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Research Paper

Proportion of oleic acid in olive oil as influenced by the dimensions of the daily temperature oscillation

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ABSTRACT

Olive fruit dry weight, oil concentration and the proportions of individual fatty acids in the oil are influenced by environmental variables, such as ambient temperatures, between flowering and harvest. An increase in mean daily temperature above 25 °C has been shown to have a negative effect on fruit dry weight, and to produce a linear decrease both in fruit oil concentration and oleic acid proportion in the oil over the range of 16–32 °C. Under natural conditions or in experiments in which mean daily temperatures are manipulated following the natural daily oscillation in temperature, mean daily maximum and minimum temperatures covary with mean daily temperature. However, variations in temperature associated with altitude, location and climate change can affect maximum and minimum temperatures differently and modify thermal amplitude. The objectives of the present study were to assess associations between changes in: i) yield variables (fruit dry weight and oil concentration) and ii) the proportions of major fatty acids in the oil, with the different dimensions of the daily temperature oscillation (mean daily minimum and maximum temperatures, mean daily thermal amplitude) experienced by the fruit during its growth from the pit-hardening stage to maturity. Five branch-level temperature treatments were applied: a control (T0) that followed the daily dynamics of ambient temperature, two levels of daytime (8–20 h) heating that increased temperature 5 and 10 °C relative to T0 during the day, and two levels of nighttime (20–8 h) heating to 5 and 10 °C more than T0. Treatments were applied for 76 days during the oil accumulation phase using transparent chambers with individualized temperature control to enclose fruiting branches of cultivar Arauco trees. The treatments successfully broke the natural covariance between the different dimensions of daily temperature variation, and achieved a broad range in mean daily temperature (~6 °C) which covered the natural range of this variable for the region. Fruit dry weight showed a tendency to decrease with increasing mean temperature, while the proportion of oil in the fruit exhibited a significant relationship (R^2 = 0.70) with mean daily thermal amplitude, and weaker −but significant- ones with mean daily maximum and minimum temperatures. The proportion of the main fatty acid in the oil, oleic acid, showed significant negative associations with mean daily minimum temperature (R^2 = 0.45) and with mean daily temperature (R^2 = 0.32), and a significant curvilinear relationship with mean daily thermal amplitude, but was not significantly associated with mean maximum temperature. Mean daily thermal amplitude in our experiment was determined mainly by mean daily minimum temperatures, a feature also found in an analysis of meteorological data for five sites and five years in the olive producing areas of La Rioja province, Argentina. Our results highlight the need to broaden studies on the temperature responses of olive fruit size, oil content and oleic acid content of the oil to include the effects of minimum temperature and thermal amplitude.

1. Introduction

Extra virgin olive oil is mainly composed of triglycerides of fatty acids, with oleic acid in the greatest proportion (55–83%), followed by linoleic acid (3.5–21%), palmitic acid (7.5–20%) and linolenic acid (≤1%) (IOOC, 2013). Oil fatty acid composition determines the nutritional and organoleptic properties of the oils. For example, oleic acid helps reduce total cholesterol and low density lipoprotein (LDL) levels in humans (Stark and Madar, 2002). On the other hand, linoleic and linolenic acid are the substrates of enzymes that generate volatile compounds responsible for oil aroma (Salas et al., 2000). The proportion of fatty acids in olive oil is influenced by effects associated with genotype, with fruit ontogeny and with environmental variables, including ambient temperature between flowering and final harvest

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(Beltrán et al., 2004; Borges et al., 2017; Dabbou et al., 2011; Orlandi et al., 2012; Rondanini et al., 2011; Tous et al., 1997). For example, in some cultivars like Arbequina and Arauco, the proportion of oleic acid in the oil decreases linearly as a function of thermal time during fruit growth, while for the cultivar Coratina the proportion of oleic acid remains constant from pit hardening to final harvest (Bodoira et al., 2016; Rondanini et al., 2014). Consequently, these patterns explain why cultivars like Arbequina and Arauco have low concentration of oleic acid at final harvest while other cultivars, like Coratina, have high concentrations (Rondanini et al., 2011). While there is abundant literature that indicates that there are variations in the proportions of fatty acids between genotypes, there is more limited information on the temperature effects on the fatty acid composition of the oils.

Olive oil accumulation in the fruit takes place in two structures, mesocarp and seed. Accumulation in the seed occurs early during the growth phase of the fruit (from fruit set until shortly after endocarp hardening), while accumulation in the mesocarp commences simultaneously with the seed accumulation but continues until fruit maturity (García-Inza et al., 2016). The endocarp hardening to maturity fruit growth subphase exhibits the highest rate of oil accumulation in the mesocarp (Conde et al., 2008). The importance of studying the impact of environmental variables during this subphase is due to the fact that oil accumulated in the mesocarp represents 95% of total fruit oil, with the remaining 5% being accumulated in the seed (Conde et al., 2008). Manipulative experiments demonstrated that oil concentration was sensitive to temperature increases during the period of active oil accumulation, decreasing 1.13 percentage points per °C of mean daily temperature increase (between 16 and 32 °C) (García-Inza et al., 2014). Correlative studies in which oil accumulation was analyzed for 6 cultivars, at three locations over two years (Rondanini et al., 2014), also showed that fruit oil concentration was negatively associated with mean temperature. In addition a negative relationship between duration of fruit oil accumulation and maximum daily temperature was found within a narrow range of temperatures (29–31.5 °C) explored in another correlative study (Trentacoste et al., 2012). The daily temperature oscillation in all these studies were the natural ones proper to each site and year, but there is a lack of information about the contribution of each dimension of the daily temperature oscillation (mean temperature, maximum temperature, minimum temperature, and thermal amplitude) on oil concentration and quality.

