Responses to temperature of fruit dry weight, oil concentration, and oil fatty acid composition in olive (Olea europaea L. var. ‘Arauco’)

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Correlative studies in olive using data from different locations or years suggest that temperature can modulate crop oil yield and oil composition. However, there are no published studies of manipulative experiments that demonstrate a direct role for temperature as a regulator of oil yield and oil quality in olive. The objectives of this study were to: i) elucidate the effect of temperature during the fruit oil accumulation phase on fruit dry weight, oil concentration and fatty acid composition; and ii) identify the developmental window within the oil accumulation phase exhibiting the greatest sensitivity to temperature and that with the highest fruit capacity to recover from the temperature treatments. Two branch-level experiments were conducted in a commercial orchard at Los Molinos (La Rioja, Argentina) using var. ‘Arauco’. Both experiments were conducted during the oil accumulation phase by enclosing fruiting branches in transparent plastic chambers with individualized temperature control. The first experiment; known as the four month long experiment, employed four temperature treatments that were applied for a single period of four months: a control at ambient temperature, two heating levels (5°C and 10°C warmer than the control), and a cooling level (5°C cooler than the control). The second experiment consisted of four separate successive one month long treatment periods, in each of which two temperature treatments were applied: control and heating (ca. 7°C higher than control). In the four month long experiment, fruit dry weight was not affected by average temperatures in the 16–25°C range, but it was reduced with further increases in temperature. Oil concentration decreased linearly at 1.1% C−1 across the whole range (16–32°C) of average seasonal temperatures explored, while oleic acid concentration decreased 0.7% C−1 over the same range. In the one month long experiment, 30 days of temperatures ca. 7°C above ambient had a permanent negative effect on oil concentration at final harvest, particularly when the exposure to high temperature took place at the beginning of oil accumulation. By contrast, oleic acid concentration at the end of the treatment interval fell with increasing temperature but it could recover after treatment was removed in all periods except the first one. These results show that high temperatures during the oil accumulation phase may negatively affect olive oil yield and quality in warm regions, particularly if the high-temperature event occurs early in the phase. Additionally, the response of oleic acid concentration (%) to temperature under our experimental conditions was found to be opposite to that of many annual oil-seed crops.

1. Introduction

Olive (Olea europaea L.) is a widely cultivated species well suited to areas with Mediterranean climate. Olive oil provides health benefits due to its content of phenols and a high percentage of monounsaturated oleic fatty acid. Oil is mechanically extracted from the mesocarp (~97% of the total), with a small contribution from the seed (~3%). The fruit growth lasts 5–7 months approximately, and includes 5 phases (Conde et al., 2008). After fruit set, the embryo cell division and growth begin simultaneously with the deposition of oil in this tissue, followed by the development of the endocarp (phases 1 and 2 respectively). Subsequently, endocarp hardening (phase 3) marks the start of the period of major cell expansion and active oil accumulation in mesocarp (phase 4). Most of the oil accumulation occurs during phase 4, although there is a small additional contribution during the ripening phase (phase 5).

The expansion of olive orchards to extra-Mediterranean regions extends the range of environmental conditions under which olive fruit growth and quality can be evaluated, and information
generated under these novel conditions may be of use in gaining insight into likely crop responses in the more traditional regions of cultivation under scenarios of future climate change. For example, in the NW of Argentina temperatures are higher than in the Mediterranean Basin particularly in winter and spring, causing earlier flowering (Gómez del Campo et al., 2010). In addition, the timing of other phenological stages is moved forward in the season, so pit-hardening occurs at the end of the spring and oil accumulation occurs under the hot summer conditions (Cherbiy-Hoffmann et al., 2013). Under these conditions oil yields in some varieties are lower than those in the Mediterranean Basin (Cherbiy-Hoffmann et al., 2012). Additionally, for some varieties the oleic acid concentration of the oil is below the limits (55–83%) proposed by the International Olive Oil Council (IOOC, 2001) for extra virgin olive oil, while palmitic, linoleic and linolenic acids exceed the proposed upper limits (20, 21, and 1%, respectively).

