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Opposite oleic acid responses to temperature in oils from the seed and mesocarp of the olive fruit

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ABSTRACT

Olive oil is mostly extracted from the mesocarp (~95%) of the fruit with the seed (endosperm and embryo, ~5%) containing little oil. There are correlative and manipulative evidence that temperature modulates fruit oil content and fatty acid composition of the oil from the whole fruit (i.e., with no distinction being made between oils derived from each oil-bearing structure) of olive. Notably, oleic acid concentration of olive oil decreases as fruit mean growth temperature increases. This response in the olive fruit is opposite to that documented in annual oil-seed crops such as sunflower and soybean. The objectives of the present study were: i) to compare temperature effects on fatty acid composition of oil derived from seed and from mesocarp; ii) to compare temperature effects on seed and mesocarp dry weights and oil concentrations. To do this, fruiting branches were enclosed in transparent plastic chambers with individualized temperature control. Temperature was manipulated during the seed growth (Period A) and during the second half of mesocarp growth (Period B) subphases. In both periods, the oleic acid proportion in mesocarp oil decreased as temperature increased, and was accompanied by increases of palmitic acid, linoleic and linolenic acids. Mesocarp dry weight did not respond significantly to temperature, but mesocarp oil concentration fell significantly as temperature increased. Seed dry weight, oil concentration and fatty acid composition exhibited responses to temperature during Period A only, with seed dry weight increasing between 20 and 25 °C with a sharp decrease at higher temperature, and oil concentration linearly falling 1.2% per °C. In contrast, seed oil oleic acid percentage increased between 20 and 28 °C, and fell slightly with higher temperature. Palmitic and stearic acids in seed oil increased sigmoidally with temperature, while linoleic acid decreased sigmoidally. Oleic acid percentage showed opposite responses in oil from the seed and the mesocarp. The response of the seed to temperature was similar to those observed in oil from embryos of annual oil-seed crops, although the abrupt fall in palmitic and stearic acid with temperature >25 °C seems to be distinctive for olive seed oil.

1. Introduction

Olive oil comes mostly from the mesocarp (epicarp and fleshy mesocarp, ~95%) with a small contribution from the seed of the fruit (endosperm and embryo, ~5%) (Conde et al., 2008). Within the seed, most of the oil is present in the embryo (Rapoport and Gómez del Campo, 2008). Fruit growth takes about 4–6 months, and seed and mesocarp growth are asynchronous, with seed growth being completed at the time the mesocarp has only achieved about half its final weight. Within the fruit growth phase, two sub-phases can be distinguished based on the intensity of cell division (Hammami et al., 2011). Period 1 (from bloom to 8 weeks after bloom) is characterized by active cell division. Approximately 66% of final cell number is generated during this period and 25% of cell size is achieved, with fruit transverse area reaching 34% of its final value. During Period 2 (from 8 to 32 weeks after bloom) cell division rate is lower and the remaining 75% of cell size increase takes place. Concomitantly, the endocarp cross-sectional area (which includes the endocarp and the enclosed seed) expands exponentially from soon after bloom to 8 weeks after bloom while the mesocarp cross-sectional area increases constantly and substantially from bloom to maturity (28 weeks after bloom).

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The parental origin of mesocarp and seed tissues is different. The pericarp (including epicarp, mesocarp and endocarp) is formed from ovary tissues of the flower (maternal tissues), while most of the seed tissues are generated after fertilization. With regard to oil storage some structural differences between these tissues have been reported. Seed oil contains oleosin, an oil-body protein that is typically present in oil from annual oil-seed crops (e.g., sunflower, safflower, maize, soybean, rapeseed, etc., Tzen et al., 1990) but this protein is not found in mesocarp oil (Ross et al., 1993). An early review (Hilditch and Williams, 1964) of fatty acid composition of oils from different species reported similar proportions of some fatty acids in both mesocarp and seed oils of olive. Palmitic was 7–15% in mesocarp and 6% in seed, while oleic was 70–85% and 83%, and linoleic was 4–12% and 7% in mesocarp and seed respectively. At a molecular level, some studies have revealed differences between these structures (i.e., mesocarp and seed) in the expression levels or in the timings of maximum expression of genes that encode fatty acid desaturase (FAD) enzymes during fruit growth (Poghosyan et al., 1999; Banilas et al., 2005;). In contrast to this considerable body of information on the adaptive, structural and enzymatic differences between these two oil-bearing fruit structures, there is a knowledge gap relating to the effects of temperature, a major environmental factor, on them. This gap is particularly marked in relation to the seed.

Correlative studies in olive, based on surveys of fruit produced in locations with different temperature regimes, have shown that whole-fruit dry weight is related more strongly to fruit growth rate than to fruit growth duration (Rondanini et al., 2014; Trentacoste et al., 2012), but the role of controlling factors, like temperature, behind this response are uncertain. A temperature manipulation study showed that final fruit dry weight is affected by temperatures higher than 25 °C, but no temperature effect was detected with average seasonal temperatures in the 16 and 25 °C range (García-Inza et al., 2014). In the oil-seed crop sunflower, temperature increases embryo growth rate up to a maximum (25 °C) while duration falls as temperature increases (Chimenti et al., 2001). In olive, fruit weight is important because of its relation with fruit caliber when the destination is Table olive, while oil accumulation is a key determinant for oil production. Correlative evidence using different varieties, years and locations showed a negative relationship between fruit oil concentration and seasonal mean daily temperature in the 23 − 27.5 °C range (Rondanini et al., 2014) and fruit oil concentration in the cultivar Arauco decreased 1.1% per °C of temperature increment in the 16 °C to 32 °C range in a temperature manipulation experiment (García-Inza et al., 2014).

Fatty acid proportion is an important attribute of edible oils and for olive oil its values for commercialization are regulated by the International Olive Oil Council (IOOC, 2013). To qualify as extra virgin olive oil, oleic acid levels in the oil must be within the limits of 55 and 83%, palmitic between 7.5 and 20%, linoleic between 3.5- and 21%, and linolenic must be ≤1%. Oil fatty acid composition is affected by the variety (Uceda and Hermoso, 2001), but the environment and genotype x environment interactions can also influence it (Rondanini et al., 2011). Temperature is one of the environmental factors that modulate fatty acid composition in olive oil and other oil-seed crops. There are correlative (Lombardo et al., 2008; Mailer et al., 2010; Orlandi et al., 2012; Rondanini et al., 2014) and manipulative evidence (García-Inza et al., 2014) in the field that show that oleic acid decreases in oil from the whole fruit as temperature increases. This response is opposite to that generally found in crops that accumulate oil in the seed. In sunflower (Canvin, 1965), maize and soybean (Zuil et al., 2012 and references cited therein) high temperature during fruit growth is associated with an increase in oleic acid concentration. This differential response to temperature could be due to the different fruit structures involved (principally mesocarp in olive and seed in oil-seed crops). The objective of the present work was to elucidate the effect of temperature on olive seed and mesocarp dry weights, oil concentrations and oil fatty acid composition of oils from both oil-bearing fruit structures. To do this, advantage was taken of the fact that seed oil accumulation is completed about a half of the way through the fruit growth phase, while oil continues to accumulate in the mesocarp almost right through to fruit maturity.