The effect of temperature on oil quality has been explored in correlative (Orlandi et al., 2012; Rondanini et al., 2014), and in manipulative studies (a-Inza et al., 2014, 2016; a-Inza et al., 2014, 2016). Correlative evidence showed that in cultivar Arbequina oleic acid concentration in the oil had a linear negative correlation with the increasing seasonal temperature (in the 23–27 °C range; Rondanini et al., 2011). Manipulative temperature experiments on fruiting branches of cultivar Arauco showed that the percentage of oleic acid in the whole fruit (i.e., seed and mesocarp) decreased by 0.7% $°C^{-1}$ with increases in average temperature (in the 16–32 °C range) during fruit growth (García-Inza et al., 2014), in contrast with the well-known increase in oleic acid with temperature described for annual oil-seed crops (e.g., Izquierdo and Aguirrezábal, 2008; Zuil et al., 2012; Baux et al., 2008).

Most of the studies that followed the changes in the proportion of fatty acids in vegetable oils as function of temperature were done on annual oil-seed crops, such as sunflower, soybean, corn, and canola. In this context, it is important to note that some studies in annual oil-seed crops show that changes in the proportion of fatty acids in the oil have a stronger correlation with minimum night temperature than with average daily temperature. Experiments with sunflower in which the average night temperature was artificially elevated (between 7 and 10 °C) for short periods (200 °C. day $^{-1}$) during fruit growth showed increases in the concentration of oleic acid (27% oleic acid in the fruits grown at control temperature *versus* 41% in heated fruits) (Izquierdo et al., 2002). In this crop, combined data from growth-chamber experiments and field experiments at two sites of contrasting thermal regime, showed correlations between oleic acid concentration in oil and the minimum night temperature (MNT). These experiments showed for an specific ontogenetic window (100–300 °C. day $^{-1}$ after flowering), that oleic acid concentration increased with increasing MNT following a sigmoidal pattern (Izquierdo and Aguirrezábal 2008). This result indicated that, for a range of MNT (between 12 and 27 °C, depending on the variety) and for this specific ontogenetic window, the proportion of oleic acid in oil had a positive linear relationship with MNT in sunflower. In soybean, oleic acid variations were also detected by modifying night temperatures during seed growth (Gibson and Mullen, 1996). In this experiment, when the night temperature increased from 20 °C to 30 °C, on plants growing at 30 °C during the daylight hours, oleic acid decreased from 23.9% to 21%. However, when the effect of the same night temperature increase (20–30 °C) was assessed on plants grown at 35 °C during daylight hours, oleic acid rose from 28% to 34%. This result suggests a more complex nighttime temperature response in soybean than in sunflower. These evidences from annual oil-seed crops indicate that the range of temperatures explored overnight affected fatty acid desaturation.

The above antecedents in annual oil-seed crops suggest that night temperature can play an important role in determining the proportions of fatty acids in the oil. However there is no information for olive and other species that accumulate oil in the mesocarp (e.g., avocado, oil palm). It has been shown that the frequency of high-temperature anomaly events is increasing (Hansen et al., 2012); a significant increase in the occurrence of warm nights in the 1951–2003 period (Alexander et al., 2006) has been detected; and an analysis of temporal temperature trends for La Rioja (Argentina) in the 1962–2013 period has showed that during the summer months the mean temperature increase is explained by the increase in the minimum temperature (R. De Ruyver, INTA-Castelar, personal communication). Simulation models suggest that expected mean temperature increases will be strongly driven by the increases in minimum temperature (Sillmann et al., 2013). Thus, it is necessary to understand the impact of the different dimensions of the daily temperature oscillation on olive yield and quality.

The aim of this study was to evaluate the relationships between changes in variables associated with yield (dry fruit weight and oil concentration) and oil quality (especially, the proportion of oleic acid) and the different dimensions of the daily thermal oscillation (mean, minimum, and maximum temperatures and thermal amplitude) experienced by the olive fruit. To achieve this objective we implemented a set of treatments aimed at breaking the natural covariance between these dimensions of the natural daily temperature oscillation.

2. Materials and methods

2.1. Experimental site and experiment design

The experiment was conducted in Los Molinos (28°43′S, 66°56′W; 1400 m above sea level), province of La Rioja, Argentina. This location was selected because of its altitude, which makes the site cooler and allowed us to attain a broader range of temperatures. The orchard was planted in 1940 at 6 m between trees and 12 m between lines. The plants were flood-irrigated every 20 days all year round, and were fertilized with 40 kg of goat manure per plant at pit hardening stage. The orchard sanitary conditions were monitored weekly, no additional pest control was required. The experimental tree canopy volume was of 25 m^3 in average and each tree yielded 80 kg on average (fresh weight). Fruit load was 400 fruit m^{-3}, intermediate for cultivar Arauco (Fernández et al., 2015). Flowering was recorded on October 19, 2012 and endocarp hardening (defined as the date at which it was no longer possible to cut the pit with a knife) occurred on December 22, 2012. We manipulated the temperature at branch level using fruiting branches during subphase IV of the fruit growth phase (Conde et al., 2008), the period of active oil accumulation in the mesocarp. At the beginning of

treatment (Feb 07, 2013) fruiting branches were enclosed in temperature-controlled chambers until harvest (Apr 24, 2013). We selected external fruiting branches of around 20 cm in length bearing between 5 and 8 fruit per branch. The chambers were thermostatted, had an internal fan that mixed the air within the chamber, had ventilation openings to allow air exchange with the environment, and were identical to those described in García-Inza et al. (2014). Leaves or fruit were thinned as necessary to ensure that the leaf/fruit (source/sink) relationship was similar in all treated branches, and all experimental trees had similar fruit loads. Five thermal regimes were applied: control (T0) that followed the natural ambient temperature oscillation; two levels of daytime (8–20 h) heating aimed at increasing chamber temperature by 5 and 10 °C relative to T0 during the day (D5 + and D10 + respectively); and two levels of nighttime (20–8 h) heating, aimed at increasing chamber temperature by 5 and 10 °C relative to T0 (N5 + and N10 +).