Temperature regulates growth and development in plants. Temperature effects on seed growth (Sofield et al., 1977; Wardlaw et al., 2002) are well documented in annual crops, including oil-seed members of this group e.g., sunflower (Chimenti et al., 2001). Also in sunflower, seed oil concentration decreased in response to high temperatures during the period of oil synthesis (Harris et al., 1978), and an even 7 days of heat stress were enough to reduce the duration of the oil synthesis phase of the fruit (Rondanini et al., 2006). In a correlative study in olive, the duration of the fruit growth phase was shown to be reduced by high temperature while no effect of temperature on fruit growth rate was detected (Tretnacoste et al., 2012). In addition, a negative relationship between oil synthesis duration and temperature was found. In this correlative study, data from different years and varieties were used, covering a narrow range of variation in average temperature (29.5–31.5 °C). Direct experimental manipulation over a broader range of temperatures would help to clarify the final oil concentration response of olive fruit to this factor.

The fatty acid profile of olive oil is an important quality attribute and is used to verify the genuineness origin (IOOC, 2001). Variety is the main determinant of fatty acid composition, but environmental factors such as climatic conditions have been linked to variations in quality. Changes in quality between years (Beltrán et al., 2004; Lombardo et al., 2008), or locations (Tous and Romero, 1994) have been attributed to climatic differences. Oleic acid concentration, the principal fatty acid in olive fruit, generally decreases as latitude or altitude decreases (e.g., Spain, Tous et al., 1997; Australia, Maier et al., 2010; Argentina, Ceci and Carelli, 2010; Italy, Orlandi et al., 2012). This behavior contrasts with what is reported for annual oil-seed crops, where lower latitudes have been associated with high oleic acid oils, a response that has been attributed to temperature effects (e.g., sunflower, Seiler and Brothers, 1999; Peryra-Irujo and Aguirrezábal, 2007; soybean, Maestri et al., 1998). When temperature was artificially manipulated, high oleic acid concentration in sunflower was shown to be associated with increased temperature (Izquierdo et al., 2006).

In olive, a negative relationship between oleic acid concentration in oil for var. ‘Arbequina’ fruit and mean seasonal temperature was found in a correlation study that included field data from different locations and years (Rondanini et al., 2011). By contrast, at the transcriptional level it has been demonstrated that the expression of three oleate acid desaturase (FAD) genes decreased after 24h of exposure of detached fruiting olive branches at high temperature (35 °C) (Hernández et al., 2011). It was expected that this decrease in transcription would be associated with an increase in the oleic acid content and the corresponding decrease in linoleic acid, but no changes were observed. More studies at both laboratory (short-term, biochemical and gene expression) and field (long-term, whole fruit) levels and combinations of these two approaches involving direct manipulations of temperature are needed to better understand olive fruit temperature responses.

Fatty acid composition can also vary depending on the timing of the occurrence of the high temperature event. For example, in sunflower (Rondanini et al., 2003), when high temperature was applied during the final portion of oil accumulation phase the proportion of oleic acid increased while that of linoleic acid decreased. On the other hand, when high temperature was applied at the beginning of the oil accumulation phase no effect on fatty acid composition was observed due to fruit capacity to recover to normal levels once the stress was removed. It is important to note that in sunflower, as in other annual crops, oil accumulates in the seed during a short period of time (between 30 and 45 days), and at a high rate. On the other hand, in the olive fruit as in those of oil-palm and avocado, oil accumulates principally in the mesocarp over a long period of time (100 to 140 days) at a low rate. Thus, it is possible that greater opportunities for recovery to normal values after a high-temperature event might exist in olive. At present, there is no information about the effects of exposures to shorter periods such as 30 days of high temperature during the long oil accumulation phase of the olive fruit.

In summary, currently available information about the effects of temperature on olive oil concentration and fatty acid composition is based on correlative field studies using data from different years and/or locations; and results from short-term transcriptional studies. There is no information available on the effects of direct exposure to high temperature over weeks or months under field conditions. The objectives of our manipulative experiments were to: 1) elucidate the effect of average temperature throughout most of the oil accumulation phase on fruit dry weight, oil concentration and fatty acid composition; and 2) identify, within phase 4, one month long windows of time exhibiting the greatest sensitivity to increased temperature of fruit dry weight, oil concentration and fatty acid composition and those with the greatest capacity for recovery after exposure.

