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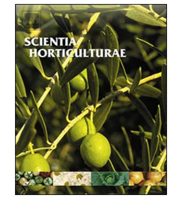

Research Paper

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

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