2. Materials and methods

2.1. Experimental site and temperature treatments

The experiment was conducted in 2011 at Los Molinos (28°43′ S, 66° 56′ W, 1400 m above sea level (masl)), 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. Mean daily solar radiation during Periods A and B of our experiments were 22.2 and 18.9 MJ m^{-2} d^{-1} respectively, similar to regional averages for equivalent periods (21.8 and 20.8 MJ m^{-2} d^{-1} for La Rioja (420 masl) and Aimogasta (800 masl), respectively, for the 2009–2012 period).

The cultivar Arauco (Barranco et al., 2000) was used for this study. More details on the experimental site and characteristics of the cultivar can be found in García-Inza et al. (2014). Full bloom was registered on 24 October 2010 and pit hardening on 30 December 2010. The latter was considered to have occurred when it proved impossible to slice the endocarp of the sampled fruit right through with a sharp knife (see García-Inza et al., 2014 for further details).

The temperature manipulation experiment involved two sub-phases of fruit growth. Period A, covered from 25 November 2010 to 23 February 2011, when most of the seed oil accumulation phase and the initial sub-phase of mesocarp oil accumulation occurred. Period B, covered from 1 March 2011 to 13 May 2011, including pit hardening to final harvest interval, thus including the second sub-phase of mesocarp oil accumulation. These treatment periods were selected to probe possible differential responses to temperature on seed and mesocarp fruit structures. During each of the two Periods four thermal levels were applied: a control at ambient temperature, two heating levels (5 °C and 10 °C warmer than control), and a cooling level (3 °C cooler than the control). 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. Once the Period A treatment was completed, the chambers were moved to different branches of the same tree to begin the Period B treatment. In both Periods, we selected external fruiting branches of around 20 cm in length bearing between 5–8 fruit per branch from the South-oriented (± 25 °) surface of the crown of the trees, at 2–3 m height. The proportion of total fruit on the trees involved in the experiment represented only about 0.34% of the fruit production of the tree (data not shown). Additionally, chambers were widely spaced in the canopy of the tree, and treated fruiting branches were positioned on different main branches (i.e., a branch older than 4 years age). Proietti and Tombesi (1996) noted that main branches on an olive tree are largely independent. Both the very small proportion of fruit involved in the experiment and the spacing between treatments make independence between treatments probable and compensation among them unlikely. Selected branches were enclosed in acrylic chambers of 22 × 22 × 10 cm (length, width and height respectively) during the treatment Period. 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 (see García-Inza et al., 2014 for further details about the chambers). 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 minutes using temperature sensors connected to a datalogger (Datalogger, Cavadevices, Argentina).

2.2. Heating and cooling systems

Temperature was manipulated inside each chamber using heating or cooling systems. The chambers and the heating/cooling system were identical to those described in García-Inza et al. (2014). Briefly, a central electronic controller (Caja controladora, Cavadevices, 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 15 minutes. The controller regulated the flow of 12 V current to Peltier thermoelectric heat pumps or to resistors in response to data from the temperature sensors and pre-set temperature differentials with respect to the control chamber. This arrangement ensured that conditions within the chambers oscillated in tune with the daily cycle in ambient temperature and, at the same time, maintained the temperature differential with respect to the control chamber, a procedure much more realistic than the constant temperatures often used in growth chambers. Previous temperature manipulation experiments have been made using whole plants of other species, but in these only small temperature increases (1–2 °C) were compared to controls (e.g. Atkinson et al., 1998; Sadras et al., 2012). Our experiment system explored a wide range of mean daily temperature (20.0–30.0 °C in Period A, and 15.0–27.2 °C Period B) as was previously done for oil-seed crops (e.g., Chimenti et al., 2001).

2.3. Response variables

Fruits were harvested at the end of each treatment Period. Whole fruit fresh weight was recorded, and the fruit was then dissected into its constituent structures (mesocarp, endocarp and seed) for further analysis. Fresh weight of each structure was recorded, and then oven dried at 60 °C to constant weight. Oil content (dry weight basis) for mesocarp and seed was determined using the Soxhlet technique (IUPAC, 1992) with hexane as solvent. To determine oil fatty acid composition, oil samples were cold-methylated in a basic medium (IOOC, 2013), 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, 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.

The values of the response variables presented here for Period B were adjusted to avoid the dilution effect generated by biomass and oil that had accumulated in the fruit structures during Period A, prior to the beginning of Period B. To do this, initial (i.e., start of Period B) values for the response variables were determined on fruit from branches similar to the treated ones (selected on the same positional and fruit/leaf relationship criteria, with 4 replications per treatment). Changes in response variable values between 1 March 2011 (just prior to the initiation of Period B temperature treatments) and the values obtained at the end of the experiment (i.e., once oil accumulation have been completed; 13 May 2011) were used to compute the changes in variable values. This procedure allowed reporting the effects of treatments based on the biomass and oil accumulated during the period of temperature treatment only.

2.4. Complementary measurements

The dynamics of fruit growth and oil synthesis in seed and mesocarp was followed in untreated fruit throughout the season. Fifty fruit were sampled every 15 days from non-treated branches equivalent to the treated ones and from the same trees. These data allowed us to follow the mesocarp and embryo oil accumulation dynamics, to determine when the plateaus of seed and mesocarp dry weight and oil concentration were achieved, and to determine timing of harvests at the end of both treatment Periods. The criteria to fix the end-point of Period A was a fall off in the rate of seed oil accumulation such that for 2 consecutive harvest dates seed oil content was the same (0.02 g, data not shown), something which took place at approximately 50 days after pit hardening (DAPH).

The possible effect of the chamber, the shading net were tested in a complementary experiment in 2011, and reported in García-Inza et al. (2014). Briefly, fruit dry weight and oil concentration were not affected by shading or by enclosure in the shaded chamber in these branch-level manipulations. As expected, oleic acid concentration of the oil was affected by the passive heating of the chamber but not by the shading alone.

