Plant Physiology and Biochemistry 139 (2019) 485–494

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Multi-environment evaluation of oil accumulation pattern parameters in olive

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ARTICLE INFO

Keywords:
Oil olive accumulation
Genetic effect
Environment effect
Genotype by environment interaction
Genotypic stability
Air temperature

ABSTRACT

The pattern of olive oil accumulation is of paramount importance in olive because its influence in determining final oil yield and optimal harvesting date. In this work, we have evaluated the genetic and environment influence on the olive oil accumulation pattern. For that purpose, a set of cultivars and breeding selections planted in a multi-environment trial was evaluated in two consecutive harvest seasons. Significant effect of the environment, genotype and their interaction were observed for the maximum oil content reached and the rate of oil accumulation. On the contrary, the date of maximum oil content seems to depend only on the environment. The two breeding selections evaluated showed, in general, high stability and adaptability in oil accumulation parameters. Among the potential environmental factors that could affect oil accumulation, PLS analysis suggests that temperature could play a determinant environmental effect in the oil accumulation parameters tested in this study. These results underline the relevance of using multi-environment trials for adequate characterization of genotypes showing either good behaviour in variable environments or only under specific environmental conditions.

1. Introduction

Maximum oil content in fruit is one of the most important component of the oil yield and the pattern of oil accumulation should be also taken into account as a main criterion to determine optimal harvesting date (Beltran et al., 2004; Mickelbart and James, 2003). High fruit oil content is considered one of the main objectives in olive breeding programs aiming at obtaining new cultivars for olive oil production. A high genetic effect has been reported for oil content in olive in studies with both cultivars (Garcia and Mancha, 1992; Mickelbart and James, 2003; Rondanini et al., 2014; Trentacoste et al., 2012) and breeding selections (de la Rosa et al., 2008; Leon et al., 2004). This together to the high heritability showed for maximum oil concentration could be expected a high response for selection in future breeding works (Leon et al., 2004, 2015). The variability of environmental conditions among years and locations, as well as cultural factors such as irrigation and crop load, have been also reported to have a strong influence on olive oil content (Lavee and Wodner, 1991; Mailer et al., 2007; Trentacoste et al., 2010). This could make olive oil accumulation process vulnerable to the extreme climatological events predicted by an imminent climate change, as reported for other fruiting parameters (Benlloch-González et al., 2018).

Recent works have simplified the oil accumulation pattern in olive to a bilinear model including a first lineal stage where oil increases at a constant rate (i.e. slope of the regression) until reach its maximum, and a second one where the oil content remain constant (Trentacoste et al., 2010). This bilinear model allows to define three critical parameters in oil accumulation: rate of oil accumulation, maximum olive oil content and date when maximum oil content is reached. Following this procedure, some studies have also shown the influence of genetic and environmental factors on the oil accumulation pattern in olive (Rondanini et al., 2014; Trentacoste et al., 2010, 2012). However, there is still little information on the effect of the genotype by environment interaction for these parameters. Moreover, these studies have been usually performed based on the evaluation of cultivar collections or commercial farms lacking appropriate experimental design, and specific comparative trials in different environments have not been reported to evaluate this interaction.

Therefore, the evaluation of specific multi-environment trials would be of great interest to perform a consistent characterization of the oil accumulation in olive. Those multi-environment trials might also be of interest to study the stability and adaptability of cultivars and breeding

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https://doi.org/10.1016/j.plaphy.2019.04.016
Received 18 January 2019; Received in revised form 22 March 2019; Accepted 11 April 2019
Available online 14 April 2019

selections to varying climatic conditions. For it, the use of different indexes (Eberhart and Russell, 1966; Finlay and Wilkinson, 1963), as well as additive main effects and multiplicative interaction (AMMI) model (Gauch and Zobel, 1997), could be the best way. AMMI model is currently used to obtain high performance genotypes in breeding programmes of other species (Ebrahimi et al., 2016; Hassani et al., 2018) in order to understand more easily the structure of the interactions and choose the best genotypes.

In the present work, we report the evaluation of the oil accumulation parameters in a set of cultivars and breeding selections in a multi-environment trial. The objectives are to: (i) assess variance components and determine the importance of genotype by environments interaction for these parameters, (ii) study stability and adaptability of several genotypes for oil accumulation, and (iii) investigate the influence of air temperature on oil accumulation.

2. Materials and methods

2.1. Multi-environment trial (MET)

The experiment was carried out in a multi-environment trial (MET) planted in 2008 in five different agro-climatic conditions of Andalusia, Southern Spain (Fig. 1). Two trials were planted in new growing areas: Gibraleón, with mild winter temperatures, and Tabernas, with arid climate (precipitation around 200 mm per year), both with drip irrigation (irrigated with 1100 and 1000 m^3/ha per year respectively). The other three were located in typical olive growing areas, Antequera and Baena under rainfed conditions, and Úbeda with drip irrigation (irrigates with 1500 m^3/ha per year). In all these locations five cultivars, 'Arbequina', 'Arbosana', 'Carrasqueño', 'Koroneiki' and 'Picual' and two advances selections from the IFAPA-University of Córdoba cooperative olive breeding program were evaluated. The two breeding selections come from an initial breeding population and were selected on the basis of their earliness of bearing and high fruit oil content (De la Rosa et al., 2006; Leon et al., 2004). In each location the cultivars and breeding selections were spacing at 7 × 6 m, arranged in a randomized design with four blocks and four trees per elementary plot. Temperature and rainfall data were obtained from weather stations located in each comparative trial.

2.2. Fruit parameters

Ripening index and fruit oil content on dry weigh basis were systematically evaluated in 2015 and 2016 in the five locations considered. For that purpose, fruit samples, around 300 g, were randomly hand-picked per elementary plot (4 trees/genotype) at 10 days intervals. Sampling started the beginning of September and finished when slight variation on oil content on fruit dry weight was observed between consecutives sample dates. Only trees with significant yield were considered and at last twelve trees per genotype-environment were necessary to be included in the study. For that, crop load was visually evaluated following a 0 (no crop) to 3 (maximum) scale. Only tress having 2 to 3 used for this study. Fruits samples were collected into polyethylene bags and preserved at 4 °C until processed. Ripening index was evaluated according to the procedure described by (Frías L. et al., 1991). Afterwards, three random sub-samples of 25 g were prepared, oven dried at 105 °C for 42 h (del Río and Romero, 1999) to ensure dehydration and oil content was measured in a NMR fat analyser.

