19 and 22 weeks after full bloom (Fig. 2).

From the analysis of the meteorological records of the four cycles, it can be highlighted that, in cycle 2, 11 days were recorded with maximum temperatures above 35°C, starting at 68 DAFB and reaching a maximum of 38.9°C at 77 DAFB. Cycles 1 and 3 respectively recorded 1 and 0 days with maximum temperatures above 35°C. In cycle 4, 5 days had a maximum higher than 35°C with only 1 peak above 38°C (Fig. 3).

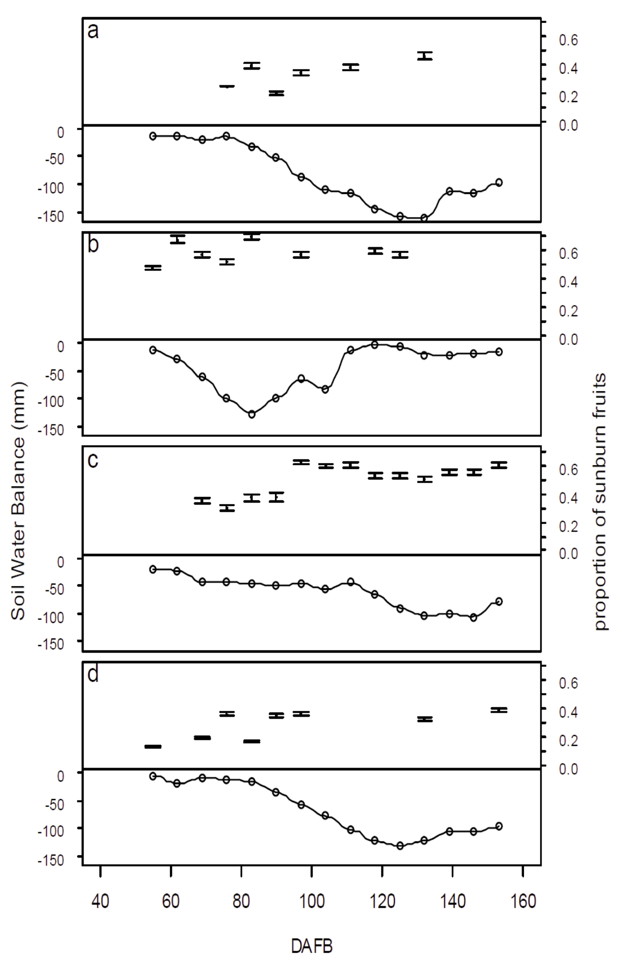


Fig. 2 - Proportion of sunburn fruits and soil water balance (SWB) by number of days after full bloom (DAFB) for four production cycles. a, cycle 1; b, cycle 2; c, cycle 3; d, cycle 4. The error bars represent standard deviation.

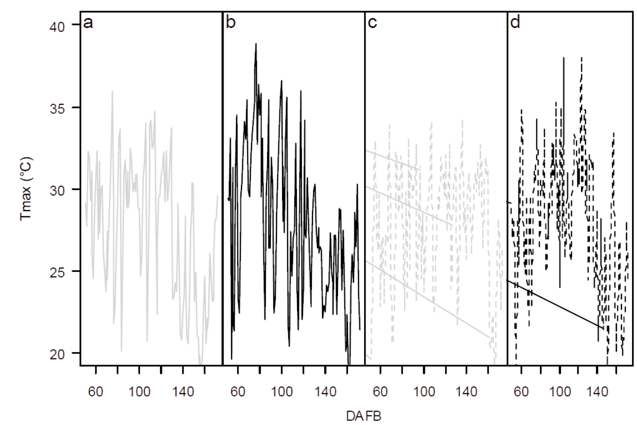


Fig. 3 - Air maximum temperature (Tmax) by days after full bloom (DAFB) for four production cycles. a. cycle 1; b. cycle 2; c. cycle 3; d. cycle 4.

Lowest values of SWB during the first 80 DAFB (about 11 weeks), in the period when damage appears, were recorded in cycles 2 and 3 showing minimum values of -123 and -46 mm respectively. On the other hand, in cycles 1 and 4, SWB decreases from week 12 (Fig. 2). Pearson's correlation coefficient between the proportion of sun-damaged fruits and the soil water balance (SWB) was -0.41, with variations between -0.37 and -0.7 depending on the cycle. The correlation with maximum temperature was 0.28, with a variation of between -0.1 and 0.51 for the different cycles (Fig. 4).