2. Materials and methods

2.1. Experimental site and temperature treatments

Two experiments were conducted in 2009 and 2010 at Los Moli- nons (28° 43’ S, 66° 56’ W, 1400 m above sea level), La Rioja province, 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 fertilized with 40 kg of goat manure per plant at pit hardening stage. The variety Arauco (Barranco et al., 2000) was selected because a previous study (Rondanini et al., 2011) found that the oil from this variety (and that of var. Arbequina) exhibited marked differences in oleic acid concentration across regions differing in temperature. This suggested that Arauco could be a useful experimental model to test the sensitivity of oleic acid concentration in oil to temperature. Two branch level temperature manipulation experiments were conducted during phase 4 of fruit growth, in which most mesocarp oil accumulation occurs (Table 1). In both experimental seasons the dates of pit hardening were determined by attempting, at two-day intervals commencing 45 days after flowering, to slice the developing fruit with a sharp knife. Pit hardening was considered to have occurred when it proved impossible to slice the endocarp of the sampled fruit right through.

A four month long experiment was performed to evaluate the responses to temperature of fruit dry weight, oil concentration and fatty acid composition. Fruiting branches approximately 20 cm long
Table 1

Experiment duration, moment of application, treatment code and mean temperature during the treatments period for the “four month long” and “one month long” experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Duration of thermal treatment</th>
<th>Interval of temperature manipulation</th>
<th>Treatment code</th>
<th>Mean temperature during the treatment (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four month long</td>
<td>4 months</td>
<td>January–May</td>
<td>T4–</td>
<td>16.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T0</td>
<td>20.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T5–</td>
<td>25.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T10+</td>
<td>30.7 ± 0.6</td>
</tr>
<tr>
<td>One month long</td>
<td>1 month</td>
<td>January–February</td>
<td>T0</td>
<td>23.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T7+</td>
<td>29.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>February–March</td>
<td>T0</td>
<td>22.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T7+</td>
<td>29.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>March–April</td>
<td>T0</td>
<td>21.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T7+</td>
<td>28.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>April–May</td>
<td>T0</td>
<td>16.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T7+</td>
<td>23.5 ± 1.1</td>
</tr>
</tbody>
</table>

were placed in controlled temperature chambers throughout the January 19 to May 13, 2010 interval (i.e., 30–144 days after pit hardening-DAPH). Four thermal regimes were applied: T0 (control), T4– (4 °C cooler than T0), T5+ and T10+ (5 and 10 °C warmer than T0, respectively).

The one month long experiment involved four separate one month long treatments applied during phase 4 of fruit growth, which lasted from January 13 to May 19, 2009 (i.e., 24–147 DAPH). Two thermal regimes were applied: T0 (control), and T7+ (between 6.2 and 7.3 °C higher than control values depending on the period) in each of four separate periods (Table 1). The first period extended from January 13 to February 16 (J–F); the second from February 16 to March 16 (F–M), the third from March 16 to April 21 (M–A), and the last from April 21 to May 19 of 2009 (A–M). At the start of each treatment two fruiting branches were enclosed in each controlled temperature chamber; one of which was harvested at the end of the treatment period (intermediate harvest) and the other at the end of the experiment (final harvest).

In both experiments, experimental design was a randomized complete block with four replicates where a tree was taken as block. A new set of trees was used each year. The treatments (four thermal regimes in the four month long and two in the one month long experiments) were represented in each block. In the one month long experiment the chambers were moved to different branches of the same tree when the previous treatment period finished. In both experiments, we selected external fruiting branches of around 20 cm in length and bearing between 5 and 8 fruit per branch from the South-oriented (±25°) surface of the crown of the trees, at height of 2–3 m. Branches were enclosed in acrylic chambers of 22 × 22 × 10 cm (length, width and height respectively) during the treatment period. The leaf/fruit ratio on the selected branches was adjusted by thinning as necessary to ensure a uniform source/sink ratio across all branches used in an experiment. Air temperature inside each individual chamber was recorded every 15 min using temperature sensors connected to a datalogger (Datalogger, Cavadavices, Argentina).

2.2. Heating and cooling systems

Temperature was manipulated inside each chamber using heating or cooling systems. The heating system consisted of an electronic resistance (Resistor 15W 4.7 Ohm, Cosmos, China) fixed to the inner side of the chamber and a 12 V fan to mix the air inside the chamber. The refrigeration system consisted of two Peltier wafers (mod. TEC1–12706, 14 V 6A 40 × 40 × 3.8 mm, China) fixed to the inner side of the chambers combined with a 12 V fan. The Peltier wafers were connected to a heat dissipater on the outer face of the chamber, and a constant flow of water running along the dissipater helped to enhance the cooling capacity of the system. Control chambers contained only a 12 V fan. All chambers had a 5 × 3 cm hole facing the air-stream produced by the fan and two smaller apertures on the opposite face of the chamber to ensure adequate gas-exchange between the inside of the chamber and the surrounding atmosphere. Sides and bases of the chambers were covered with reflective bubble wrap insulation and a shade cloth (30% transmittance) located 10 cm above the lid was used to reduce radiation receipt and improve the temperature control. Preliminary experiments showed that passive heating in otherwise similar but unshaded chambers could irreversibly damage olive fruits (provoking dehydration and senescence) within 1 h of exposure to direct radiation at midday on a clear summer day in this location. We did not find any indication of heat damage in any of the shaded chambers in any treatment condition.

