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Effects of shade screens and mulching on the color change of fruits from “Fino 49” lemon trees irrigated with water of different salinity or irrigation regimes

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ABSTRACT

The external color of lemons is one of the most important attributes for the consumer. It is known that the natural process of de-greening mainly depends on the temperature, although other agronomic factors could also affect this process. Currently, citrus orchards in Eastern Spain suffer drought and salinity problems, and growers use a series of agronomic strategies – such as the use of tolerant rootstocks, shade screens, or mulching – to mitigate them. However, the effects of all these factors on the fruit color are unknown. “Fino 49” lemon trees were used to study the effects of the rootstock, shade screens, and mulching on the color of lemon fruits in two individual trials. In one trial, trees grafted on *Citrus macrophylla* (salt sensitive rootstock) were irrigated with water of different salinity levels (CE: 0.8, 3.8, and 6.8 dS/m) and, in the second trial, trees grafted on Sour orange (a drought sensitive rootstock) were watered under different watering regimes (100% ETc, 60% ETc, and 40% ETc). Measurements of the a*, b*, and L* coordinates during the development of the fruit in two consecutive growing seasons were taken. Also, the water potential and the concentration of chloride were measured in the leaves. The results show that the start of color change was only dependent on the minimum average temperature in a period of 14 days, regardless of the rootstock. However, the final fruit color was altered by some of the factors assayed. The use of shade screens produced fruits that were less yellow than those subjected to mulching or grown in open field. For the trees under the shade screens, the drought treatments also negatively affected the de-greening process, as greenish fruits were produced. In contrast, trees grown in the open and watered with salt-treated water produced fruits that were more yellow than the fruits grown normally. The results indicate that excess solar radiation or a severe decrease in the leaf water potential diminished fruit coloration, while a high concentration of foliar Cl^- accelerated the de-greening process.

1. Introduction

Spain is the main lemon-producing country in the Mediterranean area; the crop is concentrated in the Region of Murcia and in some neighboring provinces of Alicante (Valencian Community) and Almeria (Andalusia). In the Murcia Region, lemon production is dominated by the native ‘Fino’ and ‘Verna’ varieties (Lorente et al., 2014), with lemon production reaching 686,600 t (Magrama, 2013). The harvesting of the “Fino 49” lemon fruits starts at the beginning of October and goes on until February. The size and the color of the fruit are two of the main characteristics that determine when the fruit should be harvested, as well as the market price. In their first stage of development, citrus fruits are green. The change of color starts with the decline in temperatures in the fall. It results from the degradation of chlorophylls present in the skin of the fruits (green in color) and the appearance of diverse pigments (carotenoids, flavonoids; yellow in color) that were masked by the chlorophylls in the flavedo; also, the synthesis of new pigments leads to a fast change in color (Lado et al., 2015). Besides temperature, the change of color can be affected by certain other

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environmental conditions – such as humidity, radiation, or type of soil – or by the rootstock (Gouws and Steyn, 2014; Brotons et al., 2013; Lado et al., 2015; Manera et al., 2012; Porras et al., 2014).

The weather in Eastern Spain is characterized by the lack of rain and a high evaporative demand, which frequently induce problems of drought. This lack of water resources forces the growers to use low-quality subterranean sources of water, which have excessive concentrations of soluble salts, mainly chloride and sodium (Conesa et al., 2011). Citrus crops are sensitive to salinity and drought, and such adverse environmental conditions can cause physiological alterations and nutritional imbalances that lead to diminished vegetative growth and fruit production (Al-Yassin, 2004; Syvertsen and Garcia-Sanchez, 2014).

One of the strategies that is used most to mitigate the damage caused by salinity or drought is the use of tolerant rootstocks. In Spain, the two rootstocks used almost exclusively for lemon trees are Sour orange (*Citrus aurantium* L.) and *Citrus macrophylla* Wester. The *C. macrophylla* rootstock seems to be able to exclude Na^+, while Sour orange accumulates Na^+ as well as Cl^- (Gimeno et al., 2009). As for drought, it seems that Sour orange and *C. macrophylla* are more tolerant than other citrus rootstocks (Beniken et al., 2013, 2015).

However, besides the use of rootstocks, different agronomic strategies are also used to alleviate the negative effects produced by salinity and water deficit – such as the use of shade screens and mulching of the soil, which change the environmental conditions of the citrus orchards to a certain degree (Garcia-Sanchez et al., 2015; Cerda et al., 2014; Harrison et al., 2013) and could therefore have an influence on the de-greening processes of citrus fruits.