2.5. Statistical analyses

Treatment effects for the response variables measured in Periods A and B were assessed using an ANOVA for fixed effects. Differences among mean 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). Different mathematical functions (linear, polynomial and bilinear) were fitted to the relationships between the variables of interest. We chose those functions that provided the best fits with a significant model ($P < 0.05$). These analyses and the graphs were made with GraphPad Prism version 5.01 software (GraphPad Prism Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Ambient and experimental chamber temperatures

Mean ambient air temperature during Period A (25 Nov 2010 to 23 Feb 2011) was 21.4 °C while during Period B (1 Mar to 13 May 2011) it was 15.2 °C, temperature falling as the season progressed. 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, and the resulting mean temperatures spanned ranges of between 20.1 °C to 30.3 °C in Period A and of 15.6 °C to 27.2 °C in Period B (Fig. 1). This range is wider than that of regional variations in mean daily temperature. For the equivalents of our Periods A and B, ranges in mean daily temperatures for three sites in the region and over four years were 25.8–28.2 °C (Period A), and 21.7–26.4 °C (Period B). The mean temperature differentials relative to T0 varied slightly between the Periods, reaching 4 °C and 7 °C in T5+ and T10+, respectively, in Period A, and 6 °C and 10 °C in T5+ and T10+ in Period B. Temperature values for T3-, were 3 °C cooler than T0 in Period A and 1.5 °C in Period B.

Mean daily temperatures inside the chambers followed day/night (Fig. 2) and seasonal patterns of air temperature variation (Fig. 1). Importantly, treatment temperatures also tracked the intra-day fluctuations in T0 rather well, as shown before for a similar experimental system (García-Inza et al., 2014). Over the day,

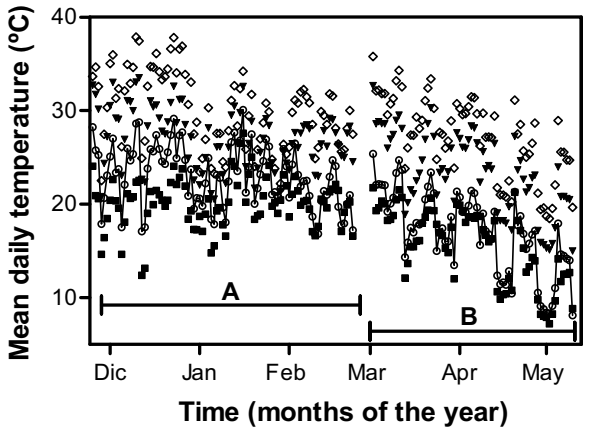

Fig. 1. Daily mean chamber temperatures during Periods A (November 25, 2010 to February 23, 2011, which includes the whole of the seed growth and the first part of mesocarp growth subphases) and B (March 1 to May 10, 2011, which includes the second part of the mesocarp growth subphase). Treatments were: control (T0 ○), cooling (T3- ■; 3 °C cooler than T0), and two heating levels (T5+ ▼ and T10+, 5 and 10 °C warmer than T0, respectively). Horizontal lines labeled A and B indicate treatment Periods. Each point represents a treatment mean (n = 4), SE values are not shown in the interests of clarity. Consecutive observations for the control treatment are linked by narrow straight lines to illustrate seasonal temperature dynamics and to act as a reference for dynamics of the heating and cooling treatments.

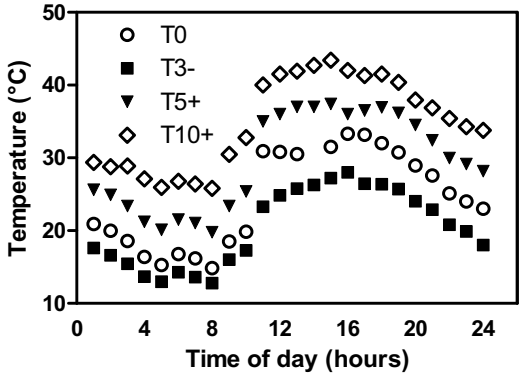

Fig. 2. Patterns of mean hourly chamber temperatures for February 20, 2011. Treatments were: control (T0 ○), cooling (T3- ■; 3C cooler than T0), and two heating levels (T5 + ▼ y T10 + ◊, 5 and 10 °C warmer than T0, respectively). Points are means (n = 4). S.E values are not shown in the interests of clarity.

temperature reached its maximum value around 15 h solar time and its minimum soon after sunrise (Fig. 2). Temperature differences between treatments were consistent throughout each 24 h period during the whole experiment (data not shown).

3.2. Seasonal dynamics of mesocarp and seed growth on untreated branches

The seasonal dynamics of mesocarp, seed and endocarp dry weight from untreated branches during Periods A and B showed that these three fruit structures had different temporal patterns and reached their maximum dry weights at different times during the experiment (Fig. 3 a). Endocarp weight increased at high rate until about 50 DAPH, with little increase after that. Seed dry weight registered a clear increase until 77 DAPH, but subsequent increments were very small (i.e., 0.01 g of increment from 77 DAPH to 130 DAPH). Although data on oil concentration in the seed were not available for the first two harvesting dates (-21 and −7 DAPH) due to small sample size, seed oil concentration was basically complete at 50 DAPH, following a growth pattern very similar to that of the endocarp. The mesocarp represented 70% of the final fruit dry

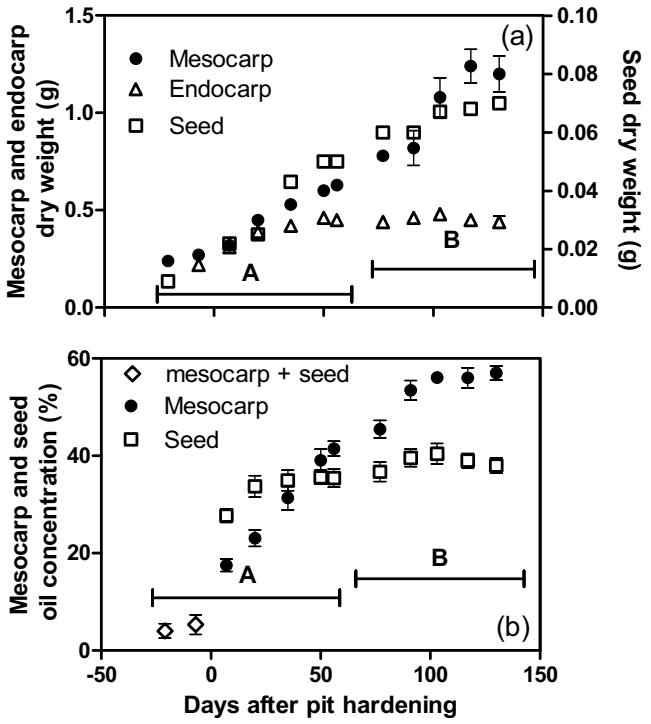

Fig. 3. Mesocarp, endocarp and seed dry weights (a); and mesocarp and embryo oil concentrations (b) as a function of time (days after pit hardening) in *Olea europaea* L. cultivar Arauco (zero corresponds to date of pit hardening, Dec. 30, 2011). Fruit were harvested from untreated fruiting branches of similar size and position to the treated ones. Horizontal lines labeled A and B indicate treatment Periods (A from −21 to 50 days from the start of pit hardening and B from 56 to 130 days after pit hardening). Symbols are means ± 1 S.E (n = 4), with S.E.s not shown when smaller than symbol.