A central electronic controller (Caja controladora, Cavadavices, Argentina) was used to control the temperature of each individual chamber. All chambers contained a temperature sensor (TC1047A, Microchip Inc., China) connected to the electronic controller that registered the temperature of the air inside the chamber every minute. The controller regulated the flow of 12 v current to Peltier wafers and the resistors in response to data from the temperature sensors. The central controller could be set to ensure a fixed temperature differential between a chamber and the control chamber, ensuring that conditions within the chambers oscillated in tune with the daily cycle in ambient temperature.

2.3. Response variables

Fruit fresh weight and maturity index (MI) was determined at the intermediate (one month long experiment) and at the final harvest (one month long and four month long experiments). MI was estimated for each sample based on an evaluation of skin color using the method of Uceda and Frías (1975) adapted to a smaller fruit sample. Fruit were oven dried at 60 °C until constant weight. Oil concentration was determined on the dry fruit using the Soxhlet technique (IUPAC, 1992) with hexane as solvent. To determine fatty acid composition, oil samples were cold methylated in basic medium (JOC, 2001), and then separated by gas chromatography (PerkinElmer Pregisely Claurus 500, USA). The carrier gas was hydrogen, the injection and detector temperatures were 240 °C and 300 °C, respectively, and the column length was 30 m. Individual fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic acids) were determined by comparison with retention times of known standards (AOCS–1, Sigma–Aldrich, St. Louis, MO) and expressed as percentage of the total amount of fatty acids.

2.4. Complementary measurements and experiment

To determine the timing of the final harvest of treated fruit, the dynamics of fruit growth and oil synthesis was followed in samples
taken every 15 days from equivalent non-treated branches from the same trees. This allowed us to follow the oil accumulation trajectory and determine when the plateaus of fruit dry weight and oil concentration were achieved.

A complementary experiment was carried out in 2011 to evaluate the possible effects of the shade cloth and/or the chamber on fruit dry weight, oil concentration and fatty acid composition. Three fruiting branches per tree (n = 4) were selected with the same criteria applied for the four month long and one month long experiments. One of them was left growing at natural ambient light, other was shaded with the same shade cloth used in both experiments, and other was enclosed in a control chamber.

2.5. Statistical analyses

Treatments effect for variables measured at the final harvest of the four month long and the intermediate harvest of the one month long experiments were assessed using an ANOVA for fixed effects. To assess treatment and period effects at final harvest in the one month long experiment, we used a mixed model ANOVA in which tree, treatment × period and treatment were considered as random effects and period was considered as a fixed effect. Differences among means of treatments were evaluated with the Tukey test (P < 0.05). All analyses were performed using SAS software v8 (SAS Institute, Cary, NC, USA 1999).

In an exploratory analysis, we compared the responses of fruit dry weight, oil concentration, and oil fatty acid composition (percentages of palmitic, palmitoleic, stearic, oleic, linoleic and linolenic) to mean daily (i.e., 24 h), nocturnal (8 pm–8 am), and diurnal temperatures (8 am–8 pm). Different mathematical models (linear, quadratic and bilinear functions) to describe these relationships between the variables were tested. Linear functions provided the best fits (higher R² values (>0.6)) and slopes significantly (P<0.05) different from zero for all variables except fruit dry weight. The fruit dry weight relationship with mean growth temperature was best described by fitting a bi-linear broken-stick function, fitted by least squares method. We found that the relationships of these variables with mean daily temperature exhibited slightly better fits than those with the other categories of temperature tested. Consequently, we report fruit and oil quality responses to mean daily temperature. These analyses and the resulting plots were made with GraphPad Prism version 5.01 software (GraphPad Prism Software, California, USA).

