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RESEARCH ARTICLE

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Phenological diversity in a World Olive Germplasm Bank: Potential use for breeding programs and climate change studies

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Abstract

Aim of study: Crop phenology is a critical component in the identification of impacts of climate change. Then, the assessment of germplasm collections provides relevant information for cultivar selection and breeding related to phenology, being the base for identifying adaptation strategies to climate change.

Area of study: The World Olive Germplasm Bank located at IFAPA Centre "Alameda del Obispo" (WOGB-IFAPA) in Cordoba (Southern Spain) was considered for the study.

Material and methods: Data gathered for nine years on flowering and ripening time of olive cultivars from WOGB-IFAPA were evaluated. Thus, full flowering date (FFD) for 148 cultivars and ripening date (RD) for 86 cultivars, coming from 14 olive growing countries, were considered for characterization of olive phenology and for calibration/validation of phenological models.

Main results: The characterization of WOGB-IFAPA has allowed the identification of cultivars with extreme early ('Borriolenca') and late ('Ulliri i Kuq') flowering as well as the ones with extreme early ('Mavreya') and late ('Gerboui') ripening dates. However, the very limited inter-cultivar variability, especially for FFD, resulted in a non-optimal simulation models performance. Thus, for FFD and RD the root mean square error was around 6 and 24 days, respectively. The limited inter-cultivar variability was associated to the low average temperatures registered during winter at WOGB-IFAPA generating an early accumulation of the chilling requirements, thus homogenizing FFD of all the analyzed cultivars.

Research highlights: The identification of cultivars with early FFD and late RD provides useful information for breeding programs and climate change studies for identifying adaptation strategies.

Additional key words: *Olea europaea* L.; *ex situ* collections; simulation models; crop phenology; adaptation measures.

Abbreviations used: DOY (day of year); FFD (full flowering date); RD (ripening date); RMSE (root mean square error); TT (parameter for considering heat requirements); TU (parameter for considering chilling requirements); WOGB-IFAPA (World Olive Germplasm Bank located at IFAPA Centre "Alameda del Obispo").

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Introduction

In the last years, numerous studies have confirmed the impact of climate change on the agricultural systems, especially in the Mediterranean environments located at Southern Europe (Koubouris *et al.*, 2009; Viola *et al.*, 2013; El Yaacoubi *et al.*, 2014; Ponti *et al.*, 2014; Dono *et al.*, 2016). Thus, the Mediterranean area is considered as one of the most impacted by climate change (Giorgi & Lionello, 2008; Giannakopoulos *et al.*, 2009), forecasting reductions in rainfall and a clear increase of heat and water stress events (Gabaldón *et al.*, 2017).

The use of crop simulation models is an innovative methodology to assess the impact of climate change on crops, especially in cereals (Pirtiojja *et al.*, 2015; Gabaldón *et al.*, 2016; Webber *et al.*, 2018). Equally, phenological models have been promoted in the last years as response to the necessity to predict the impact of climate change on critical phenological stages as flowering or ripening. Thus, for Mediterranean tree crops such as almond, vineyard or olive, advanced phenological models based on experimental data have been developed (de Melo-Abreu *et al.*, 2004; Parker *et al.*, 2011; Pope *et al.*, 2014; Gabaldón *et al.*, 2017). To carry out an accurate modelling of the impact of climate change on crops, intensive experimental activities in many growing regions with heterogeneous climatic conditions would be very useful for the development of advanced simulation tools considering all the key physiological and phenological components (Torres *et al.*, 2017; Navas *et al.*, 2018).

Olive tree has a capital importance in the economical sustainability of rural areas of most of the Mediterranean countries (Fernández-Escobar *et al.*, 2013). However, despite the crop´s importance, studies related to the impact of climate change on these systems, and the identification of adaptation measurements are still limited (Morales *et al.*, 2016; Gabaldón *et al.*, 2017; Lorite *et al.*, 2018). Previous studies evaluated the main impacts of climate change on olive phenology using experimental datasets from WOGB-IFAPA (De Melo-Abreu *et al.*, 2004; Gabaldón *et al.*, 2017) and on olive yield (Morales *et al.*, 2016; López-Bernal *et al.*, 2018). In this sense, phenology has been identified as a key component to determine the impacts of climate change and the possible adaptation measures. Thus, the evaluation of the weather conditions during flowering has allowed to identify the probability of occurrence of heat and/or water stress (Gabaldón *et al.*, 2017), the amount of flowering failure caused by the lack of chilling hours (De Melo-Abreu *et al.*, 2004; Aybar *et al.*, 2015; Morales *et al.*, 2016) or the identification of those phenological stages when irrigation must be applied considering controlled deficit irrigation strategies (Fereres & Soriano, 2007; Pierantozzi *et al.*, 2014). As a result, olive genotypes with late flowering date generated a higher probability of damage caused by heat and/or water stress, and the cultivation of genotypes with early flowering date was revealed to be a positive strategy (Gabaldón *et al.*, 2017).