In order to understand how specific edaphoclimatic conditions could affect fruit de-greening, in the present assay the color change of “Fino 49” lemon fruit was studied in two independent trials. In one trial the aim was to understand the effects that shade and mulching have on trees grafted on Sour orange under drought conditions, as this rootstock is very sensitive to this stress; in the other trial the aim was to understand the effects of shade and mulching on trees grafted on *C. macrophylla* under salt condition, as this rootstock is very sensitive to saline conditions. To perform this study, the fruit color was monitored by measuring the L*, a*, and b* values, which indicate the luminosity (L*), the variation of color from green to red (a*), and the yellow color (b*), respectively. Also, the Hue index was calculated, to show how much green and yellow coloration the fruit had (Manera et al., 2012).

2. Materials and methods

2.1. Plant materials and experimental conditions

The experimental farm is located at Santomera (Murcia, Spain), with climatic conditions characterized by the following values (averaged for 2007–2013): rainfall 285 mm, air temperature 17 °C, relative humidity 77%, and Class A pan evaporation 1230 mm. The soil is clay-loam, with 24.1% sand, 48.70% silt, and 27.2% clay, and has 1.10% organic matter, a cation exchange capacity of 14 meq $100\,g^{-1}$, 11.10% active calcium carbonate, and extractable P and K of 14 and 257 $mg\,kg^{-1}$ (Mehlich 3 extraction), respectively.

This experimental farm had “Fino 49” lemon trees available in two independent trials. One trial contained trees that were grafted on the Macrophylla rootstock (*C. macrophylla*; CM) and the other contained trees grafted on Sour orange rootstock (*C. aurantium*; SO). At the beginning of the experiment (September 2012), the trees were nine years-old and were planted at an inter-row distance of 5 m, with 4 m between the trees within each row. Drip irrigation was used, with three emitters per tree. Each emitter had a flow rate of $4\,L\,h^{-1}$. Fertilizers were added to the irrigation water according to the regional recommendations.

Each trial was divided into three blocks, where the following agronomic strategies were established: open field, shade screen, and open field + mulching. The shade treatment was carried out by draping the trees with a white-colored, spectrally-neutral HD polypropylene shade screen, which transmits about 69% of incident light (Textil Villa de Pego, S.L. Spain). The shade screen was placed 3 m above the trees using a galvanized tube structure. The mulching treatment consisted of placing black mulch-plastic film (4 mm in thickness) to cover 15 m^2 around each of the trees in the plot.

Once the agronomic strategies were put in place in each trial, the salinity or drought treatments were applied. The salinity treatments were applied to the plot containing the trees grafted on *C. macrophylla* rootstock, and the drought treatments were applied to the plot containing the Sour orange-grafted trees. The three salinity treatments were: Control (electrical conductivity, EC = 0.8 dS/m), Salt 1 (approximate EC = 3.8 dS/m, 30 mM NaCl), and Salt 2 (EC = 6.8 dS/m, 60 mM NaCl). The amount of water supplied monthly was estimated by using the reference evapotranspiration (ETo) and a crop coefficient (Kc) suitable for non-stressed citrus (0.5–0.6), based on the age, tree size, soil structure, and tree spacing (FAO, 1990). The basin irrigation water had an EC of approximately 0.8 dS/m (SAR 1.6). The three irrigation treatments were established in the following manner: control treatment (100% ETc), moderate drought (60% Etc), and severe drought (40% ETc). For each of these combinations, six trees were used.

2.2. Color measurements

During the two growing periods studied (2011/2012; 2012/2013), data on the external color of the lemon fruits were gathered. For this, 15 fruits were labeled one month after fruit set and were then used for color measurements as a function of time. Three measurements were performed along the equatorial plane of each fruit. The external color of the fruits was measured with a CM-700d portable spectrophotometer, using a view angle of 10°, standard illuminant D65, and a CIELab color space. The L^*_{10}, a^*_{10}, and b^*_{10} data were used to characterize the fruit color. The hue angle was calculated as $h_{ab,10}{}^* = \text{arc tg}\,(b^*/a^*)$ (Little, 1975).

Three readings were taken in the equatorial zone of each fruit. In each reading, the colorimetric coordinates **L***, **a***, and **b*** were measured. The L* color coordinate measures lightness (100 for white and 0 for black). The a* color coordinate corresponds to the green-red axis, where the negative values correspond to green and the positive to red (−60 green, +60 red) (Hutchings, 1994; Macdougall, 2002), and the b* color coordinate measures variations from blue to yellow (-60 blue, +60 yellow). Any decrease in the chlorophyll content of the fruit is associated with colorimetric coordinate a* (Kidsome et al., 2002; Manera et al., 2012). The hue angle (Hue) is defined as the angle between the hypotenuse and 0° on the a* (bluish-green/red-purple) axis. This indicates how much green and yellow the fruit has. It is commonly used in English-speaking countries, while the a* coordinate is more common in Mediterranean countries. Both variables are therefore used in this work.