3. Results

3.1. Ambient and experimental temperatures

Seasonal mean air temperatures were 19 °C in the four month long and 20 °C during the four treatment periods in the one month long experiments. Daily mean temperatures decreased as the season advanced, from 24 °C in January to 11 °C in May during the four month long experiment, and from 23 °C to 16 °C between the same months in the one month long experiment (Fig. 1A).

Treatments altered seasonal mean daily temperatures inside the chambers so that the differences between T0 and the remaining treatments were very close to the target differentials, spanning a range between 16.7 °C and 30.7 °C in the four month long experiment (Table 1). The temperature increment relative to T0 in the one month long experiment varied slightly between the periods, with 6.2 °C increment during J–F and 7 °C to 7.3 °C in the remaining three periods (F–M, M–A, A–M) (Table 1). In both experiments the mean temperatures in the T0 treatment were about 2 °C warmer than ambient temperature.

Temperature inside the chambers followed the seasonal pattern of air temperature, becoming cooler as the season progressed (compare Fig. 1B and C with A). Also, treatment temperatures tracked the daily fluctuations in T0 rather well, as illustrated in Fig. 2 for a single day during the four month long experiment. Across the day, temperature reached its maximum value around 15 h solar time and its minimum before dawn. Temperature differences between treatments were consistent throughout the 24 h period (Fig. 2) and during the whole experiment. Similar results were observed during the four treatment periods in the one month long experiment (data not shown).

The passive heating of the shaded chamber was enough to significantly (P>0.05) affect oleic acid concentration of the oil in the
complementary experiment (Table 2). On the other hand, shading alone did not affect oleic acid concentration with respect to that of the fruit of branches in ambient light. Fruit dry weight and oil concentration were not significantly ($P>0.05$) affected by shading alone or by enclosure in the shaded chamber in these branch-level manipulations (Table 2).

### 3.2. Seasonal dynamics of fruit growth on untreated branches

The seasonal dynamics of fruit dry weight, oil concentration, and fatty acid composition of fruit from untreated branches during the *four month long* experiment showed that during the interval between 30 and 116 DAPH, fruit dry weight increased linearly from 0.75 g to 1.75 g, oil concentration followed a similar trajectory, increasing from 17 to 44% while oleic acid percentage fell from 75% to 66% (Fig. 3). Final harvest was delayed until 144 DAPH to ensure that fruit of the T4− treatment had reached their maximum dry weight. As shown in Fig. 3, control fruit dry weight stabilized around 116 DAPH.

### 3.3. Four month long alterations in temperature regime

Treatments were applied over the whole of the interval between 30 and 144 DAPH. At harvest, maturity index was similar between treatments at around 1.5 ($P>0.05$, data not shown). Fruit dry weight was not affected by temperature in the 16–25 °C range, but as temperature increased beyond this range, fruit dry weight decreased 0.08 g °C$^{-1}$ (Fig. 4A). By contrast, fruit oil concentration was more sensitive to temperature exhibiting a fall of 1.1 percentage points of oil °C$^{-1}$ over the whole temperature range explored (Fig. 4B). Consequently, the final oil content per fruit (g oil fruit$^{-1}$) was different between treatments, with 0.35 g oil fruit$^{-1}$ in T10+ and 0.80 g oil fruit$^{-1}$ in T4− ($P<0.05$, data not shown).

### Table 2

<table>
<thead>
<tr>
<th>Fruit growing conditions</th>
<th>Dry weight (g)</th>
<th>Fruit oil concentration (%)</th>
<th>Oleic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient light</td>
<td>1.71 ± 0.13</td>
<td>31.8 ± 4.69</td>
<td>74.9 ± 0.6a</td>
</tr>
<tr>
<td>Shade cloth only</td>
<td>1.70 ± 0.09</td>
<td>31.3 ± 2.74</td>
<td>74.9 ± 0.5a</td>
</tr>
<tr>
<td>Chamber + shade cloth</td>
<td>1.69 ± 0.26</td>
<td>37.1 ± 3.34</td>
<td>72.5 ± 0.7b</td>
</tr>
</tbody>
</table>

Principal fatty acid composition of the oil at final harvest was also modulated by temperature across the whole of the temperature range explored. Oleic acid concentration decreased linearly 0.7 percentage points °C$^{-1}$ (Table 3), with an 11.2 percentage point difference between the two extreme temperature treatments (i.e., T4− and T10+) (Fig. 5A). The remaining fatty acids: palmitic, palmitoleic, linoleic and linolenic acids increased with increasing temperature, while stearic acid showed a slight decrease (Fig. 5B and C). The change in the saturated fatty acids palmitic and stearic was 0.2 and 0.05 percentage points °C$^{-1}$, respectively (Table 3), with the strong increase in palmitic outweighing the
The slight decrease in stearic (Fig. 5B and C); while the combined increase of the polyunsaturated fatty acids (linoleic and linolenic) was 0.36 percentage points °C⁻¹ (Table 3).