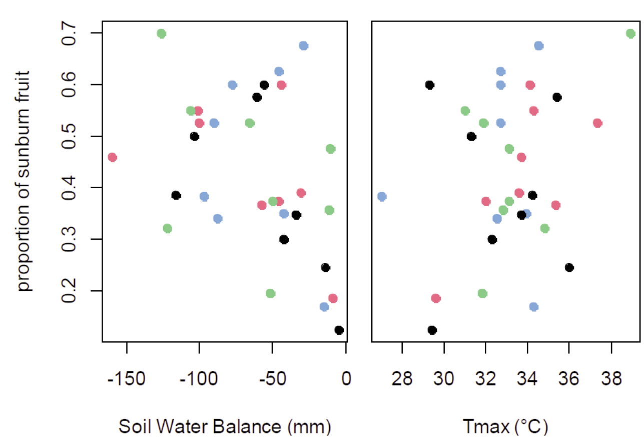


Fig. 4 - Correlations between proportion of sunburn fruits and climatic variables. a. Soil Water Balance (SWB); b. Air maximum temperature (Tmax). Colors represent each cycle: black for cycle 1, red for cycle 2, green for cycle 3,

Variables such as CHL, CAR, ANT, PRO, ΨF and Stress Index, showed significant differences for at least one of the three kinds of fruit (HF, SBF, RF) when measured in the laboratory and/or when estimated in the field, except for ΨFI and CHLb content measured by spectrophotometry (RARSb). ANT concentration, measured in laboratory, and MSR (field

CHL estimate), differed significantly between the three types of fruit. ΨFE was significantly different between SBF and the other two conditions (RF and HF). Remaining variables differed significantly between HF and RF or SBF, but not between RF and SBF. Higher PRO concentrations were measured in RF and SBF than HF fruits (Fig. 5, Table 2).

Pearson correlation coefficients calculated for the different variables assessed reached a maximum of -0.75 between ΨFE and PRO. CHL concentration measured in the laboratory had negative correlations of 0.42 with ΨF and 0.57 with PRO (Fig. 6). The relationship between ΨFE and PRO, CHLa and ΨFE, NDPI and PSRI480, 860900 and CHLa, and NDPI and CHLa have the best fit with a 2nd degree polynomial regression (Fig. 7) and a maximum $r^2$ of 0.60.

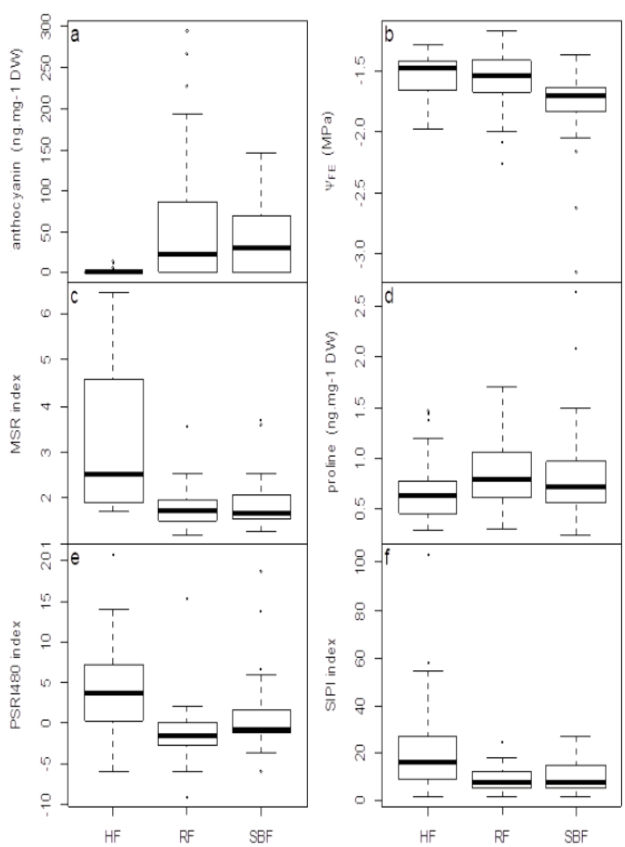


Fig. 5 - Boxplot of variables with greater discrimination capacity for the three types of fruit (HF= healthy fruit; SBF= sunburnt fruit; RF= red fruit). a) anthocyanin concentration; b) hydric potential of the exposed side of the fruit; c) MSR (modified spectral ratio, chlorophyll concentration estimate by reflectance); d) proline concentration; e) PSRI480 (Plant Senescence Reflectance Index); f. SIPI (Structural Independent Pigment Index).