weight and grew actively from the start of measurements to 117 DAPH, spanning both treatment periods. During Period A mesocarp dry weight and oil concentration increased, then oil concentration showed a lag phase at the beginning of Period B (between 56 and 77 DAPH) that was not detected for dry weight (Fig. 3 b). By the end of Period A the mesocarp had accumulated 50% of its dry weight and 52% of its oil final values, respectively.

3.3. Mesocarp and seed dry weight and oil concentration responses to temperature

Mean mesocarp dry weight at the end of Period A was 0.46 ± 0.03 g and 1.15 g ± 0.07 at the end of Period B, reflecting substantial increases in dry weight during each of the two periods. Mesocarp dry weight at the end of the treatment period was not significantly affected by temperature in either of the two periods (Fig. 4 a and b). Mesocarp oil concentration was negatively affected by temperature in both periods, decreasing 1.6% per °C of temperature increment in Period A (Fig. 4 c). Considering oil synthesized during Period B only, mesocarp oil concentration decreased 1.8% per °C (Fig. 4. d).

Seed dry weight at the end of Period A showed a curvilinear response to the temperature (Fig. 5. a) with slight (lower temperatures) and marked (higher temperatures) falls with respect to a maximum at 26 °C. Temperature had no effect on seed dry weight when treatment was applied during Period B (Fig. 5. b). Oil concentration in the seed exhibited a linear response to temperature in Period A, decreasing 1.2% per °C of temperature increment (Fig. 5. c). As with seed dry weight, seed oil concentration was not affected by temperature in Period B (Fig. 5. d).

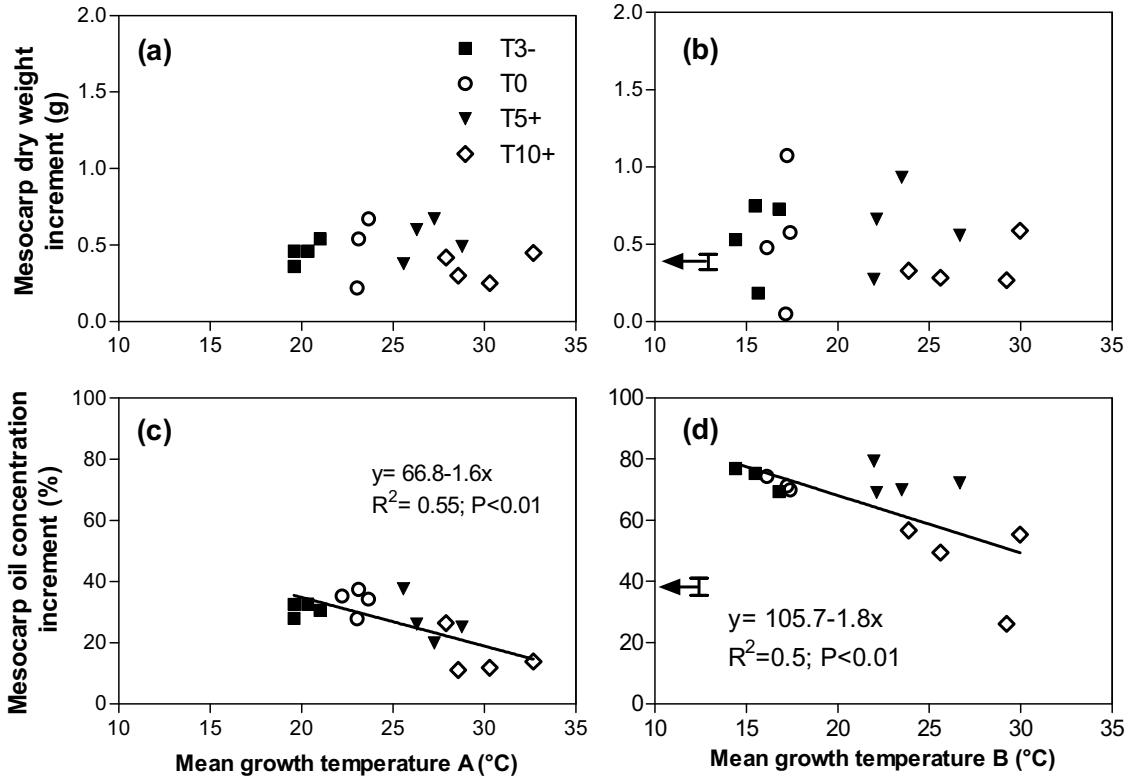

Fig. 4. Mesocarp dry weight increments as a function of mean growth temperatures (MGT) during Periods A (a) and B (b); and final mesocarp oil concentration (%) as a function of MGT during Periods A (c) and B (d). Treatments were: control (T0 ○), cooling (T3-■, 3 °C cooler than T0), and two heating levels (T5+▼ y T10+◊, 5 and 10 °C warmer than T0, respectively). Each point is the value for an individual controlled-temperature chamber. Horizontal arrows linked to error bars in panels b and d indicate mean values ± 1 S.E for mesocarp dry weight and mesocarp oil concentration at the beginning of Period B.

3.4. Mesocarp and seed oil oleic acid concentration responses to temperature

Mesocarp oil oleic acid concentration decreased linearly with increasing temperatures in both treatment periods. In Period A, oleic acid concentration fell 1.4% per °C (Fig. 6. a), reaching a 14 percentage point difference between the extreme treatments (T3- and T10+). Oleic acid synthesized during Period B decreased linearly with temperature at a slightly lower slope of 1% per °C (Fig. 6. b). While the proportion of oleic acid in oil fell from the beginning of Period B (see arrow in Fig. 6. b) until the fruits were harvested, the amount of oleic acid per unit weight of mesocarp synthesized in this treatment period increased.