The experimental design was a randomized complete block with four replicates where a tree was taken as a block, and each treatment was present within each block. Data from one (N10 +) replicate were excluded because an unusual defoliation of the branch and damage to the fruit were detected.

2.2. Heating treatments

The temperature in each chamber was manipulated with the control system described by García-Inza et al. (2014). Briefly, the temperature inside the chambers were controlled with two central electronic controllers (Caja controladora, Cavadevices, Buenos Aires, Argentina), one governing the daytime heating treatments and the other the nighttime treatments. The controllers regulated the chamber air temperature by switching on and off the 12 V power that fed heaters (resistances) inside the chambers. Each chamber was individually programmed to increase treatment air temperature by 5 or 10 °C compared to the control chamber temperature during the day or the night. The central electronic controllers were connected to clocks that switched the daytime (8–20 h) and nighttime (20–8 h) treatments on or off. Air temperature was recorded in each chamber every 15 min using integrated circuit sensors (model LM35, National Semiconductor, Dallas, TX). Data acquisition was performed with a datalogger (CR1000, Campell Scientific Inc., Logan, UT).

2.3. Response variables

The experiment started on February 7 and finished on April 24, 2013, for a total duration of 76 days. Prior to the start of the treatment period fruit were harvested from fruiting branches of equivalent size and position in the tree canopy to the treated branches and their dry weight, oil concentration and the fatty acid composition of the oil was determined. The same variables were determined, at final harvest, for the fruit from branches subjected to treatment. The oil extraction techniques and the determination of the fatty acids (palmitic, stearic, oleic, linoleic and linolenic) present in the oil by GC were the same as those detailed in García-Inza et al. (2014). The treatment effects were estimated on the basis of the biomass accumulated during the treatment period in order to avoid dilution effects arising from the fact that the fruit had a non-zero biomass and oil content values at the start of the experiment. The biomass and oil accumulation per fruit during the treatment period were calculated by subtracting the initial mean values from the corresponding value at final harvest from the same plant. The changes in the concentration of each fatty acid in the oil was determined by calculating the change in fatty acid content (between final harvest and initial value), and then were calculated as a proportion of the fatty acid accumulated during the treatment period. For example:

Change in oleic acid content in the oil (%) = [(content of oleic acid in the fruit at final harvest − content of oleic acid in the fruit at initial harvest)/(total oil content in the fruit at final harvest − oil content in the fruit at initial harvest)] × 100

The units for content are in mg/fruit.

Table 1
Mean daily values for the dimensions of the daily temperature oscillation for the different treatments: control (T0), two levels of daytime heating (D5 + and D10 +), and two levels of nighttime heating (N5 + and N10 +). Values in each cell are daily averages ± 1 SE for the treatment period (Feb 07, 2013–Apr 24, 2013). Asterisks indicate the extremes, across treatments, of the temperature range recorded for each thermal dimension. The range between extreme treatments, was calculated as the difference between values with (*) in each column.

Treatment	Mean daily temperature (°C)	Mean minimum temperature (°C)	Mean maximum temperature (°C)	Mean thermal amplitude (°C)
T0	22.4 ± 1.1 *	16.6 ± 1.3	29.9 ± 1.0	13.3 ± 1.2
D5 +	24.0 ± 0.7	15.7 ± 0.5 *	34.1 ± 1.1	18.4 ± 1.5
D10 +	25.8 ± 0.8	16.9 ± 0.5	36.2 ± 0.9 *	19.2 ± 0.4 *
N5 +	25.0 ± 0.7	20.7 ± 0.4	29.2 ± 0.9 *	9.0 ± 2
N10 +	27.2 ± 1.1 *	22.3 ± 0.3 *	31.2 ± 2.1	8.1 ± 0.8 *
Range between extreme treatments	4.8	6.6	7.0	11.1

2.4. Statistical analyses

We used ANOVA for fixed effects to evaluate treatment impacts. Differences among treatment means were evaluated with the Tukey test ($P < 0.05$). All analyses were performed using SAS software v8 (SAS Institute, Cary, NC, USA 1999). Different mathematical functions (linear, polynomial and bilinear) were fitted to the relationships between the variables of interest. We report those functions that provided the best fits with a greater level of significance ($P < 0.05$ and $R^2 > 0.2$). These analyses and the graphs were made with GraphPad Prism version 5.01 software (GraphPad Prism Software, Inc., La Jolla, CA).

3. Results

3.1. Temperature during the treatment period

The treatments were effective in changing the temperature regimes inside the chambers, as reflected in the average values of the different dimensions of the daily temperature oscillation across treatments (Table 1). It is important to note that the values in Table 1 are average daily values for each treatment over the whole of the experimental period.

Mean daily maximum and minimum temperatures and thermal amplitude in the first week of treatment were 32.0 °C, 18.9 °C and 14 °C, respectively. The maximum and minimum temperatures trended downward during the experimental period to 28.2 °C, 14.2 °C in the final week of the experimental period, while thermal amplitude remained constant during the experimental period. The daily pattern of temperature dynamics in the T0 treatment followed (± 0.6 °C) the daily and seasonal variations in temperature throughout the experiment (data not shown). As exemplified in Fig. 1 the daytime treatment (D) copied the pattern of T0 overnight and had higher temperatures than T0 during the day; while nighttime treatment (N) copied the pattern of T0 during the day and had higher temperatures than T0 overnight. As shown in Table 1 and Fig. 1, the treatments broke the natural covariance between maximum, minimum, and mean temperatures, and achieved – for each of the dimensions of the daily temperature oscillation- ranges of between 4.8 °C (mean daily temperature) to 11.1 °C