2.3. Data analysis

Oil accumulation pattern on fruit dry weight basis for each genotype/environment per elementary plot was fitted to a bilinear model with plateau as described by Trentacoste et al. (2012), using the following formula (Table Curve non-linear routine):

$$y = a + bx \text{ for } x < c$$

$$y = z \text{ for } x = z$$

where y is oil content on fruit dry weight (%), x is the date expressed as day of year (DOY), a is the intercept and b is the slope of the linear phase (rate of olive oil accumulation %/day), c is the breakpoint date over which y reaches the maximum oil content (z).

In the present work, "environment" was defined as a given combination between location and year. One of the potential combinations (Baena-2015) was excluded due to the lack of enough fruits for analysis,

Fig. 1. Geographical situation of the five environments that make up the multi-environment trial (MET) in this study situated in Andalusia, Southern Spain.

so a total of 9 environments were considered. ANOVA type III, suitable for unbalanced designs, was applied to test the genotype, environment and genotype by environment interaction (GEI) effect on olive oil accumulation parameters (date of maximum oil, maximum oil and rate of accumulation). Mean separation was conducted using Honestly-significant-difference (HSD) to test the differences between genotypes, environments and GEI for each parameter evaluated.

In those parameters having significant GEI, stability and adaptability analysis was performed. For that purpose, a single linear regression was fitted the data pooled across environments, genotypes and both growing seasons, where deviation from regression indicate the genotype stability (Eberhart and Russell's et al., 1966), $s^2d = 0$, and where the slope of the model indicate the genotype adaptability (Finlay and Wilkinson, 1963), with $bi = 1$ present average adaptability, $bi < 1$ adaptability to unfavourable environments and, $bi > 1$ adaptability to favourable environments. The third stability and adaptability index used was additive main effects and multiplication interaction (AMMI) model (Gauch and Zobel, 1997), which is being mainly performed to obtain main effects (genotype and environment) and GEI effect from MET.

Partial least square (PLS) was conducted to find possible critical periods of temperature that could cause changes in olive oil accumulation parameters. The average temperature from 1st June to 30th November per environment (Fig. 2) was subjected to 11-day running mean (Luedeling and Gassner, 2012), to obtain a reduction of the daily variation and to identify the periods more significantly affecting oil accumulation. These periods are obtained by the two main PLS outputs: the variable importance in the projection (VIP) indicate that periods are important for dependent variable (values greater than 0.8); and the model coefficients indicate the strength and direction (negative or positive correlation) of the variation of the centred and scaled data. The average per environment of the oil accumulation parameters, maximum oil and date of accumulation, were used for this relation. Additionally, the same PLS analysis was separately performed in two independent cultivars, 'Arbequina and 'Koroneiki', which presented enough fruits in all locations and seasons. Statistical analysis was performed using R software (R development Core Team, 2016), using ANOVA (type III) under car package, AMMI model and HSD test from agricolae package and PLS analysis from PLS package.

3. Results

3.1. Olive oil accumulation parameters

A bilinear model described adequately the dynamics of fruit oil accumulation (Fig. 3), with adjusted R^2 average 0.93 (between $R^2 = 0.46$ and 0.99) and the parameters derived were statistically significantly ($p < 0.05$) in the 220 curves evaluated (i.e. 55 combinations genotypes x locations x seasons x 4 replicates).

3.1.1. Maximum olive oil content on fruit dry weight

Contribution of variance components for maximum oil content was evenly distributed among genotypes, environments and their interaction, all of them showing a significant effect (Table 1). Among the nine environments analysed, BA-16, GI-15 and TA-16 showed the highest values and UB-16 the lowest, with a range of variation from 45.9 to 52.6% (Table 2). Genotypes could be divided in three groups as having high ('Carrasqueño', 'Selection 1' and 'Selection 2'), intermediate ('Picual', 'Arbosana' and 'Arbequina') and low ('Koroneiki') maximum oil content. 'Selection 2' showed the highest values for maximum oil content in five out of the nine environments where it was evaluated. Considering the interaction between genotype and environment, a wide range of variation was obtained: from 'Carrasqueño' cultivar tested in GI-15 (54.2%) and 'Picual' in TA-16 (54.4%) to 'Arbequina' in TA-15 (40.2%). The environments located in the new olive growing areas of Tabernas and Gibraleón provided both the highest and the least maximum olive oil values for most of the evaluated genotypes.

Stability estimation analysis according to Eberhart and Russell's (s^2d) showed 'Selection 1' as the most stable genotype, with the lowest s^2d (Table 2). Conversely, 'Carrasqueño', closely followed by 'Koroneiki' and 'Arbequina', were the cultivars with the lower stability (i.e. the highest s^2d values). For adaptability estimation using Finlay-Wilkinson (*bi*) model, genotypes were sorted in two groups. The first one includes 'Arbequina', 'Arbosana' and 'Picual', with *bi* values higher than one, and therefore likely having adaptability to favourable environments; the rest of the cultivars, with bi lower than 1, would have adaptability to unfavourable environments. The additive main effects and multiplicative interaction (AMMI) biplot model explained the 75.1% of GEI variability (Fig. 4A). Selection 1 and 2 are placed close to the centre, hence, present a general better stability to the different environments evaluated, while 'Carrasqueño', 'Koroneiki' and 'Arbequina' are far from it, with poor stability. AMMI biplot model also showed genotype specific adaptability to a given environment, where maximum oil content will be higher. This is the case of 'Picual' in AN-16 environment and 'Carrasqueño' in GI-16.

3.1.2. Rate of olive oil accumulation

Contrary to maximum oil content, the rate of oil accumulation depended mostly on the environment, with low genetic and GEI effect, although both being significative (Table 1). The large environmental variability of this parameter (Table 3) was mainly due to the wide differences among GI-16, BA-16 (the highest) and GI-15 (the lowest). Among genotypes, 'Selection 2' was the most differentiated genotype with the highest rate of accumulation. Then, two groups could be formed with intermediate ('Arbequina', 'Picual', 'Selection 1' and 'Koroneiki') and low ('Carrasqueño' and 'Arbosana') rate of accumulation. Comparison among the genotypes by environment interaction for rate of accumulation showed a maximum for 'Selection 2' in UB-16 and a minimum for 'Carrasqueño' in GI-15.