Seed oil oleic acid concentration in Period A showed a curvilinear response to temperature, with a maximum oleic concentration of 68.7% at 28 °C followed by a slight decrease at higher temperatures (Fig. 6. c). Consistent with the null seed-oil accumulation during Period B, oleic acid concentration at the end of that Period was not affected by temperature (Fig. 6. d).

3.5. Responses to temperature of proportions of other fatty acids in mesocarp and seed oils

Oleic acid is the principal fatty acid present in olive oil, but considerable concentrations of other fatty acids are also present. In mesocarp oil, palmitic and linoleic acid were very sensitive to temperature, increasing linearly 0.64% and 0.5% per °C of increment in temperature in Period A (Fig. 7. a). Modest differences were found in Period B for same fatty acids, with increases of 0.35% and 0.12% per °C ($P < 0.01$) for palmitic and linoleic acid, respectively (Fig. 7. b). Stearic and linolenic acids made minor contributions to the final mesocarp oil fatty acid composition (< 5%). Linolenic acid showed an increment of 0.2% per °C in Period A, while stearic acid did not respond to temperature within the range explored in this experiment (Fig. 7. c). In Period B, linolenic increased with temperature at a gentler slope of 0.02% per °C, while stearic acid, as was also the case in Period A, was not affected by temperature (Fig. 7. d).

Three seed oil fatty acids showed sigmoidal responses to temperature in Period A. Linoleic acid had high concentrations (between 21% and 25%) with temperatures below 24 °C, but decreased drastically reaching values between 1.7 and 0.3%, with temperatures greater than 25.5 °C. Conversely, palmitic and stearic acid concentrations -were low (10% and 1%, respectively) when mean temperature was below 24 °C, rising to 22% and 6.3% with higher temperatures (Figs. 8. a and c). Finally, linolenic acid decreased linearly 0.03% per °C (Fig. 8. c). In Period B, palmitic, stearic, linoleic and linolenic acid concentrations in seed oil did not change significantly in spite of the wide range of temperatures explored (15 °C–30 °C) (Figs. 8. b and d).

4. Discussion

Mesocarp dry weight was not significantly altered by temperature in either Period A or B (Fig. 4. a and b). This differs from the response reported by García-Inza et al. (2014) where temperatures > 25 °C caused a decrease in whole fruit dry weight at a rate of 0.08 g per °C. Final harvest fruit dry weight of the cultivar Arauco olives in this experiment reflected contributions from mesocarp (70%), endocarp (26%) and seed (4%) (values derived from data shown in Fig. 3). Possibly, the duration of the treatment period in the current experiment (90 days in Period A and 74 days in Period B, in contrast to a 114 day-treatment in García-Inza et al. (2014) were not long enough as to noticeably affect mesocarp dry weight. The endocarp, the second important contributor to olive

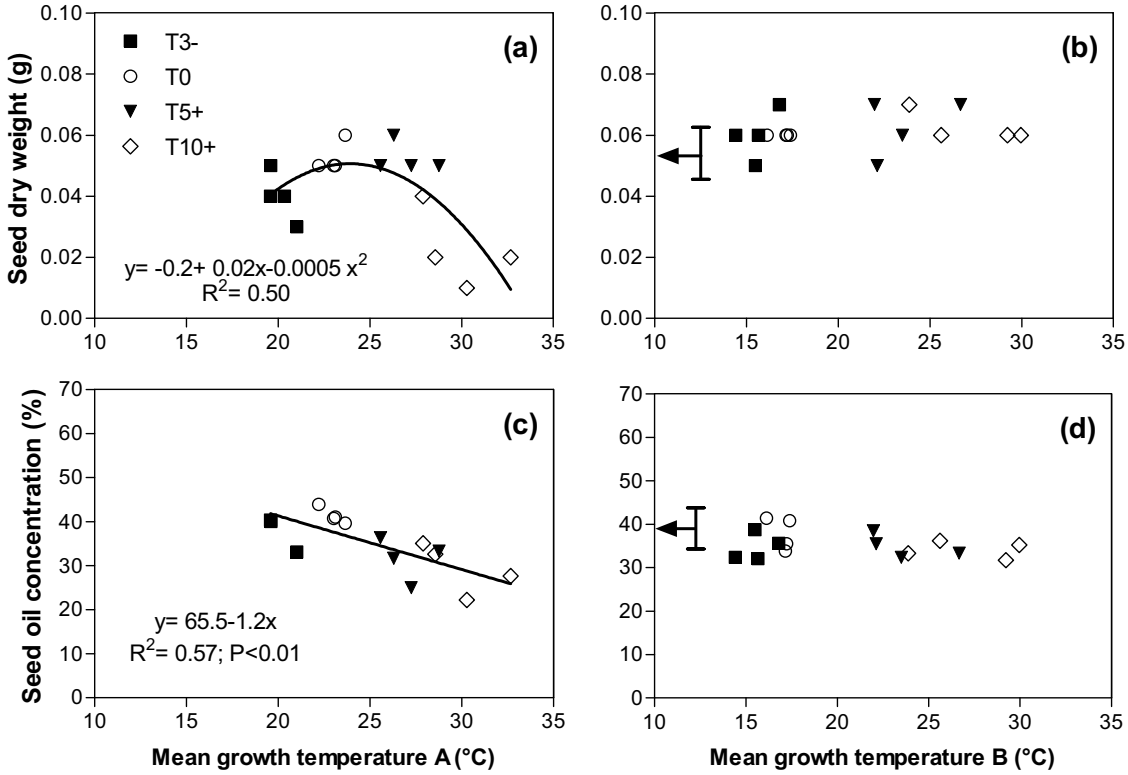

Fig. 5. Final seed dry weight as a function of mean growth temperatures (MGT) during Periods A (a) and B (b); and seed oil concentration (%) as a function of MGT during Periods A (c) and B (d). Treatments were: control (T0 ○), cooling (T3-■; 3 °C cooler than T0), and two heating levels (T5 + ▼and T10+ ◊, 5 and 10 °C warmer than T0, respectively). Each point is the value for an individual controlled-temperature chamber. Horizontal arrows linked to error bars in panels b and d indicate mean values ± 1 S.E for seed dry weight and seed oil concentration at the beginning of Period B.

fruit dry weight, did not respond to variations in mean temperature in this experiment (data not shown). Unlike the mesocarp and the endocarp, seed dry weight showed a curvilinear response to temperature with an optimum at 26 °C in the period A (Fig. 5. a). We speculate that the differences in duration of treatments may underlie the differences in response to temperature between García-Inza et al. (2014) and the current experiment. Consistent with this speculation, in an experiment involving one month long heating treatments in which fruit grown at a mean growth temperature (MGT) of 29.6 °C were compared with fruit grown at a MGT of around 22.4 °C, did not show any fruit dry weight differences (García-Inza et al., 2014).