Table 2 - Median of anthocyanins (ANT), Chlorophyll (CHL), Carotenoids (CAR) and proline (PRO) concentration, hydric potential of the exposed side of the fruit (ΨFE), Reflectance Ratio (CHL2), Chlorophyll concentration estimate by reflectance (MSR, Modified Spectral Ratio), Structural Independent Pigment Index (SIPI) and Plant Senescence Reflectance Index (PSRI480), for the three types of fruit (healthy fruit, sunburnt fruit, and red fruit)

| | Sunburnt fruit | Red fruit | Healthy fruit |
|---|---|---|---|
| ANT (ng.mg$^{-1}$ DW) | 29.871 b | 23.438 a | 0 c |
| CHL (µg.mg$^{-1}$ DW) | 0.034 b | 0.041 ab | 0.045 a |
| CAR (µg.mg$^{-1}$ DW) | 0.009 b | 0.011 ab | 0.012 a |
| PRO (ng.mg$^{-1}$ DW) | 0.724 a | 0.788 a | 0.64 b |
| $\Psi_{FE}$ (MPa) | -1.705 a | -1.54 b | -1.475 b |
| CHL2 | 1.808 b | 1.785 b | 2.658 a |
| MSR | 1.660 a | 1.716 b | 2.504 c |
| SIPI | 8.239 b | 8.052 b | 16.012 a |
| PSRI480 | -0.843 b | -1.534 b | 3.65 a |

Different letters in rows indicate significant difference between types of fruit

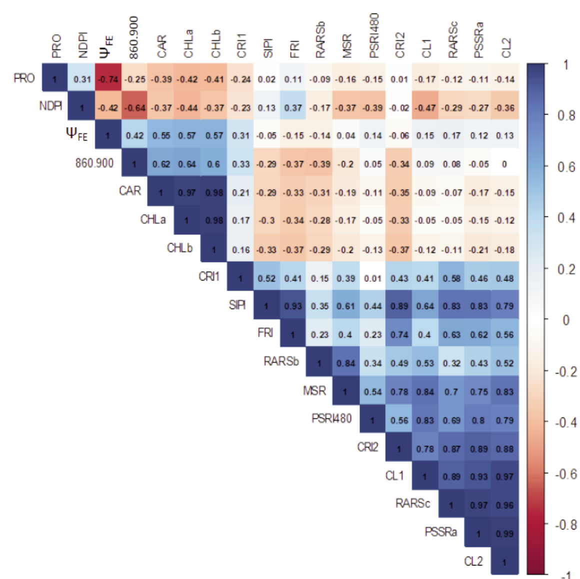


Fig. 6 - Correlations between variables with greater discrimination capacity for the different fruit conditions [Proline (PRO), Carotenoids (CAR), Chlorophylls (CHL)] measured by spectrophotometry in the laboratory, NDPI, 860-900, CRI1, SIPI, FRI, RARSb, MSR, PSRI480, CRI2, CL1, RARSc, PSSRa and CHL2, calculated based on reflectance measurements in the field.

## 4. Discussion and Conclusions

Sun damage incidence values reported in our trial reached a maximum of 70%. This was higher than reported by Racsko and Schrader (2012) in warmer climates of Australia, South Africa, Spain, Turkey and

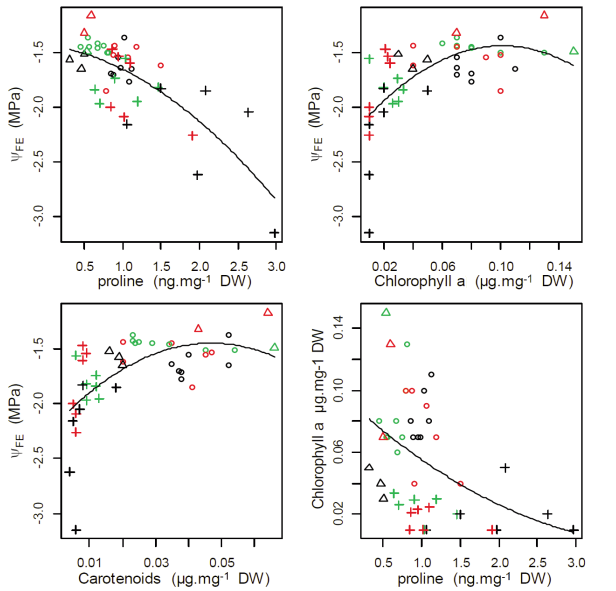


Fig. 7 - Regressions between laboratory variables. Colors represent condition of fruit: green for healthy fruit (HF), red for red fruit (RF), and black for sunburnt fruit (SBF). Symbols represent days after full bloom (DAFB): ○ for 91; Δ for 99; + for 154.

Chile, and higher than the 40% reported by Yuri and Bailey (2015) for Chilean crops. Similar sunburn values were reported during a heat wave in southeast Australia in 2009 (Darbyshire *et al*., 2015). All cycles differed, both in how the damage progressed and at the time when the maximum damage occurred. These differences can be clarified by analysing the meteorological variables, which, as expected, were also highly variable (Tiscornia *et al*., 2016). The correlation of sun damage with SWB was moderate to strong (0.37 to 0.7 depending on the cycle) and higher than that presented with maximum temperatures (Fig. 4). In cycle 2, 11 days with maximum temperatures above 35°C along with the SWB progress, could explain the occurrence of sunburn. However, in cycle 3, only the early decrease in SWB seems to be the explanatory variable of a high damage incidence (Figs. 2 and 3).