3.4. One month long alterations in temperature regime

Fruit dry weight was not significantly affected by a 30-day period of increased temperature in any of the treated periods (Fig. 6A). On the other hand, oil concentration was significantly (P < 0.05) lower in T7+ than in T0 when treatments were imposed in J-F and M–A (Fig. 6B), and the effects in the remaining two treatment periods were consistent with this response although treatment effects were not statistically significant. Oleic acid percentage was reduced (P < 0.05) by exposure to higher temperatures in the three earliest treatment periods (Fig. 6C).

Table 3: Functions fitted to the relationships between the proportions of major fatty acids in oil from fruit harvested at the end of the oil accumulation phase and mean growth temperatures (MGT) applied from January 19 to May 13 2010 (harvest). Treatments started 30 days after pit hardening. Data from the four month long experiment.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Linear regression equation</th>
<th>R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic (16:0)</td>
<td>11 + 0.20 × MGT</td>
<td>0.81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>0.3 + 0.05 × MGT</td>
<td>0.81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>2.8 – 0.01 × MGT</td>
<td>0.31</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>77.5 – 0.69 × MGT</td>
<td>0.77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>7.4 + 0.31 × MGT</td>
<td>0.60</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
<td>−0.2 + 0.05 × MGT</td>
<td>0.87</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Fig. 6. Effects of a temperature increase (6–7 °C above control) during one-month intervals on fruit dry weight (A), oil concentration (B) and oleic acid concentration in oil (C) in fruits harvested at the end of each treated interval. Treatments were: control (T0), and heating (T7+); and they were applied from January to May 2009 (24 to 147 days after pit hardening). Dotted horizontal lines indicate values at the beginning of the treatment period. Bars are means and capped vertical lines 1 S.E (n = 4). Different letters above bars indicate significant differences (P < 0.05) between treatments within a particular period. Data from the one month long experiment.
praisingly, increasing temperature by 6.2 °C (T7+) over T0 during J-F and then letting fruits grow at ambient temperature until final harvest still caused a drop of 6 percentage points in final fruit oil concentration (P < 0.05). Also, final fruit oil concentration fell 3.5 percentage points for treatment T7+ in F-M (55–80 DAPH), 6 percentage points in M-A (80–119 DAPH), and 3 percentage points in A-M (119–147 DAPH) relative to T0 (P < 0.05). These results suggest a persistent negative effect of exposure to higher temperatures on final fruit oil concentration.

Exposure to higher temperatures in the J-F period also altered final harvest fatty acid composition, with oleic acid concentration being 2.3 percentage points lower and linoleic acid 1.1 percentage points higher in T7+ than T0 when treatment were applied during J-F period (P < 0.05) (Table 4). These effects were not significant for later exposures to high temperature. The proportions of palmitic, stearic and linolenic acid were unaffected by treatment in all four treatment periods.

### 4. Discussion

As cultivation of olive expands outside of the traditional Mediterranean Basin, increased understanding of how the crop responds to environmental conditions is necessary. This greater understanding should prove useful for the prediction of possible changes in olive oil yield and quality under expected future scenarios of global warming and to explain variations in oil quality across regions. The use of 1-month and 4-month exposures to altered temperature in the two experiments allowed a more nuanced understanding of crop responses. One month alterations at any time during the main fruit growth period reduced fruit oil concentration at final harvest, but only affected the final proportion of oleic acid in oil when applied early (Table 4), even though responses to higher temperature for both variables could be detected at the end of the one-month exposure (Fig. 6). The results of the four-month exposure allowed for the construction of response curves to seasonal temperature for the entire oil accumulation period for fruit dry weight, oil concentration and fatty acid proportions in oil (Figs. 4 and 5). These can be used to predict variations in oil yield and quality for var. ‘Arauco’ between locations and seasons of differing seasonal temperature regimes.