2.3. Leaf water potential and leaf Cl^- concentration

The leaf water potential (Ψ_{leaf}) was measured periodically at midday (12:00–12:30 h) in “Fino 49” trees grafted on Sour orange, with a Schölander-type pressure chamber (model 3000; Soil Moisture Equipment Corp., Santa Barbara, California, USA), according to the Schölander et al. (1965) technique.

In August, in each growing season, leaf samples were taken for Cl^- analysis from “Fino 49” trees grafted on *C. macrophylla*. The leaf samples consisted of six samples per treatment, each from 25 fully mature leaves (five to eight months-old). The leaves were briefly rinsed in deionized water, oven-dried at 60 °C, weighed, ground

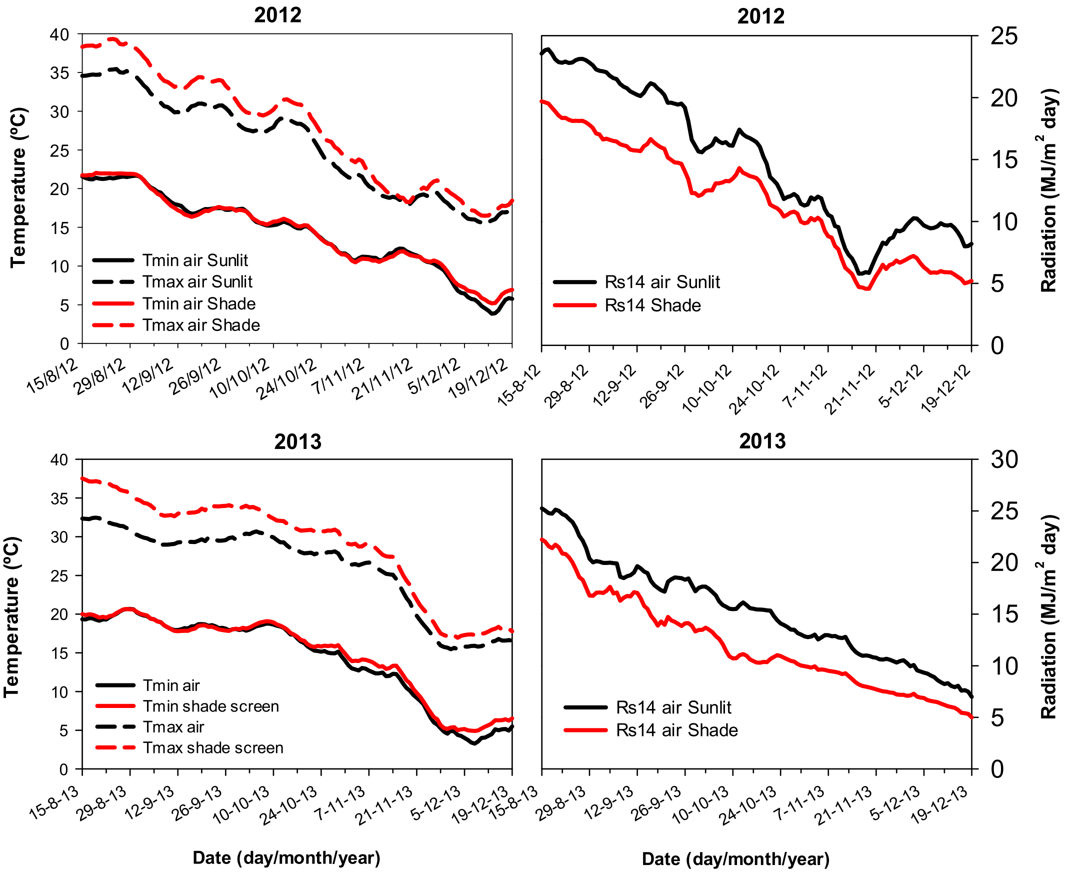

Fig. 1. Average change in maximum and minimum air temperature and solar radiation out in the open and under a screen of 14 days in the years 2012 and 2013.

with a centrifugal mill, and passed through a 5-mm-diameter sieve. The Cl^- concentration was determined by ion chromatography, using a Dionex-D100 ion chromatograph with an Ionpac AS12A 4 mm (10–32) column and guard column (Sunnyvale, California, USA).