According to s^2d stability index (Table 3) and AMMI biplot model (Fig. 4B) with 82% variance explained, 'Picual' was the most stable genotype for rate of accumulation followed by 'Selection 2', with 'Carrasqueño' and 'Arbequina', showing the lowest stability. Adaptability estimation by *bi* stability index (Table 3) showed similar and high adaptability of all genotypes in the set of environments tested (*bi* values around one). However, according to AMMI model (Fig. 4B), there was specific adaptability for some genotypes such as 'Carrasqueño' to AN-15 and 'Selection 1' to GI-16 or 'Arbosana' to GI-15.

3.1.3. Date of maximum oil content

Contrarily to what was shown for maximum oil content and rate of accumulation, date of maximum oil content only showed significant variability for environment, that accumulated most of the variance (Table 1). The range of variation among environments was 25 days, with the earliest maximum accumulation in GI-15 and latest in AN-15 (Table 4). Also, it should be noted that the date of a given environment in the two years was similar, with Gibraleón (GI-15 and GI-16) having the earliest maximum accumulation date and Antequera (AN-15 and AN-16) the latest. As the interaction was not significant, stability and adaptability analysis were no performed.

3.1.4. Average ripening index at the moment that maximum oil content is reached

Environment effect was the main contributor of variance for average ripening index, although genetic and GEI effect were also significant (Table 1). Among the environments evaluated (Table 5), GI-16 showed the lowest average ripening index when the maximum olive oil content is reached, with an average value of 0.88 (green fruits). In all the cases, maximum oil accumulation was reached with an average ripening index lower than 3. Differences for ripening index were less evident among genotypes with average values between 1.22 and 2.59. The GEI interaction (Table 5) clearly showed that the maximum oil

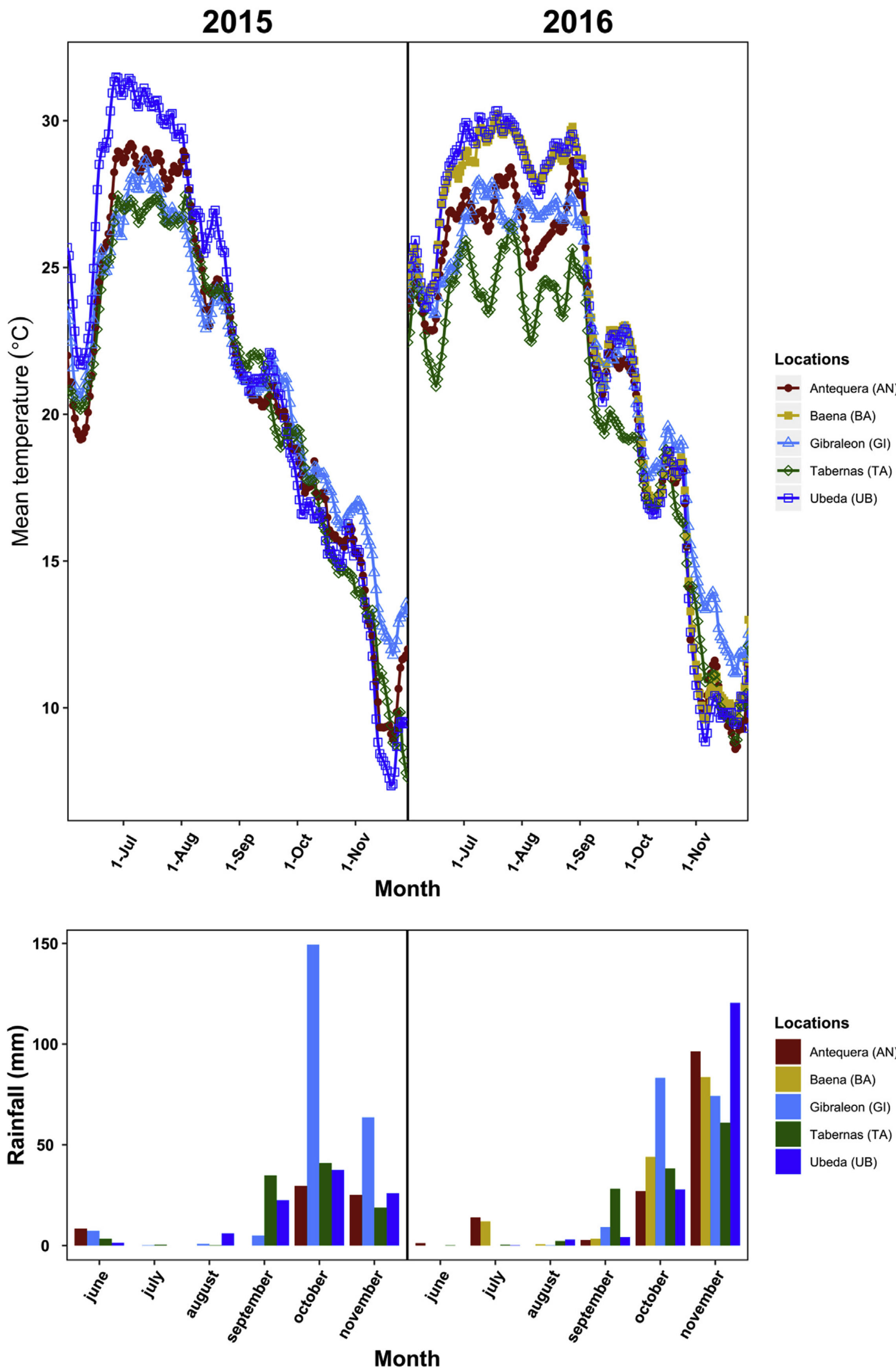

Fig. 2. Average daily temperature, recorded as 11-day running means of from 1st June to end of November, and average month rainfall, of the five locations in two years evaluated.