Both olive fruit oil-bearing structures had similar oil concentration pattern responses to temperature. Mesocarp oil concentration decreased with increasing temperature in Periods A and B, falling 1.6% and 1.8% per °C, respectively (Fig. 4. c and d) over the whole of the temperature range explored in this experiment. These responses are consistent with that reported by García-Inza et al. (2014) for the whole fruit, where oil concentration fell 1.13% per °C in an experiment in which temperature was manipulated for a period of 114 days. Seed oil concentration showed a similar response pattern to that of the mesocarp during Period A, falling 1.2% per °C, but it did not respond to temperature in Period B, having reached its maximum value prior to the beginning of the second treatment Period (Fig. 5. c and d). This absence of response is consistent with the results of a preliminary experiment which showed no seed dry weight or oil concentration changes when thermal treatments (mean temperatures ranging between 16–32 °C) were applied for four months after pit hardening (data not shown).

A key finding of our experiment is that the temperature responses of oleic acid in oils from seed and mesocarp were opposite in sign over much of the range of temperature explored during Period A (Figs. 6. a and c), and that the proportion of oleic acid in mesocarp oil maintained a similar response to temperature during Period B. The observations of the mesocarp oleic acid response to temperature in the present study are both consistent with and deepens our understanding of the results of prior correlative analyses involving putative temperature effects on oleic acid concentration in oil from the whole fruit (Rondanini et al., 2011) as well as results of manipulative experiments (García-Inza et al., 2014). The mesocarp oil oleic acid response to temperature in Period A showed higher sensitivity (decreasing 1.4% per °C) than in Period B (decreasing 1.0% per °C). This is consistent with the response observed after applying a heating treatment (6 °C warmer than the control) for a month early in the season (between mid January and mid February) compared to heating treatments imposed later during the season (mid April and mid May) (García-Inza et al., 2014). As distinct from mesocarp oil oleic acid, seed oleic acid proportion increased between 19.6 °C and 28 °C during Period A while it was unaffected by temperature during Period B (Fig. 6 c and d). These differences in temperature responses of oleic acid in oil from seed and mesocarp in Period B are consistent with those found in an exploratory experiment which involved long term temperature manipulation (4 months long, starting at 30 DAPH, similar to the beginning of the Period B). That experiment involved continuous temperature manipulation instead of the two distinct Periods used in the experiment reported here, but the experimental design and procedure was equivalent to the one described here. In the exploratory experiment, oleic acid percentages in oil from mesocarp fell 0.54% per °C ($R^2 = 0.57$; $P < 0.05$) with no response in seed oleic acid (+ 0.02% per °C, $R^2 = 0.02$; $P = 0.6$), over a range of temperature values between 16 °C and 32 °C. The olive seed oil oleic acid response to temperature in Period A, although opposite in sign to that of oleic acid mesocarp oil in both periods, is consistent with

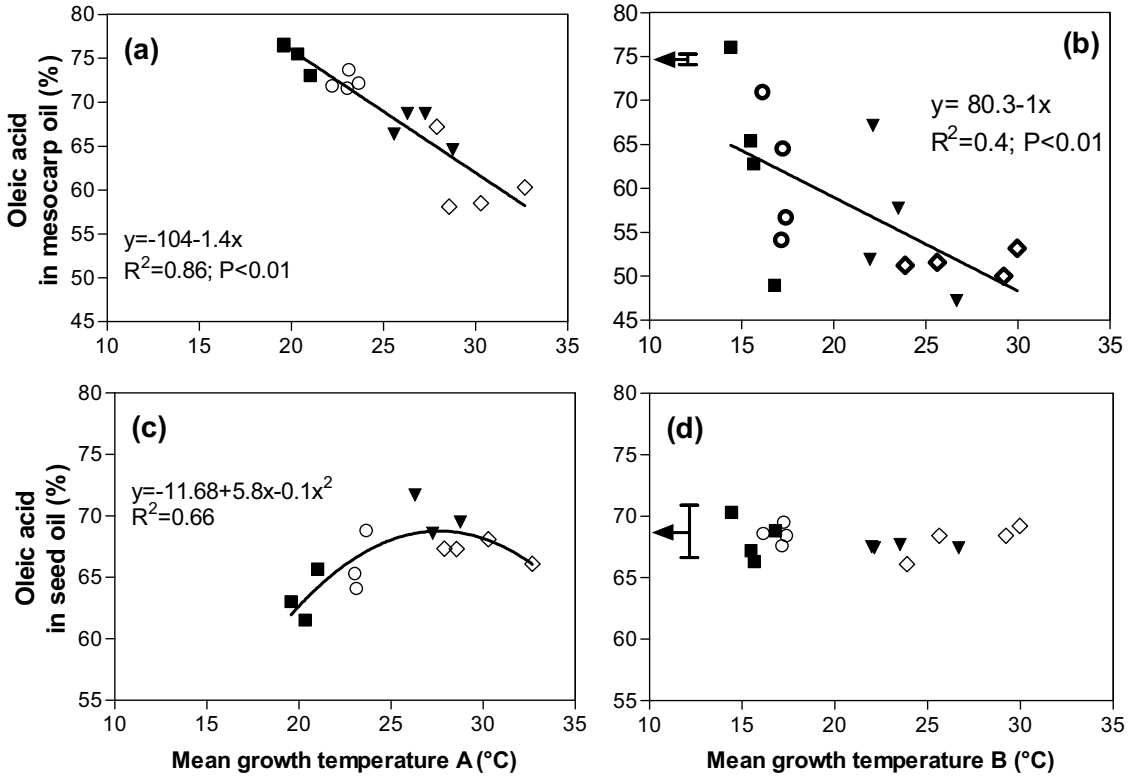

Fig. 6. Final proportions of oleic acid in mesocarp oil as a function of mean growth temperatures (MGT) during Periods A (a) and B (b); and in seed oil as a function of MGT during Periods A (c) and B (d). Treatments were: control (T0 ○), cooling (T3-■; 3 °C cooler than T0), and two heating levels (T5 + ▼ and T10+ ◊, 5 and 10 °C warmer than T0, respectively). Each point is the value for an individual controlled-temperature chamber. Horizontal arrows linked to error bars in panels b and d indicate mean values ± 1 S.E for seed dry weight and seed oil concentration at the beginning of Period B.

that of annual oil-seed crops that accumulate oil principally in seed, such as sunflower (Izquierdo et al., 2006), maize or soybean (Zuil et al., 2012). It would be interesting to check whether other species that accumulate oil in both mesocarp and seed, such as avocado or palm, exhibit responses to temperature similar to those of olive. Arauco shows a sharp decrease in oleic acid concentration over the course of the season, similar to the response shown by Arbequina (Rondanini et al., 2014) one of the varieties most cultivated for the oil industry. A need still remains to develop similar functions for other genotypes which appear to be less sensitive to temperature (e.g., Coratina, cf. Rondanini et al., 2014), at least within the ranges of temperature common in the region. In addition, oleic acid in oil temperature response functions could be used in a future model of oil quality for olive. Currently available olive models only deal with vegetative growth (Villalobos et al., 2006; Morales et al., 2016) or flowering date (De Melo-Abreu et al., 2004; Pérez-López et al., 2008), so our temperature response functions could be of use to researchers interested in extending these models to include yield and oil quality.