In four cycles studied, sunburn reached maximum values at 19, 12, 14 and 22 weeks after full bloom (Fig. 2). In all cases, minimum fruit diameter reported as necessary to absorb enough solar radiation to increase the temperature and result in damage (45 mm 7 weeks) (Racsko and Schrader, 2012) had reached. Fruit surface temperatures between 46 and 49°C, depending on the cultivar, cause browning, while temperatures over 52°C cause necrosis (Schrader *et al*., 2001). This is in accordance with fruit temperatures recorded by Darbyshire *et al*. (2015) for Australia, as well as other reports suggesting that the temperature of the exposed side of the fruit may be 12 to 15°C higher than air temperature (Woolf and Ferguson, 2000). Fruit temperatures over 52°C were recorded in our trials with thermal camera. On days with average air temperatures above 38°C (in cycle 2) were reached fruit temperatures up to 62°C (data not shown).

Variations in ΨF and pigment concentration (laboratory measurements or estimates with reflectance in the field), measured in three types of fruit (HF, RF, SBF) (Fig. 5), have been reported in previous works by Solovchenko *et al*. (2010) and Torres *et al*. (2016 a, b). Symptoms of sun damage were associated with increase in CAR and reduction in CHL and ΨF, as reported by Felicetti and Schrader (2009), Torres *et al*. (2013, 2016 b) and Yuri *et al*. (2000). CHLb estimated by reflectance did not decrease.

ΨFE was the only discriminating variable in SBF vs HF and RF (Fig. 5), in accordance with Torres *et al*. (2013). Increase of 860-900 nm reflectance, reported as a consequence of structural differences between damaged and undamaged tissue (Torres *et al*., 2016 a), could not be confirmed by spectroradiometry in our work. A 0.42 correlation between ΨFE and reflectance values in the 860-900 nm range were obtained (Fig. 6).

Although it has been widely reported that the content of PRO in vegetables tissues is an indicator of stress (Suzuki, 2015), correlations between PRO, CHL and ΨF (Fig. 6) in apples have not been reported. Research on apple trees under hydric stress conducted by Šircelj *et al.* (2005) does not report consistent patterns of change of individual free amino acids. These authors, however, mention the lack of agreement between those results and their own previous studies, as well as those by Chandel and Chauhan in 1991, which report significant increases in Pro foliar, Glu, Orn, Arg and total free amino acids under stress, evidencing active osmoregulation (Šircelj *et al.*, 1999). Our results showed an increase in PRO content in sunburn fruits. More studies should be carried out to evaluate the possibility of using this amino acid as an early indicator of sun damage. Total amino acid content and its variations should be analyzed to elucidate between de novo PRO synthesis and simple proteolysis (Šircelj *et al*., 2005; Arias-Sibillotte *et al*., 2019).

In accordance with the results of Felicetti and

Schrader (2009), the correlations found between CHL and CAR concentrations were significant and negative, whereas the correlation between the values of pigments measured in the laboratory and those estimated by reflectance reached a maximum of 0.25 (Fig. 6). Plant senescence index PSRI480 was the spectroradiometry indicator with the best capacity to discriminate between fruit types (Fig. 5). Correlation between PSRI480 and the best-performing laboratory indicators (PRO y ΨF) was 0.15 (Fig. 6). Pigment concentration expressed on the basis of surface area were lower than range cited by Solovchenko *et al*. (2010) in a 1/50 ratio, but similar to those recorded by Yuri *et al*. (2010), expressed as dry weight concentration. This difference could explain the weak correlation between the laboratory tests of pigments and field estimates in our trials.

High inter-annual variability of sunburn in apple fruit was observed in terms of magnitude and moment of occurrence. The inter-annual variability of rainfall combined with insufficient irrigation to meet crop water demand was more associated with sunburn during the period studied than the maximum temperature. Regarding non-destructive prediction, the PSRI480 senescence reflectance index was the best discriminator between undamaged and damaged fruits, however, like other recent studies, we were unable to devise a method of non-destructive prediction that could be used for commercial production. ΨFE is the main variable that discriminates the degree of damage to the fruits, so it could be the consequence of the effects of high irradiance and temperature. The association between low hydric potential and PRO contents in sunburn fruits suggests the possibility of using this amino acid as an early indicator of this damage in apples; however, more information is necessary to establish a cause and effect relationship.

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