Despite the relevance of the phenology in olive characterization, available experimental data on flowering date of different olive cultivars are still limited (Orlandi *et al.*, 2006; Aybar *et al.*, 2015; Torres *et al.*, 2017). Very few studies have been focused on the olive cultivar variability under the same reference environmental conditions (Sanz-Cortés *et al.*, 2002; Caballero *et al.*, 2006; Trentacoste & Puertas, 2011). In this sense, *ex situ* germplasm collections play an important role as they permit acquiring, maintaining, documenting and assessing the genetic and agronomic diversity of many cultivars in the same environmental conditions (Belaj *et al.*, 2012; 2016). This provides the opportunity for a general view of their diversity (Caballero *et al.*, 2006). However, information on the agronomical behavior of olive cultivars (Ozkaya *et al.*, 2006; Taamalli *et al.*, 2006; Hannachi *et al.*, 2008; Trentacoste & Puertas, 2011; Alba *et al.*, 2012; Di Vaio *et al.*, 2013; Ruiz-Dominguez *et al.*, 2013; Bodoira *et al.*, 2015, 2016) in germplasm collections is still limited. The use of olive germplasm for identifying adaptation measures to climate change has not been reported yet, being limited for the rest of crops (Egea *et al.*, 2017).

To carry out studies related to the crop behavior under future weather conditions, the consideration of simulation models has been frequent (Webber *et al.*, 2018). For phenology simulation, the consideration of dynamic models overlapping chilling and heat accumulation stages (Pope *et al.*, 2014) focusing on dormancy release (Andreini *et al.*, 2014) has been frequent. However, phenological models for olive are limited and focused on a small number of cultivars (de Melo *et al.*, 2004; Gabaldón *et al.*, 2017). The simulation of two independent stages (endodormancy and ecodormancy), or the consideration of mainly heat accumulation (Fornaciari *et al.,* 1998; El Yaacoubi *et al.*, 2014) have been the most common methodologies considered for olive.

The World Olive Germplasm Bank located at IFAPA Centre “Alameda del Obispo” (WOGB-IFAPA) in Córdoba (Spain), was established around 50 years ago and represents the first international attempt of conservation and management of the olive germplasm through a FAO-INIA project and with the International Olive Council (IOC) support (Caballero *et al.*, 2006; Belaj *et al.*, 2016). At present, this collection accounts around 900 accessions from 26 countries (Belaj *et al.*, 2016) and is an international reference on olive germplasm due to the high number of accessions included and their high degree of identification and evaluation (Barranco *et al.*, 2005; Belaj *et al.*, 2012; 2016; Trujillo *et al.*, 2014). The agronomical evaluation of olive cultivars maintained in the collection has shown that they may be a useful source of diversity for important traits related to vigor, production, fruit characters (Barranco *et al.*, 2005; Caballero *et al.*, 2006; Belaj *et al.,* 2012) as well as oil content and composition (Beltrán *et al.*, 2016; León *et al.*, 2018).

The objective of this study was to evaluate the phenological data of a set of 150 olive cultivars (148 for full flowering date [FFD], and 86 for ripening date [RD] characterization) grown under the same environmental condition*s* in the WOGB-IFAPA, during 9 years in the period 1994-2008 (2002, 2003, 2004, 2005, 2006 and 2008 for calibration, and 1994, 1995 and 1999 for validation of the models). This characterization will enable to get a better knowledge on the olive cultivar variability in terms of phenology, and it will allow thus to classify them depending on the phenology, identifying the cultivars with potential use for breeding programs and for the development of adaptation measures to climate change. Besides, a spatial analysis of the phenology for each of the cultivars under study was also carried out. Finally, two flowering models (De Melo-Abreu *et al.*, 2004; Gabaldón *et al.*, 2017), and a ripening model have been parameterized with the experimental data for the 150 olive cultivars analyzed in this study.