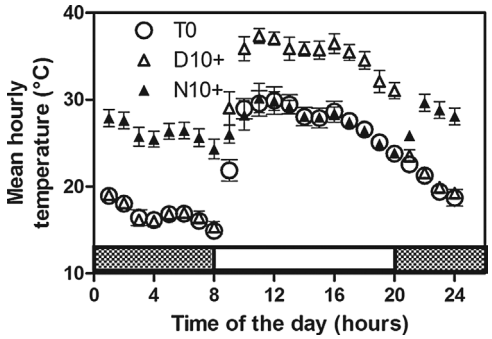

Fig. 1. Patterns of mean hourly chamber temperatures for February 20, 2013. Treatments were: control (T0, ○), daytime (8–20 h) heating (D10 +, Δ, 10 °C warmer than T0 during the day) and nighttime (20–8 h) heating (N10 +, ▲, 10 °C warmer than T0 during the night). Data points are mean values ± 1 S.E (n = 4).

(mean daily thermal amplitude) across treatments.

3.2. Fruit dry weight, fruit oil content and oil concentration responses to the dimensions of the daily thermal regime

During the treatment period (February 7–April 24, 2013), the fruit accumulated something in the order of one-quarter of the final fruit dry weight and one-half or more of the fruit final oil content, with these increments in both absolute and relative terms tending to be largest in T0 and smallest in N10 + (Table 2). Nighttime warming treatments (N5 + and N10 +) resulted in most of the fruit dry weight increment during treatment being attributable to an increase in oil (90% and 92%, respectively; Table 2), while daytime heating treatments tended to reduce the contribution of the oil to the dry weight increment of the fruit with respect to the control values. Linear functions were fitted to the relationships between these response variables and the dimensions of the daily temperature oscillation, since it was not possible to use multivariate techniques for this purpose because increases in oil concentration covaried with increases in fruit biomass and oil content. The increments in fruit dry weight and oil content during the experiment showed weak and non-significant relationships (R^2 = 0.15 and 0.17 respectively; P > 0.05) with mean temperature (Fig. 2). The relationship between these two response variables with the remaining dimensions of the daily temperature oscillation were substantially weaker than with mean daily temperature (data not shown). However, the decreases in both variables between the extreme treatments for mean daily temperature (T0 and N10+, Table 1) were significant (Table 2).

By contrast, changes in the oil concentration as a proportion of the fruit dry weight increment during the treatment period as functions of mean minimum and mean maximum temperatures and mean thermal amplitude were robust and statistically significant (Fig. 3a–c). Oil

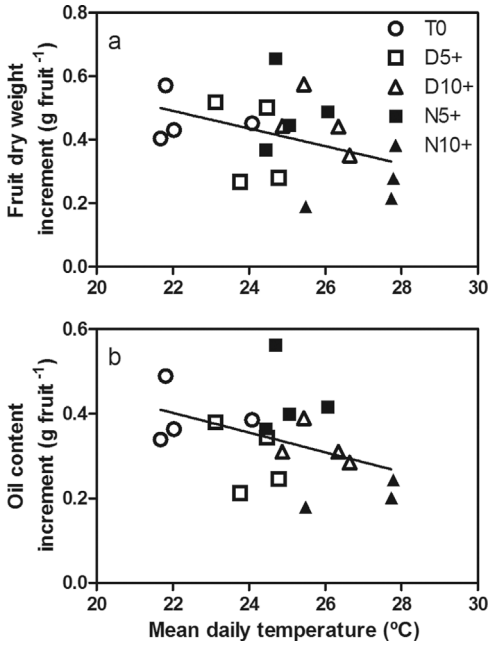

Fig. 2. Increments during the treatment period (February 7–April 24, 2013) of fruit dry weight (a) and oil content (b) as a function of mean daily temperature during the same period. Treatments were: control (T0, ○), two levels of daytime heating (D5 +, □ and D10 +, Δ) and two levels of nighttime heating (N5 +, ■ and N10 +, ▲). Each point is the value for an individual controlled-temperature chamber. The slope of the functions fitted to the data for fruit dry weight and oil content are not significantly different to zero, but are shown to illustrate the trend of the observed responses.

concentration increased 2.3% °C $^{-1}$ with minimum temperature (Fig. 3a), the same rate but with opposite sign (−2.3% $^{-}$°C^{-1}) as a function of mean maximum temperature (Fig. 3b). Finally, the fruit oil concentration fell linearly with increasing thermal amplitude (Fig. 3c).

3.3. Variations in the proportions of oil fatty acid proportions in response to the different dimensions of the daily thermal oscillation

Changes in the proportion of oleic acid in the oil accumulated during the experiment were significantly associated with mean temperature and minimum temperature, while it was not associated with maximum temperature (Table 3). The proportion of oleic acid had significant quadratic relationship with the thermal amplitude (R^2 = 0.36, P < 0.05) (Table 3). The driving force variable that best explained changes in the proportion of oleic acid was the minimum temperature (Table 3). The relationships between the proportions of oleic and linoleic acids in the oil with mean minimum temperature were robust and could be fitted with statistically significant linear

Table 2
Fruit dry weight and oil content at final harvest (April 24, 2013), absolute fruit dry weight and oil increments during the treatment period (g), the proportion (%) of final biomass and oil accumulated during the experimental period, and the proportion of oil (%) in the fruit dry weight increment during the treatment period. Treatments were: control (T0), two levels of daytime heating (D5 + and D10 +), and two levels of nighttime heating (N5 + and N10 +). Fruit dry weight and oil content at the start of treatments were 1.17 ± 0.03 g fruit^{-1} and 0.23 ± 0.01 g oil fruit^{-1}, respectively. Values are means ± 1 SE (n = 4). Different letters after values within each column indicate significant differences (P < 0.05) between treatments.