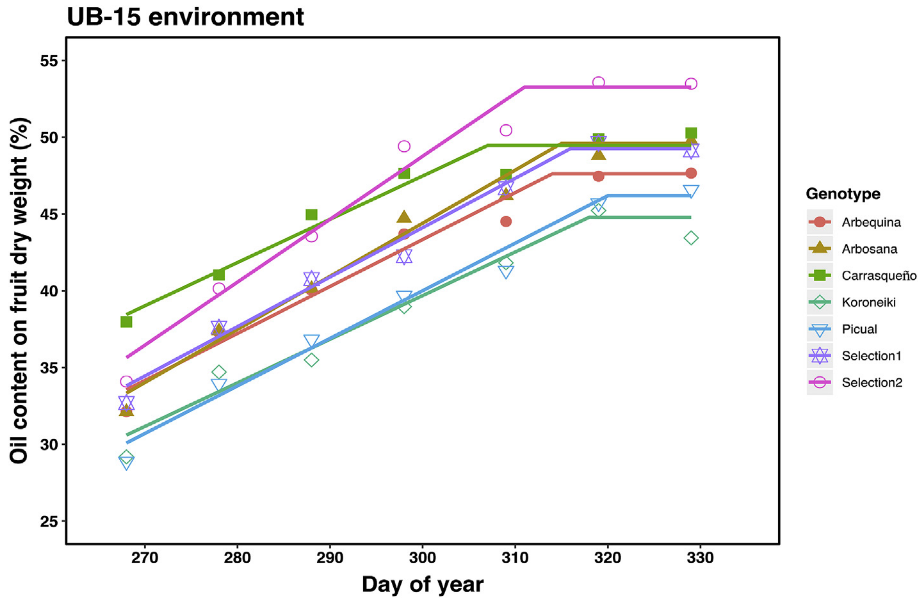

Fig. 3. Olive oil accumulation on fruit dry weight and its bilinear model for seven genotypes in UB-15 environment.

accumulation occurs at a different average ripening index for each cultivar-environment combination.

'Arbosana' and 'Koroneiki' were the most stable cultivars for average ripening index according to s^2d (Table 5) and AMMI model (Fig. 5), with similar average ripening index when the maximum oil is reached in all environments, while 'Selection 2' was the most unstable. 'Koroneiki' also showed high adaptability to all environments, according to *bi* (Table 5). AMMI model, in this case, showed specific adaptation to a give environment as 'Carrasqueño' in BA-16, where its average ripening index will be the highest when the maximum oil is reached.

3.2. Air temperature and its influence on oil accumulation

Average daily temperatures were different among 5 locations, more evident in 2016 than 2015 year (Fig. 2). July was warmer in 2015 than 2016 for Úbeda and Tabernas, with similar average temperature for Antequera and Gibraleón in both years. August showed differences depending of the location, with higher temperatures during 2016 in Úbeda and Gibraleón and lower in 2016 than 2015 in Tabernas. This latter location showed the lowest average temperature throughout the summer and beginning of autumn in 2016. However, in the other four locations, the beginning of Autumn was hotter in 2016 than in 2015, showing the greatest variation between years for September.

Average data per environment of maximum oil content and date of maximum oil content was correlated with 11 days running mean temperature from 1st June to the end of November using partial least squares (PLS) (Fig. 6). Maximum olive oil content showed high correlation with temperature on July, August and September (Fig. 6A) with values of variable importance in the projection (VIP) above 0.8. Model coefficients were negative, indicating that high temperatures on these months will cause of lower maximum olive oil content. Similarly, for date of maximum olive oil content, temperatures in June, July and beginning of August showed also high VIP but positive model coefficients (Fig. 6B). Therefore, a temperature increase in this period could be associated to a delay the date of maximum oil reached. On the contrary, negative correlation of October and November temperatures with maximum oil date indicates that high temperatures during this period can accelerate the oil accumulation process.

To check if those correlations might differ among genotypes, independent analysis for 'Arbequina' and 'Koroneiki' were performed (Fig. 7). Maximum oil content showed correlation with the temperature in different periods depending on the genotype. 'Arbequina' showed negative correlation with temperature in August, September and beginning of October. For 'Koroneiki', temperature in all months considered showed a negative correlation with maximum olive oil. On the contrary, the critical periods of average daily temperature for date of maximum oil were similar for both cultivars, and similar to the obtained for genotypic average per environment (Fig. 6B).

4. Discussion

The expansion of olive tree to new environments and the possible climate change effects reinforce the need of investigating olive cultivars behaviour under different environmental conditions to gain some knowledge on the genetic and environmental influence on the expression of important agronomic parameters. For this reason, the use of multi-environment trails (MET) could be a useful tool to: evaluate the influence of genotype, environment and their interaction; determine the environmental limits of cultivation of each genotype; and to

Table 1
Percentage of variance components and significance in the ANOVA for olive oil accumulation parameters. $**P < 0.01$; $***P < 0.001$.

	Olive oil accumulation parameters			
	Maximum oil content	Rate of oil accumulation	Date of maximum oil content	Average ripening index at maximum oil content
Environment	17.5***	28.5***	44.7***	30.7***
Genotype	20.3***	4.4***	1.8	19.7***
GEI	23.7***	16.4***	2.7	15.88**
Residual	38.4	50.5	50.6	33.6

GEI (genotype by environment interaction).

Table 2

Comparison of means among the seven genotypes tested at the nine environments (ENV) for maximum oil content on fruit dry weight (%). Adaptability (*bi*) and stability (s^2d) analysis for genotypes are also indicated. Different letters for genotype (Mean[1]), environment (Mean[2]) and genotype-environment interaction ([3]) represent significant difference at $p < 0.05$ level based on Honestly-significant-difference (HSD).