2.4. Environmental conditions

Throughout the two growing seasons, the air temperature and the solar radiation were monitored in both plots from the middle of August until the end of the year. These data were gathered out in the open as well as under the screen, with a data logger (Model 100 Watchdog Data Logger, Spectrum Technologies, Inc., Plainfield, IL, USA) placed about 2 m off the ground. The air temperature and solar radiation were recorded every 30 min, every day. Fig. 1 shows the minimum average temperature from a period of 14 days. Manera et al. (2013) indicated that the color change of lemon fruits is related to this parameter.

2.5. Statistical analysis

The results were analyzed using the SPSS Statistics 22 program (Chicago, IL). The differences between treatments ($p < 0.05$) for the different parameters studied were evaluated by analysis of variance (ANOVA), followed by Duncan's test of comparison of means. The color changes and temperature pattern during 2012 were similar to those of 2013; therefore, the figure only shows data corresponding to 2012.

3. Results

3.1. Air temperature and solar radiation

As air temperature is one of the main factors that marks the start of the de-greening process, the daily maximum and minimum temperatures as well as the solar radiation were measured from the middle of August until the end of the year (Fig. 1). When analyzing the maximum temperature data, we observed that from August until the beginning of November, when the air temperature is usually higher than 25 °C, the maximum temperature under the shade screen reached values higher (by up to 5 °C) than those recorded out in the open. When the temperatures were below 25 °C, in November and December, the differences were smaller. On the other hand, in the years researched (2012 and 2013), the minimum temperatures out in the open as well as under the shade screen were very similar during the summer and fall. In November, however, the minimum temperatures were below 10 °C. The daily solar radiation was always lower under the screen than out in the open during the fall months, in both years (2012 and 2013).

3.2. a* coordinate and Hue index

3.2.1. Rootstock

During the de-greening process of the fruits, the a* coordinate and the Hue index increased and decreased, respectively, with time (Figs. 2–4). As for the effect on the a* and Hue index parameters of the rootstock on which "Fino 49" was grafted on, Fig. 2 shows that the start of de-greening as well as the final color of the fruit were not altered by the rootstock used. However, the fruits from the trees

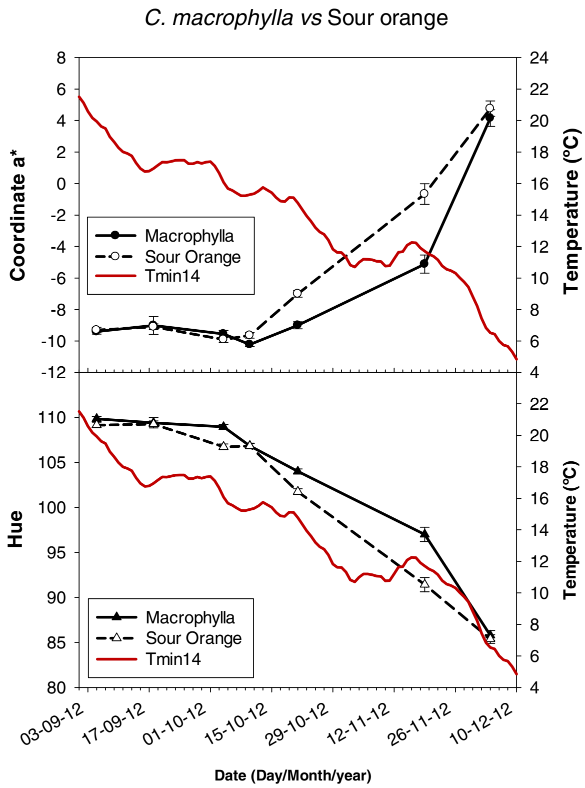

Fig. 2. Change in minimum temperature (14 day average) and the a* coordinate and Hue of lemon fruits from trees grafted onto *Citrus macrophylla* and Sour orange. The trees were grown out in the open and watered with non-saline water at 100% ETc. The values are means of n = 6 ± SE.

grafted on Sour orange rootstock had a greater a* and lower Hue than those from *C. macrophylla* rootstock.

3.2.2. Salinity experiment

In the plot containing the trees grafted on *C. macrophylla* rootstock, neither the growing strategy (open-air, shaded, or open-air + mulch) nor the salinity treatment affected the start of the de-greening of the fruits (Fig. 3). However, at the end of the harvest period, the agronomic strategy and the water treatment (salinity) did provoke some alterations in the color of the fruits. The fruits from the trees under shade screens, independently from the salinity treatment, had a lower a* and a greater Hue index than the fruits from trees with mulch or out in the open (Fig. 3; Table 1). As for the salinity treatments, fruits from the trees out in the open under treatments S1 and S2 had a greater a* coordinate and a lower Hue index than the no-salt (control) treatment.