Material and methods

The World Olive Germplasm Bank of "Alameda del Obispo" (WOGB-IFAPA)

The phenology data came from the WOGB-IFAPA, located in Córdoba, Southern Spain (37º 51' 39" N, 4º 48' 30" W). The data set included only previously identified cultivars by means of both morphological and molecular markers (Atienza *et al.*, 2013; Trujillo *et al.*, 2014; Belaj *et al.*, 2018). These cultivars were introduced at different moments in the collection (1987-2002). Trees were planted at 7 × 7 m and grown in the same edaphoclimatic conditions, using drip irrigation and standard cultural practices. The selection of these cultivars was carried out based on data availability during each one of the six years considered for calibration in the period 2002-2008. Thus, the present study included FFD and RD for 148 and 86 olive cultivars respectively, being 150, the total number of cultivars evaluated (Table S1 [suppl.]). All these cultivars had Mediterranean origin except one, native to Southern America (Fig. 1 and Tables S1, S2 and S3 [suppl.]): Albania (ALB), Chile (CHL), Croatia (HRV), France (FRA), Greece (GRC), Israel (ISR), Italy (ITA), Lebanon (LBN), Morocco (MOR), Portugal (PRT), Spain (SPA), Syria (SYR), Tunisia (TUN), and Turkey (TUR).

For all the cultivars considered, at least two trees were analyzed for every year, although, due to varietal

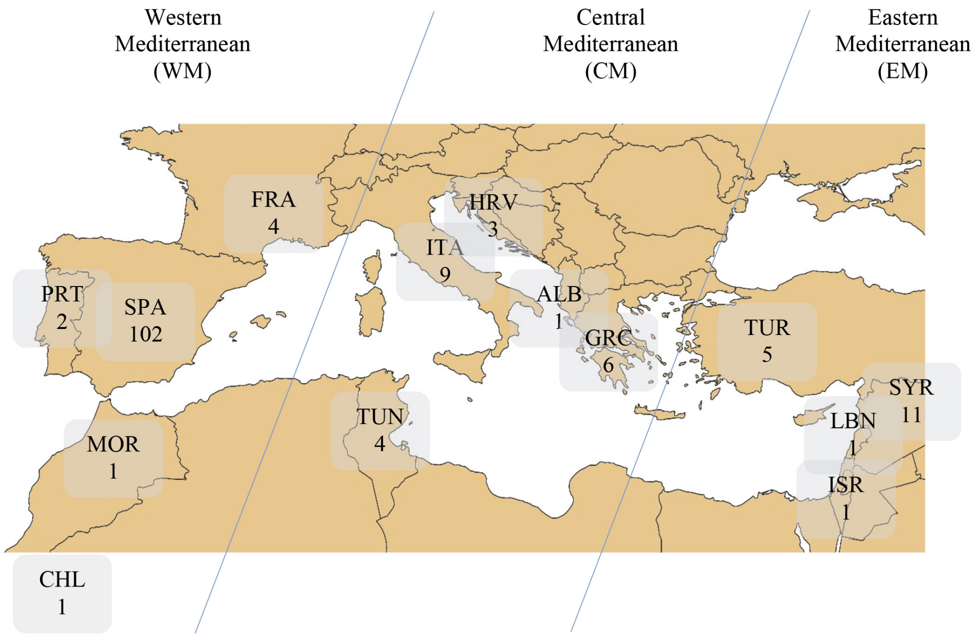

Figure 1. Map showing the origin of the 150 cultivars considered in this study (Albania, ALB; Chile, CHL; Croatia, HRV; France, FRA; Greece, GRC; Israel, ISR; Italy, ITA; Lebanon, LBN; Morocco, MOR; Portugal, PRT; Spain, SPA; Syria, SYR; Tunisia, TUN and Turkey, TUR), number of cultivars analyzed by country, and zones delimitated within the Mediterranean basin. The olive live cultivar from Chile was not considered in the spatial analysis.

Table 1. Average full flowering date (FFD) and ripening date (RD) for representative cultivars. DOY indicates the day of the year.

Flowering group	Representative cultivar	FFD (DOY)	Ripening group	RD (DOY)
F1	Borriolenca	114.9	R6	304.0
F2	Empeltre	118.6	R4	292.4
F3	Arbequina	119.6	R8	314.5
F4	Manzanilla Cacereña	119.8	R5	298.3
F5	Nevado Azul	120.6	R4	292.1
F6	Picholine Marocaine	121.0	R9	320.8
F7	Frantoio	121.4	R5	301.4
F8	Bolvino	122.1	R2	284.9
F9	Picual	123.2	R7	312.3
F10	Blanqueta	123.8	R5	300.1