Maximum olive oil content								
ENV	Genotypes							Mean[2]
	Arbequina	Arbosana	Carrasqueño	Koroneiki	Picual	Selection1	Selection2	
AN-15	49.1 abcdef[3]	45.6 cdef	48.1 abcdef	45.9 bcdef	45.5 cdef	48.1 abcdef	50.2 abcde	47.4 cd
AN-16	45.7 bcdef	45.7 cdef	44.8 cdef	47.9 abcdef	49.8 abcde	49.8 abcde	47.8 abcdef	47.3 cd
BA-16	47.8 abcdef		50.9 abcde	45.8 bcdef	52.8 abc	52.1 abcd	52.2 abc	50.2 ab
GI-15	50.8 abcde		54.2 a	45.3 cdef	50.7 abcde	53.5 ab	52.2 abc	51.1 a
GI-16	42.4 ef	43.9 def	53.6 ab	40.3 f	42.5 ef	48.4 abcdef	51.9 abcd	46.1 d
UB-15	47.7 abcdef	49.6 abcde	49.5 abcde	44.5 cdef	46.3 bcdef	49.4 abcde	53.5 ab	48.6 bc
UB-16	44.4 cdef	45.8 bcdef	49.3 abcde	40.6 f		49.6 abcde	46.3 bcdef	45.9 d
TA-15	40.2 f	51.2 abcd		48.5 abcdef		49.3 abcde	49.6 abcde	47.6 bcd
TA-16	52.7 abc	53.3 abc	53.4 abc	49.1 abcdef	54.4 a			52.6 a
Mean[1]	46.6 b	47.7 b	50.3 a	45.2 c	48.7 ab	50.1 a	50.4 a	
s*^{2}*d [4]	6.7	4.5	9.4	7.3	5.1	1.1	4.6	
bi [5]	1.3	1.2	0.6	0.8	1.5	0.8	0.7	

Nine environments (ENV): AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016.

[4] Stability according to Eberhart and Russell's.

[5] Adaptability using Finlay-Wilkinson index.

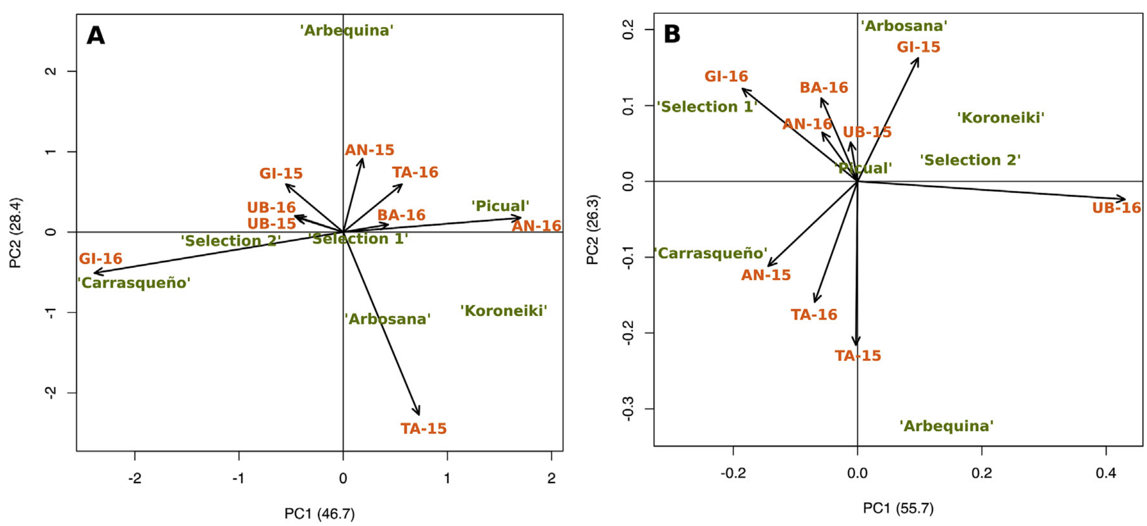

Fig. 4. AMMI model biplot for IPC1 vs IPC2 of maximum oil content on fruit dry weight (A) and rate of oil accumulation (B) for seven olive genotypes evaluated in nine environments (AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016).

elucidate which are the best genotypes for specific parameters in relation to stability and adaptability analysis. However, there are few reports on olive cultivar comparative trials and very scarce knowledge on the differential behaviour of olive cultivars under different environmental conditions. In this study, we use a set of comparative trials planted in different environmental conditions to evaluate the relative influence of genotype, environment and their interaction in olive oil accumulation parameters. To the best of our knowledge, this information from specifically designed comparative trials has not been previously reported for olive.

4.1. Environment, genotype and its interaction effects for olive oil accumulation

Previous reports have showed the significant influence of environmental pattern using years (Beltran et al., 2004; Mailer et al., 2007) or locations (Lavee and Wodner, 1991; Rondanini et al., 2014). In the present work, the effect of location and year, combined into a single environmental factor, showed significant differences for all the oil accumulation parameters tested. Interestingly, no clear separation by water availability (rainfed vs irrigation) was observed between environments. The genetic variance was also significant for all of the oil accumulation parameters except for date of maximum oil content. Consequently, all cultivars here studied could be harvested at same optimal time when planted in a given environment.

The highest genetic influence was observed for maximum oil content, with ‘Carrasqueño’ and the two breeding selections showing the highest values. Similar genetic effect for oil content has been previously reported for olive in cultivar trials in Italy (Camposeo et al., 2013), Argentina (Rondanini et al., 2014; Trentacoste et al., 2012), New Zealand (Mickelbart and James, 2003) or Tunisia (Allalout et al., 2011). On the other hand, lower genetic variance was observed for rate of accumulation, with ‘Selection 2’ having the highest rate of oil accumulation. Previous reports on that were contradictory, showing

Table 3
Comparison of means among the seven genotypes tested at the nine environments (ENV) for rate of accumulation of olive oil on fruit dry weight (%/day). Adaptability (*bi*) and stability (s^2d) analysis for genotypes are also indicated. Different letters for genotype (Mean[1]), environment (Mean[2]) and genotype-environment interaction ([3]) represent significant difference at $p < 0.05$ level based on Honestly-significant-difference (HSD).