During the de-greening process, the fruits from trees irrigated with the S1 treatment, under the shade screens or out in the open, exhibited greater a* and lower Hue values than the rest of the treatments. In the case of the fruits from the mulch treatment (Control, Salt 1, and Salt 2), their values were intermediate between the values of the fruits of the Salt 1 and Control treatments out in the open.

3.2.3. Drought experiment

In the plot containing the trees grafted on Sour orange the start of de-greening was not altered by the factors studied in this experiment. At the end of the growing cycle, the only significant effect with respect to the other treatments studied was found in the case of the shaded trees under the irrigation treatment at 40% Etc: the fruit had a negative a* value and a Hue value that was above 90 (Fig. 4, Table 1).

Table 1
Average values of the a* coordinate and Hue at the end of the fruit's development in "Fino 49" trees grafted onto *C. macrophylla* or Sour orange grown out in the open, under a shade screen or with mulching, and irrigated with water with different salinity (EC: 0.8, 3.8 and 6.8 dS/m) or under different irrigation regimes (100% ETc, 60% ETc and 40% ETc).

	Screen	Mulching	Control
Citrus Macrophylla			
a* coordinate			
Control	$^{y}3.09^{a}$	$^{x}5.29^{a}$	$^{xy}4.16^{a}$
Sal 1	$^{x}3.58^{a}$	$^{y}5.49^{a}$	$^{y}6.01^{b}$
Sal 2	$^{x}3.74^{a}$	$^{y}6.02^{a}$	$^{y}5.80^{b}$
HUE			
Sal C	$^{x}86.77^{a}$	$^{y}84.62^{a}$	$^{xy}85.78^{a}$
Sal 1	$^{x}86.40^{a}$	$^{y}84.45^{a}$	$^{y}83.98^{b}$
Sal 2	$^{x}86.26^{a}$	$^{y}83.98^{a}$	$^{y}84.28^{b}$
Sour orange			
a* coordinate			
100% ETc	$^{y}4.52^{a}$	$^{y}3.67^{a}$	$^{y}4.76^{a,b}$
60% ETc	$^{y}5.20^{a}$	$^{y}4.81^{a}$	$^{y}5.86^{b}$
40% ETc	$^{x}-0.57^{b}$	$^{y}4.98^{a}$	$^{y}3.99^{a}$
Hue			
100% ETc	$^{y}85.42^{a}$	$^{y}86.16^{a}$	$^{y}85.38^{a,b}$
60% ETc	$^{y}84.60^{a}$	$^{y}85.13^{a}$	$^{y}84.00^{b}$
40% ETc	$^{x}90.90^{b}$	$^{y}84.99^{a}$	$^{y}85.92^{a}$

Means in the same column followed by the same letter (a, b) are not significantly different at the 5% level of significance according to Duncan's *t*-test. Numbers in the same row preceded by the same symbol (x, y) are not significantly different at the 5% level of significance according to Duncan's *t*-test.

During the de-greening process, when comparing the data obtained for the trees under shade screens and those out in the open, the a* values followed the following tendency, from greater to lesser: in the open + 100% ETc = Shade + 100% ETc > Shade + 60% ETc = in the open + 60% ETc = in the open + 40% ETc > Shade + 40% ETc. For the mulching treatment, the a* value was greatest for out in the open + 100% ETc fruits and lowest for mulching + 40% ETc, with the rest of the treatments having values between these two extremes (Fig. 4).

3.3. Leaf water potential and leaf Cl^- concentration

To discover the plant water status in the plot where drought was applied and the concentration of toxic ions in the plot where salinity was applied, the water potential in August and the concentration of Cl^- were measured, respectively. In the plot where salinity was applied, and for all the agronomic strategies used, when the concentration of NaCl was increased there was an increase in the Cl^- concentration in the trees. Meanwhile, for each salinity treatment (Salt 1 and Salt 2), the trees grown with shade screens or mulching showed a lesser concentration than the trees that were grown out in the open. As for the plot where the drought treatments were applied, in all the agronomic strategies used, when the amount of water provided to each tree was reduced the water potential of the leaves decreased. Nevertheless, for the 60% ETc and 40% ETc drought treatments, the water potential was lower for the trees grown under shade screens, followed by trees grown in the open, and, lastly, trees grown with mulch.