redundancies in the collection (Atienza *et al.*, 2013; Belaj *et al.*, 2018), the number of trees considered for some cultivars such as 'Frantoio', 'Gordal de Granada', 'Manzanilla', 'Mollar de Cieza', 'Ocal', 'Picholine Marocaine' or 'Verdial de Badajoz' reached more than 10. Cultivars were pooled in ten groups (Fig. 1 and Fig. S1, Table S1 and Table S2 [suppl.]) depending on their full flowering (F1 to F10) or on their ripening date (R1 to R10) and including all the ranges from the earliest flowering and ripening date (Groups F1 and R1) to the latest ones (Groups F10 and R10), respectively. 'Borriolenca', 'Empeltre', 'Arbequina', 'Manzanilla Cacereña', 'Nevado Azul', 'Picholine Marocaine', 'Frantoio', 'Bolvino', 'Picual' and 'Blanqueta' were the representative cultivars of these groups (Table 1).

Data collection

Weather data were collected by a weather station included in the Agroclimatic Information Network of Andalusia (RIA) and located in the experimental field IFAPA-Alameda del Obispo (Gavilán *et al.*, 2006), the same farm where the WOGB orchard is grown, and by an additional weather station managed by AEMET (State Agency of Meteorology) in Córdoba Airport.

The flowering data were provided from the WOGB-IFAPA database (Del Río & Vallejo, 2005). These phenology data were recorded following the procedure described by Barranco *et al.* (2005). Thus, flowering data were recorded every three days identifying successive phenological stages from winter rest period to fruit set. In every scoring day, the most delayed, frequent and advanced phenological stage was recorded for each tree. The BBCH scale was used for the phenological data analysis (Sanz-Cortés *et al.*, 2002). For that, the original data recorded according to De Andrés (1974) phenological scale were translated to the BBCH scale using the already established correspondence (Sanz-Cortés *et al.*, 2002). Thus, FFD was calculated as the number of days between the day when phenophase 61 (beginning of flowering, 10% open flowers) appeared as most common for the first time, until the last day when phenophase 65 (full flowering, at least 50% open flowers) was found as most common. Full bloom date was then calculated as the average date found on full bloom period. Data for ripening stage of fruits were recorded according to the ripening index described by Frías *et al.* (1991). This method is based on color changes of peel and pulp classified into eight groups or categories: green intense (0), yellow or yellowish green (1), green with reddish spots (2), reddish or light violet (3), black with white pulp (4), black with <50% purple flesh (5), black with ≥50% purple flesh (6) and black with 100% purple flesh (7). Ripening observations were carried out around the canopy at weekly intervals from September and characterized, as in the case of flowering, by three numbers representing the most delayed, abundant and advanced categories observed, respectively. From these determinations, RD was calculated as the date in which the most abundant category observed change from 2 to 3 (De la Rosa *et al.*, 2008).

Only trees with similar flower intensity and crop load were evaluated in this work to minimize as much as possible the potential influence of alternate bearing. Moreover, the early harvest date and the irrigation management carried out in the experimental fields of the WOGB-IFAPA are designed also to reduce potential effects of alternate bearing.

As the consideration of correct experimental data is critical for achieving accurate simulation models, a detailed evaluation of available experimental data is required. For our study, detailed evaluation of metadata of the whole dataset was done, removing those years with poor flowering or with doubts about the quality of the measurements. The removal of these years was done to avoid including in the simulation models wrong components that could mask relevant processes.

Variability analysis

ANOVA analysis was performed to evaluate the relative contribution of cultivar and year on FFD and RD variability. Spearman Rank correlation was used to determine the stability of the cultivar rank order among years for both variables. Correlation (Pearson) was done between the two variables under study.

To determine the influence of the cultivar origin on the phenology, cultivars under study were grouped in West, Centre and East- Mediterranean geographic areas (Fig. 1). These three Mediterranean areas have been defined as the main gene pools in olive germplasm (Haouane *et al.*, 2011; Belaj *et al.*, 2012; Besnard *et al.*, 2013). Besides, a clustering of olive cultivars according to their putative geographical distribution has also been evidenced (Belaj *et al.*, 2016). The olive cultivar from Chile was not considered in the spatial analysis.