The experimental system used allowed daily and seasonal variations in temperature to be followed in the field, something much more realistic than the constant temperatures often used in growth chambers. Some temperature manipulation experiments have been made in other fruit species for whole plants, but in these only small temperature increases (1–2 °C) were compared to controls (e.g. Atkinson et al., 1998; Sadras et al., 2012). The experimental system explored a wide range of temperature (from 16 °C to 32 °C mean growth temperature) as was previously done for oil-seed crops (e.g. Chimenti et al., 2001). The chamber design permitted the enclosure of two branches with 5–8 fruit each, which was enough to quantify the measured variables accurately. It could be argued that, due to shading, the leaves within the chamber had lower carbon assimilation rates than those associated with fruit growing outside the chamber, but a complementary experiment showed no inherent disadvantage of the methodology for fruit final dry weight or oil concentration (Table 2). As inferred from published olive leaf net carbon assimilation light response curves, leaf photosynthesis inside the chamber was likely within 50% of photosynthesis at light saturation (Bongi and Long, 1987). It is also possible that photosimilates from nearby shoots could have also contributed to fruit growth (Proietti, 2003).

Under our experimental conditions, fruit dry weight was stable across a wide range of mean growth temperature (16–25 °C), but was negatively affected above 25 °C (Fig. 4A). This is consistent

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with findings from Trentacoste et al. (2012) based on an analysis of fruit growth in ten varieties over two years. They found no relationship between fruit fresh weight and temperature across the narrow range of mean seasonal temperature explored (18.9–19.7 °C). Over a broader range, no temperature response was seen in our study below 25 °C (Fig. 4A). A correlative study that analyzed different varieties and environments (a range of mean seasonal temperatures between 23 °C and 27 °C) showed that variations in final fruit weight were better explained by variations in growth rate than in duration (Rondanini et al., 2014) and no relationship between fruit growth rate and temperature were observed. It would be useful, in future experiments, to investigate whether the relationships between the responses to temperature of final fruit weight which we found (Fig. 4, Table 4) can be linked to variations in duration and rate of fruit growth. These variables have been shown to respond to temperature in other species (sunflower, Chimenti et al., 2001; Rondanini et al., 2006; soybean, Egli and Wardlaw, 1980; wheat, Wardlaw and Moncur, 1995; apple Atkinson et al., 1998; Warrington et al., 1999). Final fruit dry weights in the control treatments varied between years (Fig. 4A and Table 4), but no significant differences were found in final fruit dry weight from the non-treated branches (data not shown). Those inter-year variations in fruit weight observed in the controls may reflect differences in the fruit growing phase at which treatments were applied and the duration of the treatments.

The experiments showed that oil concentration was sensitive to temperature increases during the period of active oil accumulation, under both long (Fig. 4B) and short (Fig. 6B, Table 4) exposures to high temperature. Oil concentration decreased slightly per °C increment in the range between 16 and 32 °C. This finding is consistent with the results of a correlative analysis in which oil accumulation was analyzed for 6 varieties, at three locations and over two years (Rondanini et al., 2014), which showed that fruit oil concentration was negatively associated with average temperature. Trentacoste et al. (2012) also found a negative relationship between duration of fruit oil accumulation and maximum daily temperature, within the narrow range of temperatures (29–31.5 °C) explored in their correlative work. Because the experiments involved direct manipulation of temperature, excluding other site- or year-influenced factors that might affect the results of correlative analyses, temperature appears to be a crucial factor in determining oil yield and quality. In our work, the negative effect of high temperatures during most of the mesocarp oil accumulation phase was further confirmed by the results of the one-month exposure. Surprisingly, reductions in oil concentration after a short-term exposure to higher temperature persisted until final harvest after three (J-F exposure), two (F-M exposure) or one (M-A exposure) months under normal ambient temperature (Table 4). Exposure during J-F produced the biggest drop in oil concentration at final harvest despite the fact that only 12% of final oil content (g fruit−1) was synthesized by the middle of February (Fig. 6B). This result is in agreement with observations in sunflower where final oil concentration was reduced when temperature was >35 °C for 7 days in the early phase of oil accumulation (Rondanini et al., 2003).