4. Discussion

In the figures showing the changes in color as a function of time (Figs. 2–4), it can be observed that the a* coordinate started to increase at the same time that the Hue index started to decrease, on the same date (end of fall) for all the factors assayed in this experiment: rootstock, shade screen, mulching, salinity, or drought. This happened when the average temperature of the previous 14 days was below 14 °C. This means that the start of de-greening was

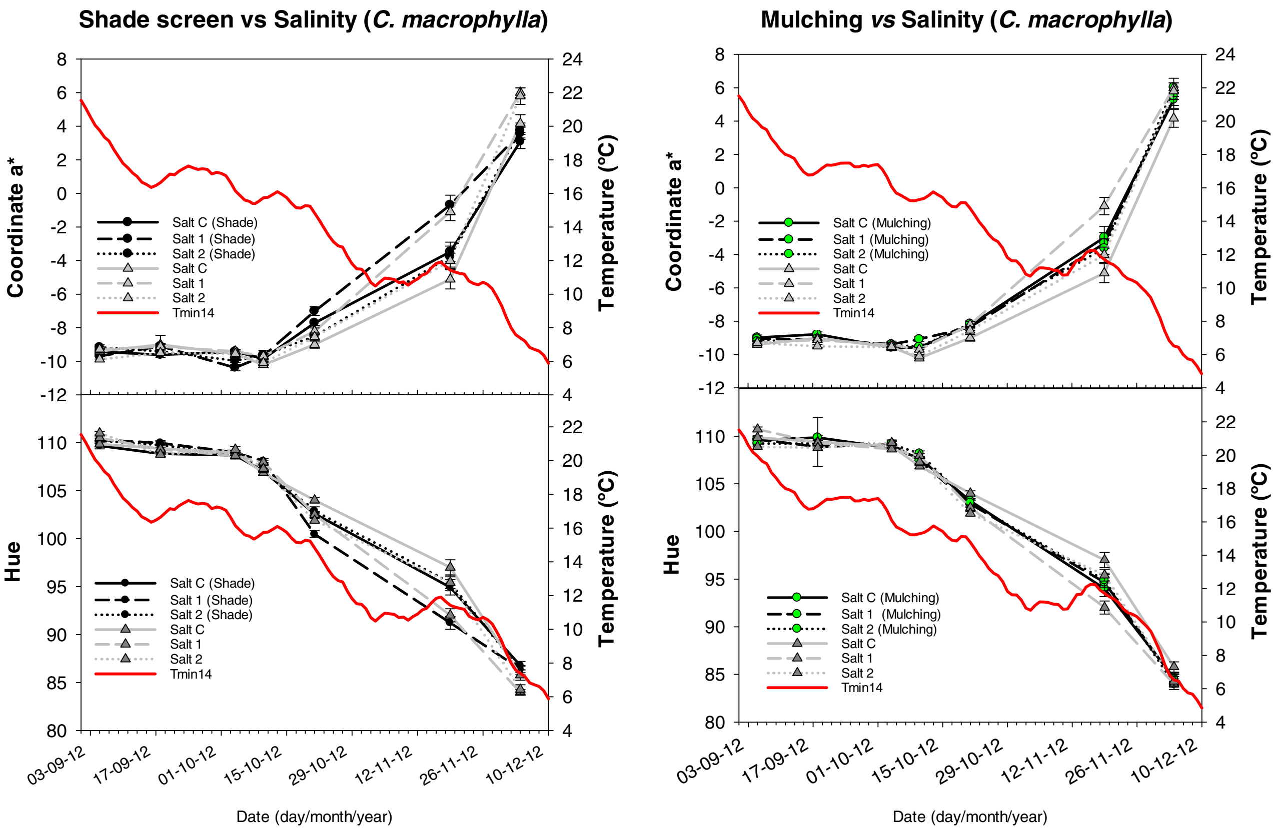

Fig. 3. Change in minimum temperature (14 day average) and the a* coordinate and Hue of lemon fruits from trees grafted onto *Citrus macrophylla*. The trees were grown out in the open, under a screen or with mulching, and watered at 100% ETc with water containing different concentrations of salt (Control 0.8, Salt 1 3.8, and Salt 2 6.8 dS/m). The values are means of n = 6 ± SE.

dependent solely on the minimum temperature, and not on the other environmental factors assayed in this experiment, such as salinity and soil moisture, water status of the plant, foliar concentrations of Cl^- and Na^+, maximum air temperature, or solar radiation.

From the start of the de-greening process until the end of the fall, a progressive increase in the a* coordinate and a decrease in the Hue index were observed during the experiment, reaching their respective maximum and minimum values in the month of December (Figs. 2–4), which indicates that the lemon fruits had reached their characteristic yellow color (Brotons et al., 2013). In Fig. 1, we can see that the type of rootstock did not influence the final color of the fruit, as a* and Hue were similar for both *C. macrophylla* and Sour orange (4.76, 85.38). However, the cultivation strategy (out in the open, shade screen, and mulching) and the irrigation treatment (drought and salinity) did have an effect on the final color of the fruits (Table 1).