Rate of olive oil accumulation

ENV	Genotypes							Mean[2]
	Arbequina	Arbosana	Carrasqueño	Koroneiki	Picual	Selection1	Selection2	
AN-15	0.28 cde[3]	0.21 cde	0.32 abcde	0.28 cde	0.29 bcde	0.29 cde	0.28 cde	0.27 cd
AN-16	0.26 cde	0.29 bcde	0.24 cde	0.28 cde	0.26 cde	0.31 abcde	0.29 cde	0.27 cd
BA-16	0.34 abcde		0.34 abcde	0.41 abc	0.41 abc	0.44 abc	0.44 abc	0.39 a
GI-15	0.18 de		0.15 e	0.32 abcde	0.28 cde	0.25 cde	0.32 abcde	0.24 d
GI-16	0.31 abcde	0.39 abc	0.42 acb	0.37 abcd	0.42 abc	0.43 abc	0.42 abc	0.39 a
UB-15	0.31 abcde	0.34 abcde	0.28 cde	0.28 cde	0.31 abcde	0.32 abcde	0.41 abc	0.31 bc
UB-16	0.42 abc	0.37 abcd	0.23 cde	0.49 ab		0.24 cde	0.49 a	0.35 ab
TA-15	0.44 abc	0.28 cde		0.33 abcde		0.35 abcde	0.41 abc	0.35 ab
TA-16	0.36 abcd	0.24 cde	0.34 abcde	0.32 abcde	0.33 abcde			0.33 abc
Mean[1]	0.31 bc	0.29 bc	0.27 c	0.33 ab	0.32 abc	0.32 bc	0.37 a	
s^2d [4]	0.0041	0.0025	0.0042	0.0033	0.0003	0.0029	0.0022	
bi [5]	1	1.09	1.02	0.84	1.03	0.95	1.09	

Nine environments (ENV): AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016.
[4] Stability according to Eberhart and Russell's.
[5] Adaptability using Finlay-Wilkinson index.

significant (Rondanini et al., 2014) or non-significant (Tentracoste et al., 2012) genetic influence on rate of olive oil accumulation. Those discrepancies might be caused by the different genetic materials and environments used in the different works reported.

Genotype by environment (GEI) interaction was significant for maximum oil content and rate of accumulation, with similar or even higher variance component than the genetic variance. In a previous report (Rondanini et al., 2014) the raltive importance of GEI for maximum oil accumulation in olive tree was commented, but this was based on commercial orchards and no on systematic comparative trials. As in other fruit crops, where significant GEI has been found for many agronomic parameters (Badenes and Byrne, 2012; Finn et al., 2003), the need of MET evaluation for an accurate description of the cultivars and breeding selections has been underlined (Hardner et al., 2016). For that, analysis of stability and adaptability indexes such as Eberhart and Russell's, Finlay-Wilkinson and AMMI models can be used for statistically testing the particular behaviour of each cultivars and breeding selections across environments and,thus, select the best genotype for the given environments, as widely used in annual crops (Coutino-Estrada and Vidal-Martinez, 2003; Ebrahimi et al., 2016; Gonzalez-Barrios et al., 2017). The results obtained for maximum oil content showed poor stability for some traditional cultivars such as 'Carrasqueño', 'Koroneiki' and 'Arbequina'. Low stability has been also reported for 'Arbequina' regarding oil quality parameters such as fatty acid composition (Torres et al., 2017). Altogether, these results underlined the potential risk associated to cultivation of traditional cultivars in new areas. On the contrary, high stability and large adaptability was obtained for the two advanced selections tested for maximum oil and rate of accumulation, which suggest the potential of breeding works to obtain new cultivars with improved suitability to wider environmental conditions. In any case, the distribution of cultivars and environments in the AMMI plot, for all the oil parameters measured indicates the need for testing the cultivars on a local basis during several years in order to really accurately determine the best cultivar for a given environmental conditions.

The relative importance of the genotype, environment and their interaction for the oil parameters evaluated could be of interest to improve the selection strategies in breeding programs. High genetic variance has been found for maximum oil content and low for the parameters related to the oil accumulation pattern. This indicates that it

Table 4
Comparison of means among the seven genotypes tested at the nine environments (ENV) for date (DOY) of maximum oil content on fruit dry weight. Different letters for environmental average (Mean[1]) represent significant difference at $p < 0.05$ level based on Honestly-significant-difference (HSD).

Date of maximum oil content

ENV	Genotypes							Mean[1]
	Arbequina	Arbosana	Carrasqueño	Koroneiki	Picual	Selection1	Selection2	
AN-15	320.7	327.7	317.1	329.2	320.7	322.9	324.3	323.2 a
AN-16	318.7	315.7	328.8	323.4	314.9		313.8	319.2 ab
BA-16	309.7		306.7	307.1	302.3	304.4	316.6	307.7 cde
GI-15	291.5		302.7	298.8	295.6	306.3	296.1	298.4 f
GI-16	307.6	299.8	298.4	302.2	295.6	302.7	304.4	301.5 ef
UB-15	314.5	315.4	307.4	318.7	320.3	316.6	311.6	314.9 bc
UB-16	303.6	313.8	326.3	302.8		326.3	302.3	312.3 bcd
TA-15	297.5	321.1		307.6		314.5	310.9	310.2 bcde
TA-16	301.2	309.7	301.6	300.7	300.5			302.7 def
Mean	307.1	314.6	310.9	309.9	307.0	313.3	309.9	

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016.

Table 5
Comparison of means among the seven genotypes tested at the nine environments (ENV) for average ripening index at the moment that maximum oil content is reached. Adaptability (bi) and stability (s2d) analysis for genotypes are also indicated. Different letters for genotype (Mean1), environment (Mean2) and genotype-environment interaction (3) represent significant difference at $p < 0.05$ level based on Honestly-significant-difference (HSD).

Average ripening index								
ENV	Genotypes							Mean[2]
	Arbequina	Arbosana	Carrasqueño	Koroneiki	Picual	Selection1	Selection2	
AN-15	2.65 abcdefg[3]	1.39 cdefgh	2.98 abcde	2.11 abcdefgh	2.73 abcdef	2.01 abcdefgh	3.24 abcd	2.44 abc
AN-16	3.97 a	1.39 cdefgh	2.84 abcdef	3.01 abcde	3.13 abcde	2.04 abcdefgh	3.87 ab	2.92 a
BA-16	3.02 abcde		3.61 abc	2.41 abcdefgh	3.37 abc	2.75 abcdefgh	2.31 abcdefgh	2.87 a
GI-15	2.55 abcdefgh		2.78 abcdef	1.11 efgh	2.05 abcdefgh	1.73 abcdefgh	1.42 cdefgh	1.94 cd
GI-16	1.43 cdefgh	0.84 fgh	1.36 cdefgh	0.66 gh	0.65 gh	0.37 h	1.29 defgh	0.88 e
UB-15	1.77 bcdefgh	1.24 defgh	2.11 abcdefgh	1.19 defgh	1.61 cdefgh	1.09 efgh	2.38 abcdefgh	1.62 d
UB-16	0.87 fgh	0.81 fgh	1.81 abcdefgh	1.29 defgh		1.71 abcdefgh	1.8 bcdefgh	1.42 de
TA-15	1.64 bcdefgh	1.26 defgh		2.31 abcdefgh		2.41 abcdefgh	2.72 abcdefg	2.11 bcd
TA-16	2.43 abcdefgh	2.03 abcdefgh	2.71 abcdef	2.32 abcdefgh	3.92 ab			2.74 ab
Mean[1]	2.32 a	1.22 c	2.59 a	1.81 b	2.51 a	1.66 bc	2.38 a	
***s^2d* [4]**	0.26	0.09	0.09	0.11	0.26	0.22	0.43	
***bi* [5]**	1.17	0.41	0.86	1.05	1.42	0.76	0.91	