The proportions of non-oleic fatty acids in oils from mesocarp and seed of Arauco exhibited some differences, a situation which contrasts to some degree with the conclusions of Hilditch and Williams (1964) who reported similar proportions of some fatty acids between mesocarp and seed in olive oil. For example, in oils from untreated fruit in our experiments showed differences in the proportions of palmitic acid (14% mesocarp, 9% seed), linoleic (12% mesocarp, 18% seed) and linolenic acid (0.9% mesocarp, 0.3% seed), but no differences in oleic acid proportion was detected. In correspondence with the temperature-induced reductions in the proportions of oleic acid in mesocarp oil, those of several other fatty acids increased. These effects were especially notable for palmitic, linoleic and linolenic acids in Period A, and were much less marked in Period B (Fig. 7). As a consequence of the changes in the proportion of fatty acids in the oil in both periods in our experiment, the limits proposed by the IOOC for the extra virgin oil were exceeded for palmitic acid and linolenic acid. In the case of palmitic acid, the limit is 20% and was exceeded with temperatures above 28 °C in Period A and temperatures > 25 °C in Period B. The IOOC limit for linolenic acid of 1% was exceeded throughout the explored temperature range during Period A and in some samples during Period B with temperatures > 25 °C.

The proportions of non-oleic fatty acids in seed oil also responded to temperature, but only in Period A and with response patterns that differed clearly from those observed in the mesocarp oil (compare Fig. 7 a and c with Figs. 8 a and c). Taken together, the temperature response patterns of the main fatty acids (ie, oleic, linoleic, palmitic and stearic, Figs. 6 c, 8 a and c) suggest that 25 °C is a critical temperature for fatty acid synthesis chain. Temperatures > 25 °C seem to have affected the desaturation of stearic acid to oleic acid so that the proportion of oleic acid was reduced, as well as those of the fatty acids downstream in the synthesis pathway (linoleic and linolenic). At the same time, the precursors (palmitic and stearic acids) accumulated (Fig. 7 a and c). These responses of these fatty acids to temperature observed in olive seeds are novel and different from those reported for annual oilseed crops. The effect of temperature on fatty acid composition of oil from the olive seed was not limited to the oleic/linoleic ratio as occurs in sunflower (Izquierdo et al., 2006), soybean or maize (Zuil et al., 2012), but there was a strong effect on saturated fatty acids (specifically palmitic, Fig. 8. a). Oleic and linoleic acid proportions in seed oil showed a similar response to temperature in both olive and annual oilseed crops. In corn and soybeans a positive linear relationship was reported between oleic acid and temperature (Zuil et al., 2012), whereas sunflower had a bilinear response (Izquierdo

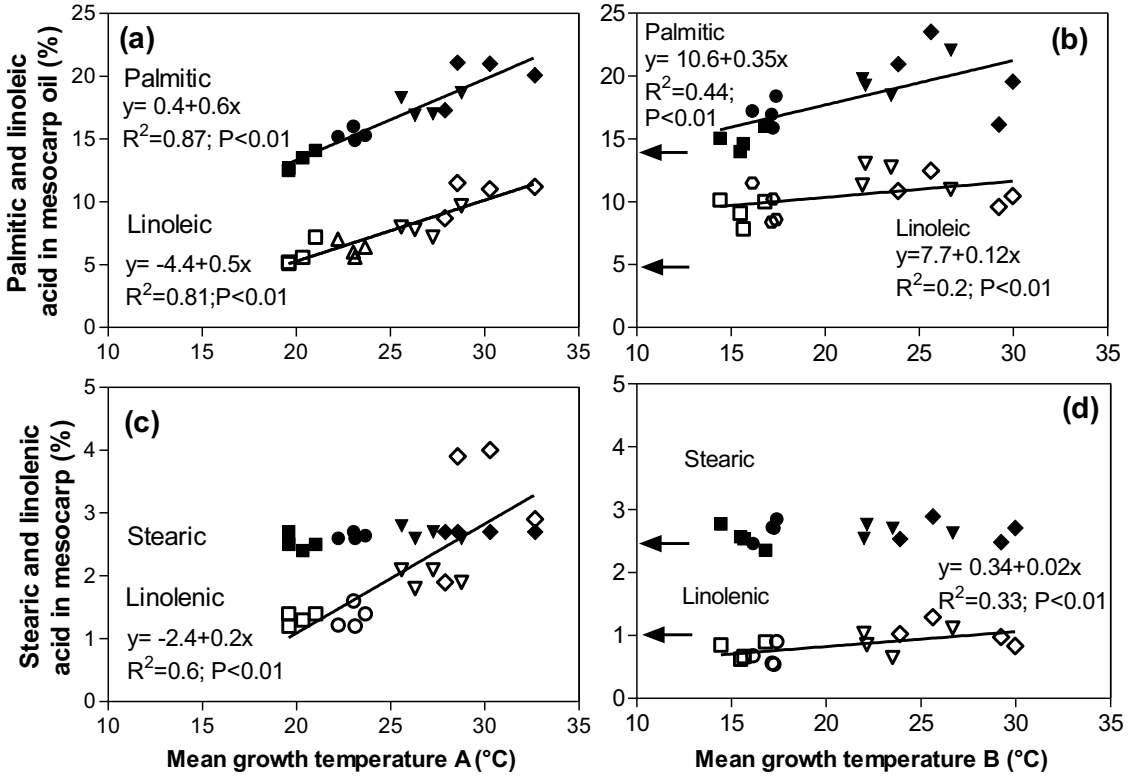

Fig. 7. Relationships between the final proportions of selected major fatty acids in mesocarp oil and mean growth temperatures (MGT) during Periods A (left-hand panels) and B (right-hand panels). Upper panels (a and b) show proportions of palmitic and linoleic acid, lower panels (c and d) show proportions of stearic and linolenic acids. Treatments were: control (circle), cooling (square), and two heating levels (T5+, triangle and T10+, rhombus). Each point is the value for an individual controlled-temperature chamber. Horizontal arrows in panels b and d indicate mean fatty acid proportion at the beginning of Period B in mesocarp oil, S.E.'s are not shown because they are smaller than symbols.