Regarding the plots containing the trees grafted on *C. macrophylla*, the data show that the shade screens, independently of the irrigation treatment, produced fruits that were less yellow than the fruits that had grown out in the open. The screen used did not alter the minimum temperatures during the fall and winter, but led to an increase in the maximum air temperature and a reduction of solar radiation of about 30%. Some authors have observed that the reduction of the light incident on the fruit due to different causes – such as the use of shade screens, its position on the tree, or plots with a high density of plants – can reduce the color of the fruits (Jifon and Syvertsen, 2001). Our data, along with the data reported by these authors, suggest that the differences in response observed between the fruits without screening and the fruits with screens could be due to the decrease in solar radiation, more than to a temperature effect. In our experiment, the maximum average temperature increased with the use of the shade screens, while in the previous work the reduction in solar radiation led to a decrease in temperature. This indicates that solar radiation could be responsible for the decrease in the yellow color of the fruits. In this same plot, the application of salinity (S1 and S2) to the trees grown out in the open produced fruits that were more intense (in yellow color) than in non-saline conditions (Fig. 3, Table 1), corresponding at the same time with trees that had a greater concentration of Cl^- in their leaves (Table 2). In citrus, salinity degrades chlorophyll molecules and chlorophyll-protein complexes in the leaves (Srivastava et al.,

Table 2
Average values of the foliar Cl concentration and water potential in "Fino 49" trees grafted onto *Citrus Macrophylla* or Sour orange grown out in the open, under a shade screen or with mulching, and irrigated with water with different salinity (EC: 0.8, 3.8 and 6.8 dS/m) or under different irrigation regimes (100% ETc, 60% ETc and 40% ETc).

	Screen	Mulching	Control
Citrus Macrophylla			
Leaf Cl^- (% dw)			
Control	$^{x}0.16^{a}$	$^{x}0.14^{a}$	$^{x}0.15^{a}$
Sal 1	$^{x}0.64^{b}$	$^{x}0.7^{b}$	$^{y}0.94^{b}$
Sal 2	$^{x}1.04^{c}$	$^{x}1.1^{c}$	$^{y}1.64^{c}$
Sour orange			
Leaf water potential (-MPa)			
100% ETc	$^{x}1.66^{a}$	$^{x}1.54^{a}$	$^{x}1.57^{a}$
60% ETc	$^{x}1.92^{b}$	$^{y}1.75^{b}$	$^{y}1.84^{b}$
50% ETc	$^{x}2.54^{c}$	$^{z}2.02^{c}$	$^{y}2.20^{c}$

Means in the same column followed by the same letter (a, b, c) are not significantly different at the 5% level of significance according to Duncan's *t*-test. Numbers in the same row preceded by the same symbol (x, y, z) are not significantly different at the 5% level of significance according to Duncan's *t*-test.