Nine environments: AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016.
[4] Stability according to Eberhart and Russell's.
[5] Adaptability using Finlay-Wilkinson index.

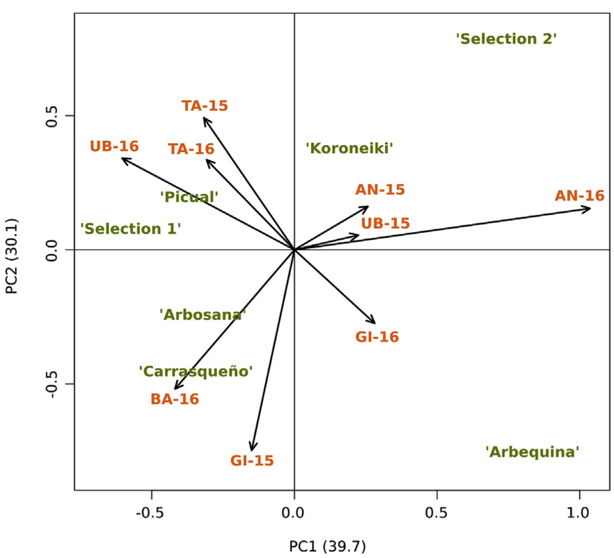

Fig. 5. AMMI model biplot for IPC1 vs IPC2 of average ripening index at the moment that maximum oil content is reached for seven olive genotypes evaluated in nine environments (AN-15: Antequera-2015; AN-16: Antequera-2016; BA-16: Baena-2016; GI-15: Gibraleón-2015; GI-16: Gibraleón-2016; UB-15: Úbeda-2015; UB-16: Úbeda-2016; TA-15: Tabernas-2015; TA-16: Tabernas-2016).

would be easy to get new breed cultivars with final high oil content, as already reported in some breeding programmes (Lavee et al., 2003; Rallo et al., 2008), but it would be more complicated to breed for cultivars with early or delayed oil accumulation pattern. However, the germplasm here evaluated is reduced to 7 cultivars. Probably, it might be possible to identify a stronger genetic variability on the pattern of oil accumulation by broadening the genetic base, as for example collecting data from a Germplasm Bank.

4.2. *Average ripening index as factor for optimum harvest time*

On the other hand, ripening index based on the colour of the skin and pulp, has been traditionally associated to the oil accumulation pattern and, therefore, used to determine the optimum harvest time. In our work, relationship between average ripening index and moment of oil maximum reached was not found. In fact, average ripening index in maximum oil content was very variable among environments and genotypes and also among their interaction. The environment was the main variation effect for average ripening, with values from green to veraison olives for the same cultivar. In fact, 'Arbequina' or 'Selection 2' showed the lowest stability for ripening, showing average ripening indexes from 1 to 4 at the time of maximum oil content. This indicates the limited usefulness of this index to determine the optimal time for harvesting. High influence of cultivar on the ripening index has been previously reported (Beltran et al., 2004).

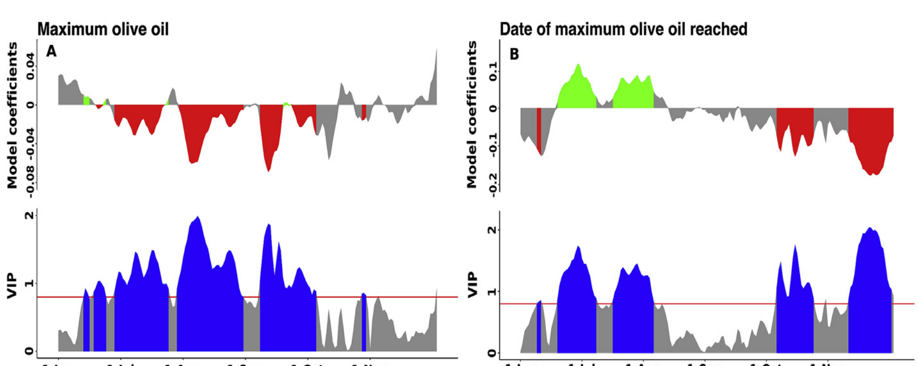

Fig. 6. Partial least square (PLS) regression of maximum olive oil (A) and date of maximum olive oil (B) with 11-days running mean of daily temperatures from 1st June to 30th November. Regression has been calculated with the means for each of the nine environments. Top plots indicate model coefficients, with red color being the negative coefficient and green-light color positive one. Bottom plots represents variable importance in the projection (VIP), being the values above 0.8 in blue color and the rest in grey. This 0.8 threshold for VIP is marked with a red line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