Treatments	Fruit dry weight at final harvest (g.fruit^{-1})	Fruit oil content at final harvest (g oil.fruit^{-1})	Increment in fruit dry weight during the treatment period (g.fruit^{-1})	Proportion of final fruit dry weight accumulated during the treatment period (%)	Increment in fruit oil accumulated during the treatment period (g.fruit $^{-1}$)	Proportion of final fruit oil content accumulated during the treatment period (%)	Increment in fruit oil during the treatment period as a proportion of the corresponding fruit dry weight increment (%)
T0	1.63 ± 0.07 a	0.62 ± 0.04 ab	0.46 ± 0.04 a	28.0 a	0.39 ± 0.03 ab	62.7 ab	84.8 ab
D5 +	1.56 ± 0.08 a	0.53 ± 0.04 c	0.39 ± 0.07 a	25.0 a	0.29 ± 0.04 c	55.7 b	77.2 bc
N5 +	1.66 ± 0.06 a	0.67 ± 0.04 a	0.48 ± 0.07 a	29.0 a	0.43 ± 0.05 a	65.0 a	89.9 a
D10 +	1.62 ± 0.04 a	0.55 ± 0.01 bc	0.44 ± 0.05 a	27.5 a	0.32 ± 0.02 bc	57.9 ab	72.3 c
N10 +	1.38 ± 0.02 b	0.43 ± 0.01 d	0.22 ± 0.02 b	16.5 b	0.20 ± 0.01 d	47.7 c	91.8 a

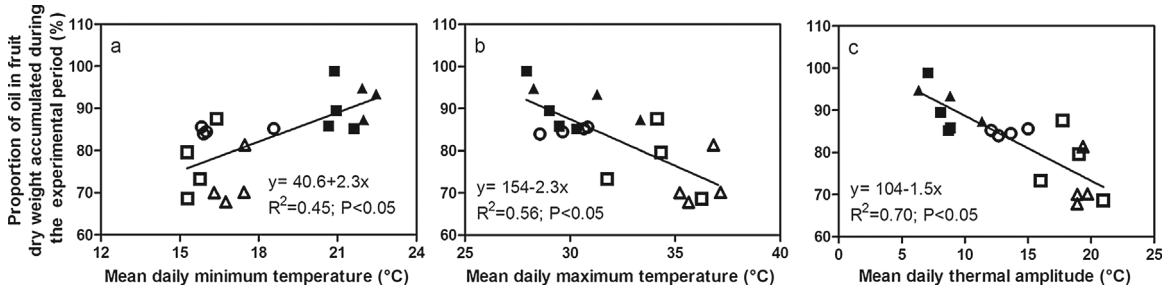

Fig. 3. Fruit oil increment as a proportion of fruit dry weight increment during treatment period as function of mean daily minimum temperature (a), mean daily maximum temperature (b) and mean daily thermal amplitude (considered as the difference between mean of daily differences in maximum and minimum temperatures) (c). Treatments were: control (T0, ○), two daytime heating levels (D5 +, □ and D10 +, Δ) and two levels of nighttime heating (N5 +, ■ and N10 +, ▲). Each point is the value for an individual controlled-temperature chamber.

Table 3
R^2 and P values for the functions fitted to relationships fitted between the proportions of major fatty acids (oleic and linoleic) in the oil and the specified dimensions of the daily temperature oscillation (mean, minimum and maximum temperatures) during the experimental period (Feb 07, 2013–Apr 24, 2013).

Thermal dimension	Variable	Fitted function	R^2	P value
Minimum temperature	% Oleic	86 − 1.3x	0.45	< 0.05
	% Linoleic	4.4 + 0.7x	0. 40	< 0.05
Mean temperature	% Oleic	103 − 1.6x	0.32	< 0.05
	% Linoleic	−1.7 + 0.7x	0. 20	< 0.05
Maximum temperature	% Oleic	53.2 + 0.3x	0.03	0.44
	% Linoleic	24.4 − 0.2x	0.06	0.31
Thermal amplitude	% Oleic	$38.5 + 3.4x - 0.1x^2$	0.36	< 0.05
	% Linoleic	$36\text{-}2.8x - 0.1x^2$	0.51	< 0.05

functions (Fig. 4c and d). By contrast, the proportions of palmitic and linolenic acids did not change significantly with increasing minimum temperature and the proportion of stearic acid was stable across all the minimum temperature range (Fig. 4a, b and e).

The proportions of oleic and linoleic acids in the oil generated during the treatment period were significantly associated with mean temperature (Fig. 5c and d), but these associations were weaker ($< R^2$) than those registered for the associations with minimum temperature (Fig. 4c and d). The association between the proportion of linolenic acid and mean temperature was significant but had a low slope value, increasing 0.07% $^{-1}$ °C with the increase in mean temperature (Fig. 5e). As with the minimum temperature response, the proportion of palmitic acid had a non-significant tendency to increase with mean temperature while the proportion of stearic did not change with variations in mean temperature (Fig. 5a and b).

The main fatty acids in the oil, oleic and linoleic, had non-significant associations with maximum temperature (Table 3), showing tendencies to increase and decrease with maximum temperature, respectively. The proportions of oleic and linoleic acids in the oil accumulated during the treatment period had a significant but nonlinear relationships with thermal amplitude (Table 3). The proportion of oleic acid increased with increasing amplitude between 6 and 15 °C, but amplitudes greater than 15 °C did not generate changes in the proportion of this fatty acid. The linoleic acid had an inverted parabola type response, falling in the range of 6–15 °C followed by a slight increase between 15 and 20 °C (Fig. 6c and d).