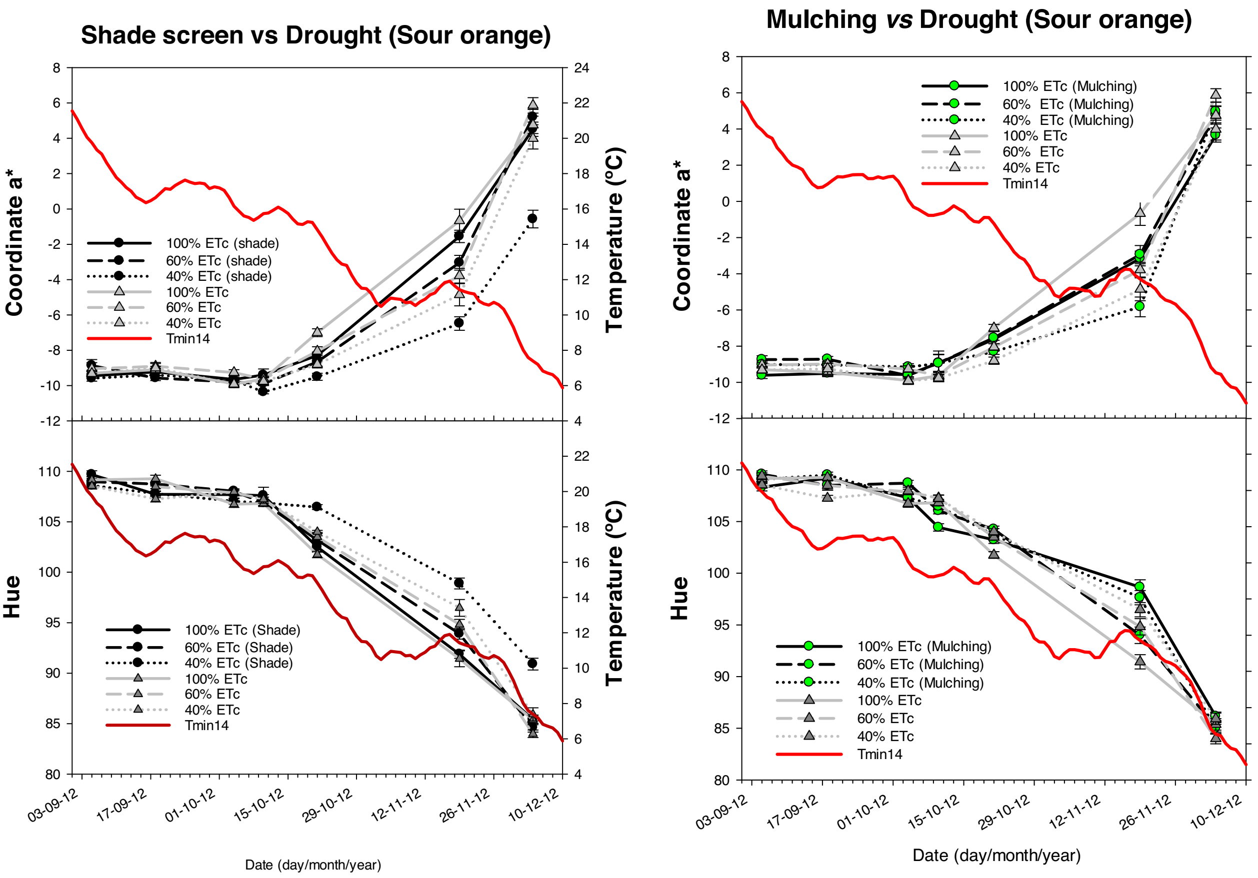

Fig. 4. Change in minimum temperature (14 day average) and the a* coordinate and Hue of lemon fruits from trees grafted onto Sour orange, grown out in the open, under a screen or with mulching, and watered with 0.8 dS/m water at 100%, 60% or 40% ETc. The values are means of n = 6 ± SE.

1988; Sheng et al., 2008; Navarro et al., 2010), due to suppression of specific enzymes responsible for the synthesis of photosynthetic pigments (Mukerji et al., 2006) or to the antagonistic effect of Na on Mg uptake, needed for chlorophyll biosynthesis (Giri and Mukerji, 2004). Therefore, the greater accumulation of Cl^- in trees grown out in the open, with respect to the shaded trees or those with mulching, could have accelerated the degradation of chlorophylls and hence the disappearance of the green color of the fruits.

In the case of the plot containing the "Fino 49" trees grafted on Sour orange, the irrigation treatment of 40% under a screen produced fruits having a yellow-green coloration, not reaching the characteristic intense yellow of lemons (Fig. 3, Table 1). The trees in this treatment (under the screen, 40% ETc) were the only ones that reached a leaf water potential at noon below −2.5 MPa (Table 2). In the case of the trees in the open, the leaf water potential did not decrease below −2.2 MPa in the drought treatments, while the trees with the mulch had values above −2.0 MPa. This suggests that the water status of the plant is an important factor to take into account in the natural process of de-greening of lemon fruits. In this sense, Kallsen and Sanden (2011) observed, in Navel orange trees, that drought treatments severely diminished the color of the fruit's skin at the end of November, retarding their natural de-greening. Navarro et al. (2013), in research performed on Clemenule mandarins, observed that the season in which irrigation is stopped can differently affect the change in color of the fruits. Therefore, when drought was applied from June to October the fruits had a lesser coloration that when the period of drought was during October and November. As for mulching, in other experiments we found that a reduction of the soil temperature, produced by covering the soil during the two months previous to harvesting through the use of a white reflective plastic, brought forward the change of color of the skin of Clementina (Clemenpons) fruit, allowing earlier harvesting (Mesejo et al., 2012). However, we hardly saw an effect on the change of the color in our current experiments.

5. Conclusion

In the research presented here, we have studied the color changes in the natural process of de-greening of lemon fruits, and we can conclude that the start of the de-greening process depends exclusively on the average air temperature, and not on other factors such as rootstock, shade screens, mulching, salinity, or drought. However, some of these factors could determine the final color of the fruit, which is very important for the consumer. Also, the data suggest that drought periods should be avoided when the trees are under shade screens, as the fruits will not develop the characteristic yellow color. Likewise, a decrease in solar radiation is also a negative factor that should be avoided if good fruit coloration is to be attained. As for salinity, it seems that it is not a negative factor for coloration, as its effect favors the degradation of the green color when certain Cl^- concentration thresholds are exceeded in the leaf.

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