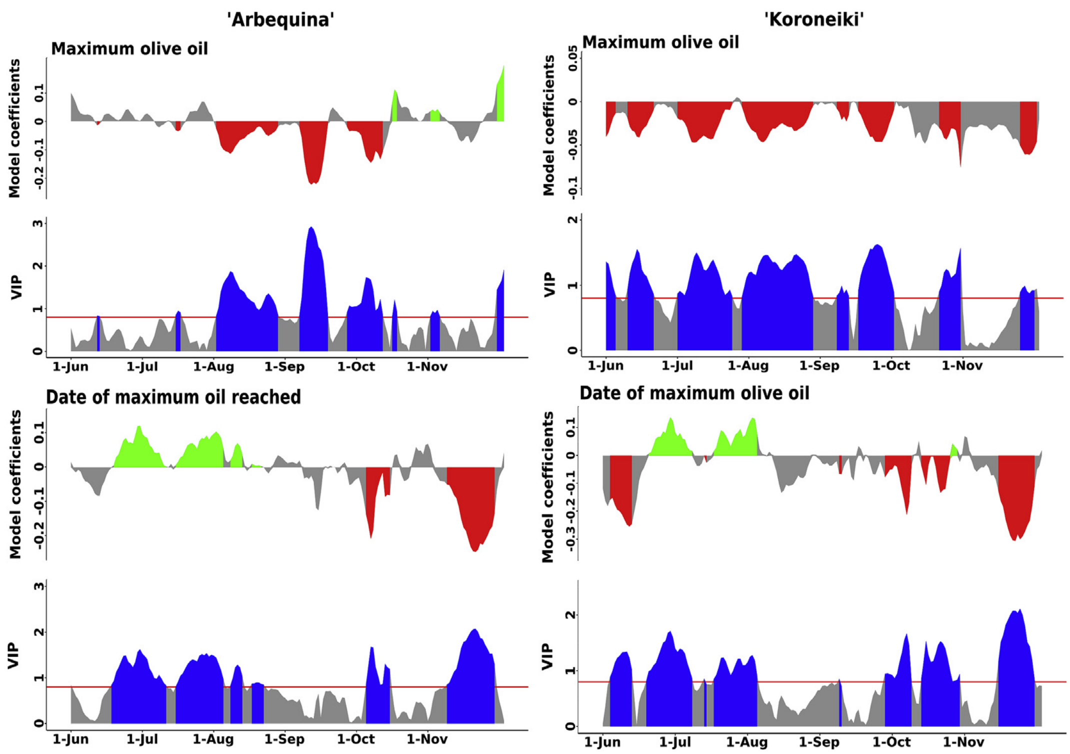

Fig. 7. Partial least square (PLS) regression for maximum oil content (top plots) and date of maximum (down plots) of 'Arbequina' (left plots) and 'Koroneiki' (right plots) with 11 day running mean daily temperatures from 1st June to 30th November. Top of each plot indicate model coefficients, with red color the negatives coefficients and green-light color positives. In the bottom of each plot is represented variable importance in the projection (VIP) with blue color indicate values above 0.8, the threshold for variable importance, marked with a red line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.3. Air temperature effects on olive oil accumulation

The high environmental variance found for most of the characters evaluated indicates the interest of modelling the oil accumulation pattern as affected by the climatic conditions. In particular temperature, which has been indicated to influence the oil accumulation process in several crops such as olive (Garcia-Inza et al., 2014; Rondanini et al., 2014; Trentacoste et al., 2012) or soybean (Rotundo and Westgate, 2009). Partial least square (PLS) is a useful statistical tool to relate climatic factors with phenological events, which has showed a great environmental effect. This approach has been previously followed mainly to associate daily average temperature with phenological events related to flowering (Luedeling and Gassner, 2012; Luedeling et al., 2013; Oteros et al., 2013). This information may be critical to forecast the possible phenological behaviours caused by temperature variation (El Yaacoubi et al., 2014), particularly due to climate change. In our study, PLS analysis showed that average temperature in summer months had a negative correlation with maximum oil content and positive with date of maximum. Negative effect of higher temperatures during lipogenesis on the final oil concentration has been previously reported (Garcia-Inza et al., 2014; Rondanini et al., 2014). Similar behaviour occurs for the oil synthesis in oilseed crops such as soybean, rapeseed or sunflower, where a high temperature effect has been also reported (Rotundo and Westgate, 2009; Triboi and Triboi-Blondel, 2002). However, the influence of summer temperature in maximum oil concentration seems to be cultivar dependent as is much stronger in 'Koroneiki' respect to 'Arbequina'. This agrees with the high GEI found for this character and seems to indicate higher potential heat susceptibility for some cultivars. Similar cultivar dependent heat effects have been reported for other important agronomic parameters relate to flowering (Gabaldon-Leal et al., 2017) and should be taken into account particularly considering projected temperature increases associated to current climate change (IPCC et al., 2018). On the contrary, the influence of the summer temperature on the date for maximum oil concentration is more consistent among cultivars, having this trait not significant GEI. Those results stress the usefulness of developing climatic models to forecast the pattern of oil accumulation on olive, although more data points including more environments and cultivars are needed to produce consistent modelling. We use the simplest calendar day approach to model oil accumulation trajectories, but the results suggest that a thermal time model could be more appropriate (Duchene et al., 2012). Nevertheless, positive and negative effect of temperature on both date and maximum oil content parameters indicates that non-linear thermal model would be needed (Zapata et al., 2015) which require further studies.

5. Conclusions

The high environmental effect found for all the olive oil accumulation parameters, as well as significative effect of genotype by environment interaction in most of them, indicate that multi-environment cultivar trials are essential tools for accurate selection of the best cultivars and new bred selections for a given environment and to test their stability across environments. Moreover, this type of trials will give a great opportunity to identify the best environments for olive cultivation as well as the potential effect of climate warming in the olive oil accumulation. In particular, the high genetic variance found for maximum oil content indicates that this character would easily respond to breeding selection while the high environment variance for the pattern of oil accumulation encourages the need for developing climatic models to predict their variability across environments. Future works should be done to more specifically investigate the relative influence of year and site on the parameters studied.

Authors' contribution

J.F. Navas-López: Writing- Original and Final draft preparation. L. León: Writing- Reviewing and Editing, Supervision. E. R. Trentacoste: Writing – Review & Editing, Methodology. R. de la Rosa: Writing-Reviewing and Editing, Supervision, Funding Acquisition.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This work has been partly supported by projects AVA201601.2, TRA201600.2 (IFAPA, Andalusian Institute of Agricultural Research and Training) and RTA2012-00018 (INIA, National Institute of Agricultural Research), partly funded by European Regional Development Fund. J.F. Navas-Lopez thanks to INIA-RTA2012-00018 for giving the opportunity to do this kind and important research study in Spain. It also thanks to co-author E. R. Trentacoste for sharing his bilinear model for this study.

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