4. Discussion

During the experimental period (subphase IV of fruit growth) and in the control treatment, 28% of the final fruit dry weight and 63% of the final fruit oil content were accumulated (Table 2). These changes were associated entirely with mesocarp growth because the seed had previously completed its growth (García-Inza et al., 2016). The fact that important proportions of final fruit dry weight and oil were accumulated during the experimental period increases confidence in the observed associations between responses and treatments.

The novel aspect of this study was that the treatments ruptured the covariance between the different dimensions of the natural daily temperature oscillation over an extended period under field conditions (Fig. 1). We were able to achieve a substantial range of thermal amplitude, a dimension of the daily temperature variation that had not been explored in previous manipulative experiments (a-Inza et al., 2014, 2016; a-Inza et al., 2014, 2016). In previous correlative studies in olive (e.g. Orlandi et al., 2012; Rondanini et al., 2014; Trentacoste et al., 2012), in which the effects of temperature variations were explored by analyzing different sites and/or years, this dimension was not substantially altered. In previous studies in which temperature was manipulated under field conditions for long periods, either increased temperature copied the natural daily pattern (apple: Atkinson et al., 1998; olive: a-Inza et al., 2014, 2016; a-Inza et al., 2014, 2016) or temperature was increased only during the nighttime (sunflower: Izquierdo et al., 2002; wheat and barley: García et al., 2015). Only in grape have experiments been conducted in which temperature was increased during daytime and nighttime periods (Cohen et al., 2008, 2012; Sadras and Soar, 2009; Spayd et al., 2002).The manipulations of temperature in grape were applied during two subphases of the fruit growth phase. Ranges of 4.5 °C in mean temperature, 5.4 °C in maximum temperature, 4.9 °C in minimum temperature were achieved (Cohen et al., 2008), somewhat narrower than the ranges explored in our experiment (Table 1). The range of temperatures achieved in our work exceeded the ranges recorded for three different olive production areas of La Rioja Province, Argentina, namely La Rioja (2009–2013), Aimogasta and Chilecito (both sites 2007 and 2008). Thus, the temperature ranges recorded for these three sites, for the same subphase of fruit growth as the one analyzed in our experiment were: 23.0–23.8 °C for mean daily temperature, 30.1–31.0 °C for maximum temperature, 15.8–16.8 °C for minimum temperature, and 13.3–14.9 °C for thermal amplitude. All these ranges fall within and are much narrower than those achieved in our experiment (Table 1), increasing the confidence in the relevance of our results for regional olive production environments.

The tendency of the increments of fruit dry weight and oil content to decrease during the experimental period with increasing mean daily temperature were not significant (Fig. 2), although values for these variables showed a significant decrease between extreme treatments (T0 and N10+, Table 2), a response consistent with the effects found for mesocarp dry weight in response to fixed increments in temperature during the full 24-h period during a slightly later sub-phase of fruit growth (García-Inza et al., 2016). The associations between the increments of fruit dry weight and oil content with other dimensions of the daily temperature oscillation (maximum, minimum, thermal amplitude) showed no significant correlations. Consequently, ruptures in the natural correlations between the dimensions of the daily thermal regime in our experiment did not provide new information about the

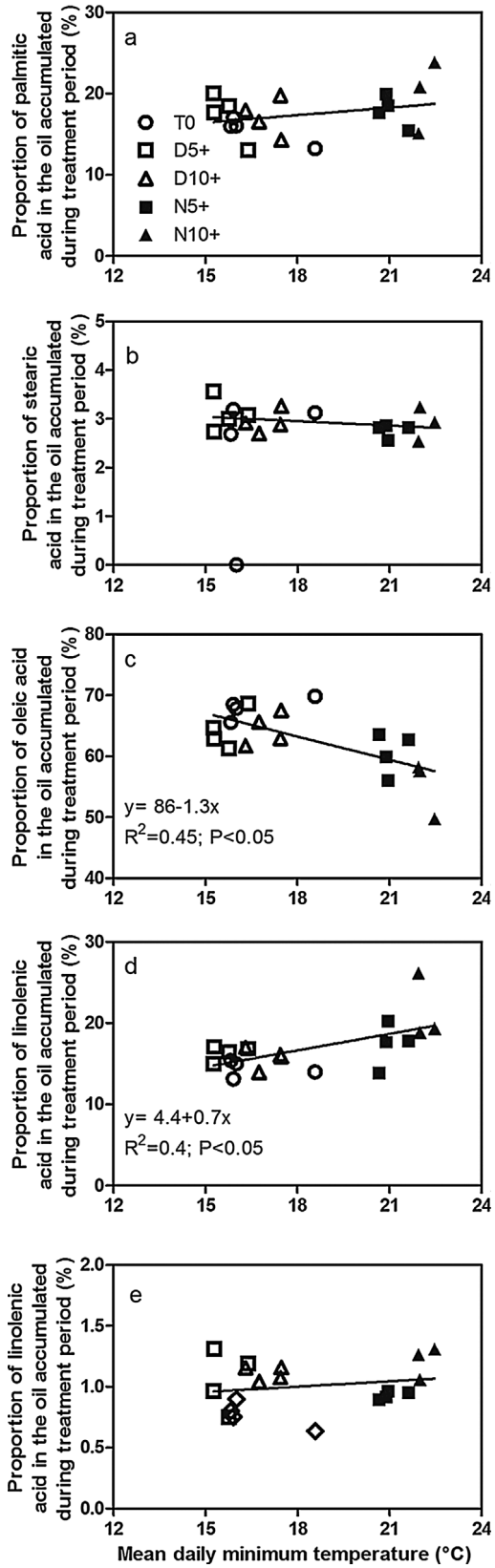

Fig. 4. Proportion of the major fatty acids in the oil accumulated during the treatment period as a function of mean daily minimum night temperature. The fatty acids were: palmitic (a), stearic (b), oleic (c), linoleic (d), and linolenic (e). Treatments were: control (T0, ○), two daytime heating levels (D5 +, □ and D10 +, Δ) and two levels of nighttime heating (N5 +, ■ and N10 +, ▲). Each point is the value for an individual controlled-temperature chamber. The slope of the functions fitted to the data for palmitic, stearic and linolenic were not significantly different to zero, but are shown to illustrate the observed trends.