Little is known about the enzymatic activity or gene expression linked to lipid biosynthesis in the fruit. In olive, increasing the temperature of callus cultures from 20 to 30 °C accelerated the synthesis of diacyl and triacylglycerol (Ramali et al., 2002), but further increases in temperature strongly reduced the synthesis of TAG in olive mesocarp (Salas et al., 2000). Taken together, the results from our two experiments show that final fruit oil concentration decreased linearly with heat load, which is a measure of heat stress exposure in plants (Rondanini et al., 2003), estimated as the number of hours with temperature >30 °C. Processes indirectly linked to oil synthesis such as photosynthesis or fruit respiration could also be simultaneously modulating the oil concentration decrease with increasing temperatures. Although it was not measured in our experiments, photosynthesis of both leaves and fruit are likely to have been negatively affected by exposure to high temperatures. Increases in leaf temperature above 32 °C in the chambers likely resulted in a decline in photosynthetic rate (Bongi and Long, 1987). Concomitantly, respiration could increase with temperature as the fruit exhibits an active respiratory rate during ripening (Ranalli et al., 1998). Further work is required to elucidate possible causal relationships between the response of oil concentration to temperature and these candidate processes.

Olive oil fatty acid profile is mainly determined by genotype although environmental factors do influence it. Differences between varieties in the dynamics of oleic acid content during oil accumulation have recently been reported under our climatic conditions (Rondanini et al., 2014), and a direct effect of temperature on oil fatty acid composition was found in this study for var. ‘Arauco’ (Fig. 5). Arauco shows a sharp decrease in oleic acid content over the course of the season, similar to Arbequina, which may suggest that Arbequina would respond similarly to temperature (Rondanini et al., 2014). However, it is likely that a range of responses among different varieties occurs. This possibility is supported by observations of differences in transcript levels of FAD genes between varieties in response to environmental factors (Hernández et al., 2009, 2011). It should be noted that many previous studies have suggested a negative correlation between fatty acid content and temperature based on data from different locations or years (Maier et al., 2010; Ceci and Carelli, 2010; Rondanini et al., 2011; Lombardo et al., 2008; Orlando et al., 2012). Increasing temperature reduced the oleic acid concentration 0.7% per °C and increased that of palmitic, palmitoleic, linoleic and linolenic acid, while slightly lowering that of stearic (Table 3). Moreover, it was found that periods of 30 days at high temperature were enough to reduce the oleic acid proportion at the end of the treatment period in the three earliest treatment periods (Fig. 6C). Interestingly, a recovery was found after the treatment was removed in all periods, except J-F for which the effect persisted (Table 4). A similar “memory effect” was reported for oleic acid in sunflower after an early temperature treatment (from flowering to 200 °C day), although the sign of the response was opposite to that found in olive (Izquierdo et al., 2002).

It is important to highlight the contrast in the responses of oleic acid to temperature in olive and those of annual oil-seed crops. For example, the observed reduction in FAD transcripts in response to short term (i.e., 24 h) exposure to high temperature in olive is consistent with the responses found in oil crops that accumulate oil in embryo tissues (Hernández et al., 2011), but contrary to the observed long-term responses in the field for olive which are reported here. Thus, it is possible that the mechanism by which temperature modulates fatty acid composition in the olive mesocarp in the field would be better explained by post-transcriptional changes and/or by substrate availability. The results of this study could be a starting point for future research that could help understand the mechanism behind this novel fatty acid temperature response in the olive fruit. Some potential questions are: 1) does the olive embryo show an oil quality temperature response similar to that of herbaceous oil-seed crops?; 2) do other mesocarp oil crops (e.g., avocado, oil-palm) show an oil quality temperature responses similar to that of olive?. In addition, in annual oil-seed crops it has been demonstrated that night temperatures better explain the oil fatty acid composition than daily mean temperature (sunflower: Izquierdo et al., 2006; Peryra-rujo and Aguirrezabal, 2007; soybean: Gibson and Mullen, 1996). The results reported here only tested fixed temperature increases with respect to the control throughout the day (i.e., the daytime and night-time temperatures covaried). Further experiments in which day and night temperatures are manipulated independently are currently underway.
In summary, the two experiments in this study directly manipulated temperature during the growth of the olive fruit and demonstrated that temperature during fruit growth in the 16–32 °C range, negatively impacted fruit oil concentration and the proportion of oleic acid in the oil. The various one-month exposures to increased temperatures reduced oil concentration as did the longer 4-month treatment, but changes in oleic acid proportion were limited to exposures in the early stages of rapid oil accumulation. These results point to the existence of some persistent effects of high temperature after its removal. Fruit dry weight exhibited a threshold (>25 °C) response to prolonged exposure to temperatures in the 16–32 °C range, with a sharp fall in weight above the threshold, but short-term exposures evoked no response in fruit dry weight. Along with previous correlative results, our experiments corroborate that the responses of oil quality to temperature in olive are different than those found in some annual oil-seeds.

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