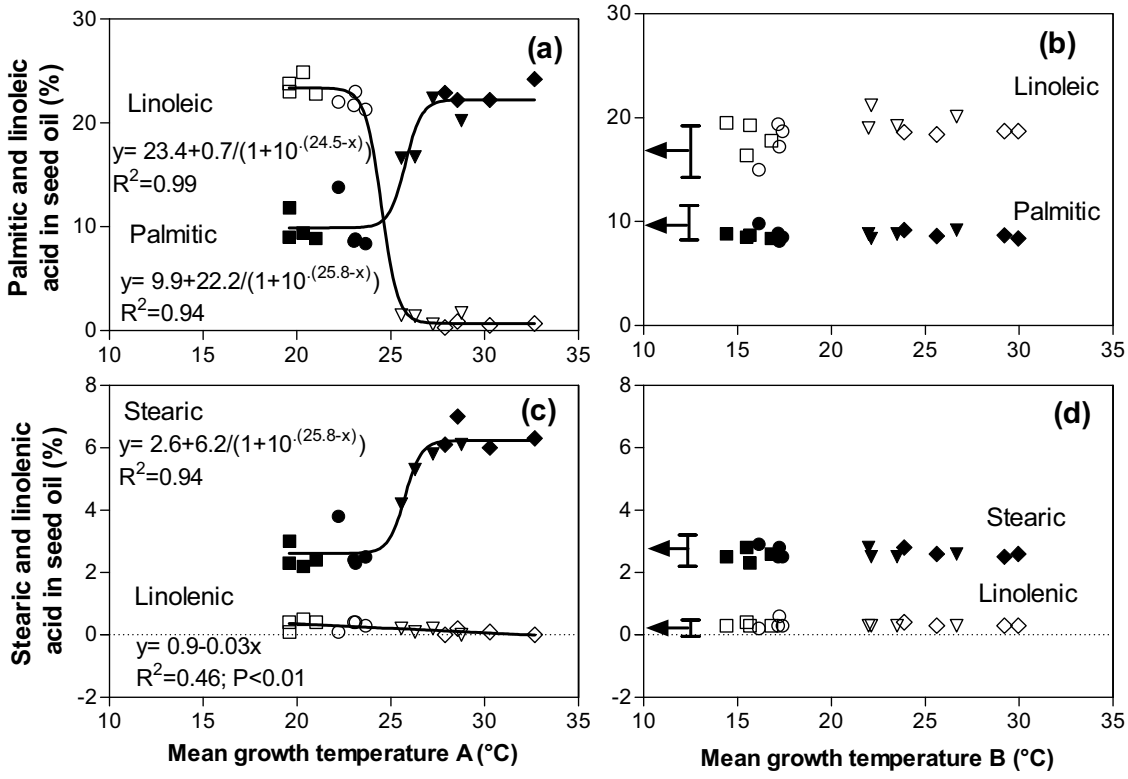

Fig. 8. Relationships between the proportions of major fatty acids in seed oil and mean growth temperature for Period A (left panels) and B (right panels). Upper panels show palmitic and linoleic acid proportion (a and b), lower panels show stearic and linolenic acids proportion (c and d). Treatments were: control (circle), cooling (square), and two heating levels (T5+, triangle and T10+, rhombus). Each point is the value for an individual controlled-temperature chamber. Horizontal arrows linked to error bars in panels b) and d) indicate mean values ± 1 S.E for palmitic and linoleic acid and stearic and linolenic acid in seed oil at the beginning of Period B.

et al., 2006), consistent with our finding over a similar thermal range (15 – 30 °C). The linoleic acid proportion in oil from olive and sunflower seeds showed a similar sharp decline at high temperatures. In olive seed oil temperatures > 25 °C caused a drop of 20 percentage points (Fig. 7 a), the same response was found in sunflower with seven days of temperatures > 25 °C during the seed growth (Rondanini et al., 2003), or comparing seed growing at 30/20 °C with 20/10 °C (Martínez-Force et al., 1998). There is no information about the effect of temperature on the expression of genes that encode enzymes involved in fatty acid desaturation or the effect on the regulation of their activity in olive seeds. In sunflower, the oleate desaturase activity is partially and reversibly inhibited by high temperature (30 °C), reducing the linoleic acid concentration in the oil (Sarmiento et al., 1998). This response in the proportion of fatty acids is consistent with our findings in olive seed oil but opposite that found for mesocarp oil. Furthermore, in sunflower, increases in temperature reduce the oxygen concentration inside the seed. Lower levels of oxygen reduce the ATP/ADP ratio (energy status) and the FAD enzyme activity with a corresponding increase in the proportion of oleic acid in oil (Rolletschek et al., 2007). This mechanism, known in sunflower, has not been studied in olive seeds or mesocarp. The information about the desaturase enzymes in olive is very limited. It is known that the expression of the genes that encode desaturase enzymes, such as stearate desaturase (responsible for the transformation of stearic acid to oleic acid) (Haralampidis et al., 1998; Parvini et al., 2015) or oleate desaturase (linked to passage of oleic acid to linoleic) (Banilas et al., 2005; Hernández et al., 2009 and Parvini et al., 2015) occur at different times of mesocarp and seed growth. However, we are still lacking information about the responses of the activity of these enzymes to temperature.

To summarize, our results have shown that oils for the mesocarp and the seed of olive have different responses to temperature in terms of fatty acid composition and dry weights but both structures had similar responses in terms of oil concentration. Previous information was based on data from the whole fruit and was dominated by mesocarp behavior which masked the seed responses. We have also shown that the seed is sensitive to temperature during Period A only, while the mesocarp response to temperature persisted during both periods. Mesocarp response was consistent with that previously reported for oil from the whole fruit (García-Inza et al., 2014), which showed that fatty acid proportion response to temperature different from the one reported for annual oilseed crops. Finally, our results show that the olive seed oil fatty acid composition responses to temperature were similar to those of annual oilseed crops for oleic and linoleic acid but different in terms of palmitic and stearic acid. The contrast between seed and mesocarp fatty acid proportion response to temperature suggests a differential regulation of fatty acid synthesis in both oil-bearing structures of the fruit. Further studies are needed to compare enzymatic function between species and between the olive mesocarp and seed in order to elucidate the contrasts between species and between oil-bearing structures of the olive fruit which our results have served to highlight.

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