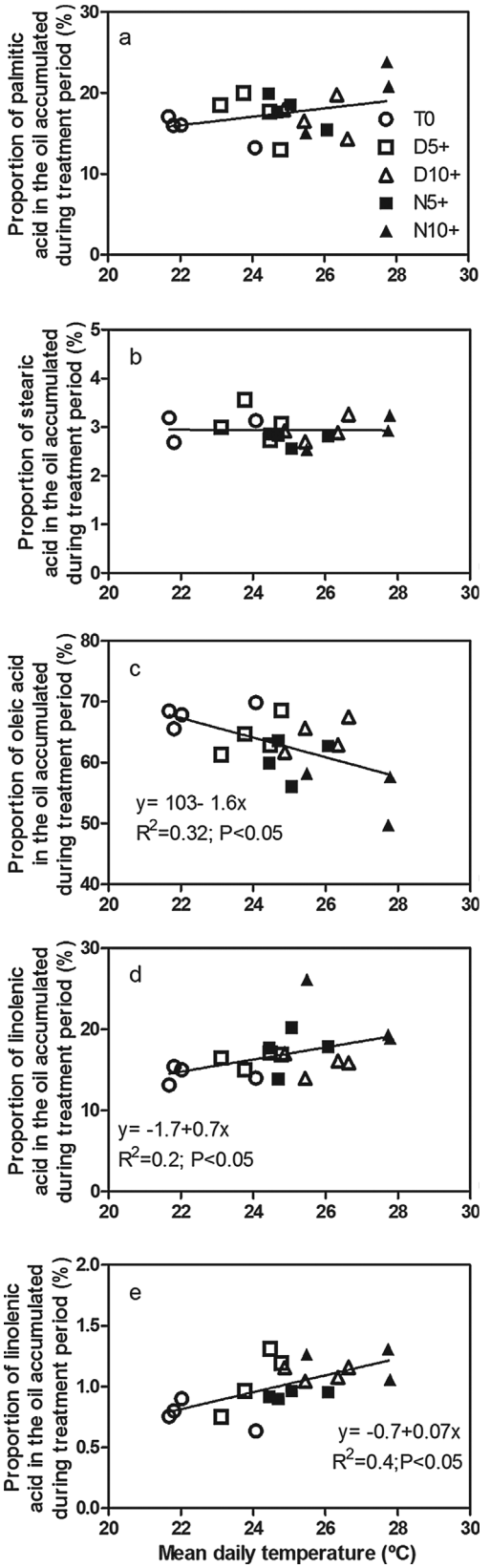

Fig. 5. Proportion of the major fatty acids in the oil accumulated during the treatment period as a function of mean daily temperature. The fatty acids were: palmitic (a), stearic (b), oleic (c), linoleic (d), and linolenic acid (e). Treatments were: control (T0, ○), two levels of daytime heating (D5 +, □ and D10 +, Δ) and two levels of nighttime heating (N5 +, ■ and N10 +, ▲). Each point is the value for an individual controlled-temperature chamber. The slope of the functions fitted to the data for palmitic and stearic were not significantly different to zero, but are shown to illustrate the observed trends.

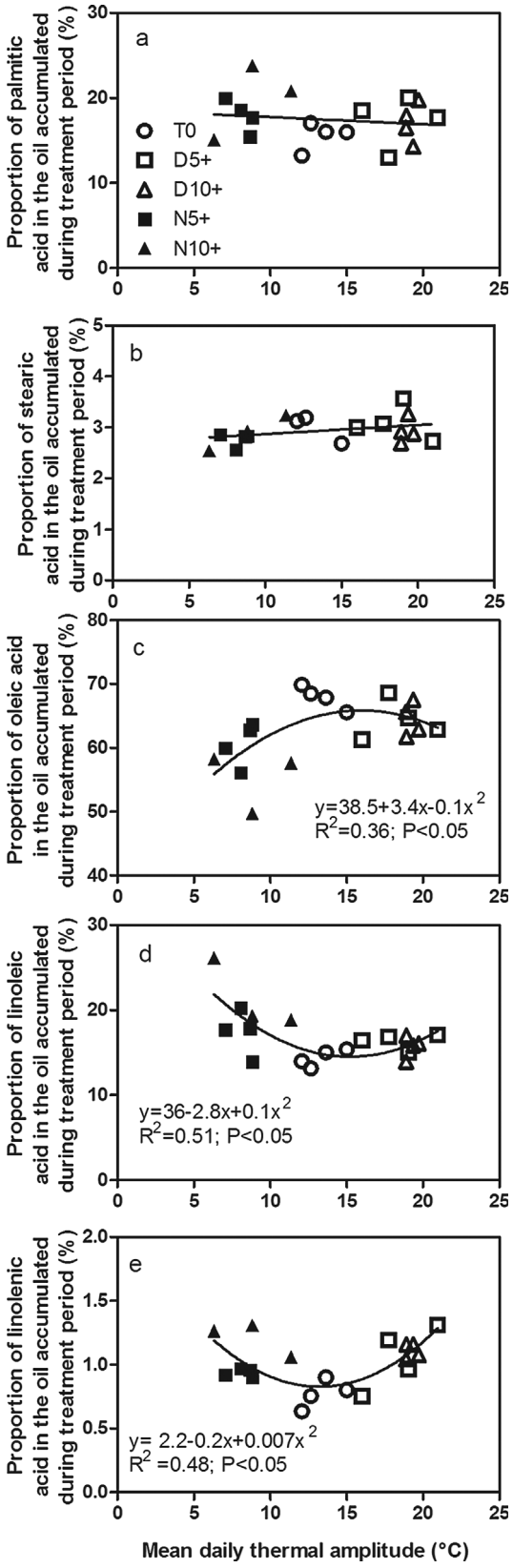

Fig. 6. Proportion of the major fatty acids in the oil accumulated during the treatment period as a function of mean daily thermal amplitude. The fatty acids were: palmitic (a), stearic (b), oleic (c), linoleic (d), and linolenic acid (e). Treatments were: control (T0, ○), two levels of daytime heating (D5 +, □ and D10 +, Δ) and two levels of nighttime heating (N5 +, ■ and N10 +, ▲). Each point is the value for an individual controlled-temperature chamber. Thermal amplitude was calculated as mean of daily differences in maximum and minimum temperatures. The slope of the functions fitted to the data for palmitic and stearic were not significantly different to zero, but are shown to illustrate the observed trends.

thermal signals capable of affecting this pair of variables.

By contrast with the above pair of variables, the proportion of the oil accumulated during the experimental period in the corresponding increment in fruit dry weight showed a strong negative association ($R^2 = 0.70$, $P < 0.05$) with the thermal amplitude and weaker associations with maximum ($R^2 = 0.56$, $P < 0.05$) and minimum ($R^2 = 0.45$, $P < 0.05$) temperatures (Fig. 3). The response to maximum temperature is consistent with the results of previous correlative analyses in olive based on years (Trentacoste et al., 2012) or years and sites (Rondanini et al., 2014). Both studies showed a negative association between fruit oil concentration and maximum temperature, although the thermal ranges explored were more limited than that in our work. Thus the range of mean maximum temperatures explored in our work was 7.0 °C (Table 1), contrasting with values of 3.0 °C in Trentacoste et al. (2012) and 4.4 °C in Rondanini et al. (2014). Rondanini et al. (2014) found a stronger negative association between oil concentration and maximum temperature ($R^2 = 0.50$, $P < 0.05$) than with mean or minimum temperatures, but they did not analyze the relationship between oil concentration and thermal amplitude.

Changes in the proportions of oleic and linoleic acid in the oil were associated with minimum temperature ($R^2 = 0.45$, $P < 0.05$) (Fig. 4c and d) and more weakly with mean temperature ($R^2 = 0.32$, $P < 0.05$) (Fig. 5c and d). This is consistent with observations for annual oilseeds such as sunflower or rapeseed (Baux et al., 2008; Izquierdo and Aguirrezábal, 2008; Echarte et al., 2010), except that in those species the response to minimum temperature is opposite to that observed in olive. In olive, a negative correlation was found between the proportion of oleic acid in the oil and minimum temperature (mean of eleven fruit- growth seasons) (Orlandi et al., 2012); unfortunately, the range of minimum temperatures explored in that work was not specified. Our results also highlight the associations between the proportions of oleic and linoleic acid in olive oil with thermal amplitude (a positive association, $R^2 = 0.36$, $P < 0.05$ for the proportion of oleic, and a negative association, $R^2 = 0.51$, $P < 0.05$ for linoleic acid) over a wide range of amplitude values (8.1–19.2 °C, Table 1), although these associations were not linear (see text related to Fig. 6 in Section 3.3). A multiple regression analysis of daily thermal amplitude as a function of daily minimum and maximum temperatures in our experiment showed that the minimum temperatures explained 69% of the model, while the maximum temperature contributed only 30% (data not shown). A re-analysis, using multiple linear regression of the relationships between thermal amplitude and minimum and maximum temperatures of the database that included five sites and years in La Rioja Province used by Rondanini et al. (2011, 2014) and for the fruit growth subphase equivalent to the one used in our experiment, showed that minimum temperature contributed with 87% to the variability in the thermal amplitude, compared with a contribution of only 12% for maximum temperature. This contrast suggests that the contribution of maximum and minimum temperatures to thermal amplitude in our experiment were consistent with those found in olive-producing areas of the region. Thermal amplitude is of interest in the light of the frequent producer comments that locations or years of greater thermal amplitude are related with a higher quality of the oils obtained.

In summary, our results contain robust pointers to the fact that mean daily thermal amplitude and mean daily minimum temperature are dimensions of the daily temperature oscillation that influenced the proportion of oil in the dry weight increment of the fruit in sub-phase IV of fruit growth, although mean daily maximum temperature was also influential (Fig. 3). By contrast, the proportions of oleic and linoleic acids in the oil accumulated during that subphase were strongly influenced by thermal amplitude and specially by daily minimum temperature, with daily mean temperature showing a weaker relationship (Figs. 4–6), and no effect of daily mean maximum temperature was found. In this context, recent reports indicating that the enzymatic reversion of the far-red form of phytochrome to its red form may act as a thermo sensor for night temperatures (Legris et al., 2016; Jung et al.,

2016) may be significant. Both minimum temperature and thermal amplitude would affect the range and the trajectory of the temperatures experienced by the plant during the nighttime.

We suggest that further work on the temperature responses of olive fruit dry weight accumulation, oil content and fatty acid content of the oil should pay due attention to the issues of daily minimum temperature and daily thermal amplitude. The results presented here also need to be expanded to cover the whole of the fruit growth phase, at a whole-plant rather than fruiting branch level (something which would allow the responses of rates of fruit dry weight increment and duration of fruit growth to be measured), and explore these issues in a range of cultivars of known differential response to temperature. If the indications of the importance of minimum daily temperature and of daily thermal amplitude in determining crop responses are replicated at whole-plant level for some or many olive varieties, then this would allow for a better selection of planting areas on the basis of historical meteorological records and allow for an improved understanding of the effects of abnormally high temperature events on fruit oil content and quality.

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