Phenological models

For assessing full flowering date, two simulation models were calibrated and validated for the 148 cultivars previously described. The first one was developed by De Melo-Abreu *et al.* (2004) and is based in the computation of chilling and heat accumulation units. When the required accumulated chilling hours (TU) and the required thermal time until flowering (TT) are achieved, the flowering happens. Thus, TU is computed depending on the hourly temperature considering the rate of increase described in Fig. 2a and TT considering the mean daily temperature with the rate of increase described in Fig. 2b. This model is called thereafter Chilling+Heat flowering model. The second one was developed by Gabaldón *et al.* (2017) and uniquely considers heat accumulation units until a threshold is achieved (TT parameter) and then, flowering happens. TT parameter is computed following the rate of increase described in Fig 2b. This model is called thereafter Heat flowering model. For assessment of ripening date, a simple model was developed computing heat accumulation until a threshold (TT parameter), when ripening of the fruits is achieved as defined above. Similarly, TT parameter is computed following the rate of increase described in Fig 2b.

TU and TT parameters for Chilling+Heat flowering model and TT for Heat flowering and Ripening models were calculated for each cultivar, minimizing the error in the flowering/ripening date assessment using Root Mean Square Error (RMSE) as statistic. Due to the small number of parameters considered, the calibration procedure to obtain the parameter set that minimized the RMSE did not consider any computational procedure.

For the calibration of phenological models, six years in the period 2002-2008 were considered (year 2007 was not included in the study due to a very poor flowering and fruiting set observed). For the validation of the phenological models three years in the period 1994-1999 were considered (years 1996, 1997 and 1998 were not included for the same reason than 2007).

In order to assess the performance of flowering and ripening models by comparing simulated and observed

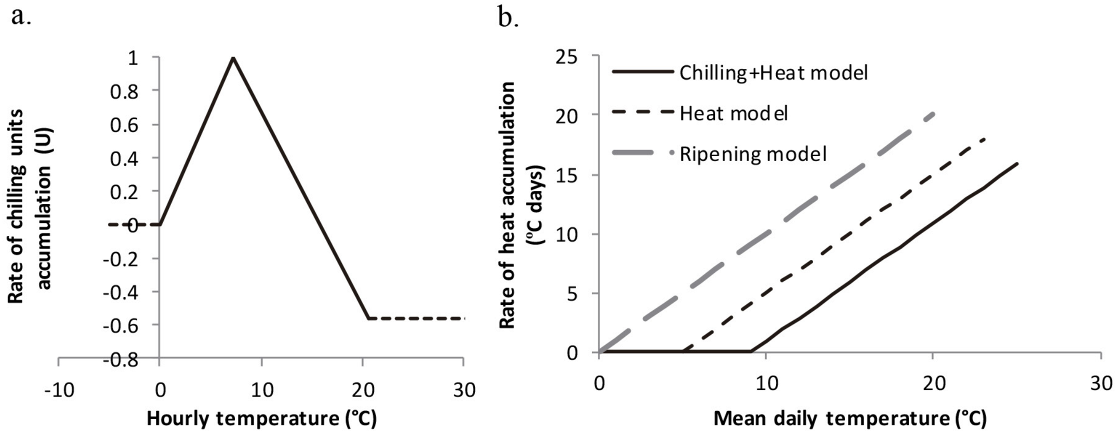

Figure 2. Rates of chilling unit accumulation for Chilling+Heat model (a) and rates of heat accumulation for Chilling+Heat, Heat and Ripening models (b).

values, the RMSE statistic was considered. This statistic is defined as:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(S_i - 0_i)^2}{n}} \quad [1]$$

where S_i is the simulated full flowering/ripening date value, O_i is the observed value, and n represents the number of data. De Melo-Abreu *et al.* (2004) reported RMSE values around 2.4 days using phenological models to assess flowering dates with satisfactory performance.

Results

Full flowering (FFD) and ripening date (RD)

The analysis of variance indicates that the three factors evaluated, *i.e.*, cultivar and year, and their interaction, had significant effect in both FFD and RD (Table 2). However, in the case of FFD, most of the variance was due to year variability, even though the number of cultivars was much higher than the years. The average FFD values by year ranged from DOY 118 (2008) to DOY 124 (2005) with mean average value of DOY 121 (May 1st) (Table 3). On the contrary, a narrow variability for FFD was observed for the 148 cultivars under study (Table 1, Table 3 and Fig. 3, and Table S1 [suppl.]) during the 2002-2008 period. The low cultivar variability in FFD was confirmed by the fact that, for 97% of the cultivars analyzed, the average FFD ranged between DOY 117 and 124. Even more, the FFD of 84% of the cultivars was concentrated in the range of 4 days (DOY 119-123). However, some exceptions with earlier (‘Borriolenca’, ‘Canetera’, ‘Vallesa’ and ‘Figueretes’) and later (‘Ulliri i Kuq’) flowering cultivars were also observed (Table S1 [suppl.]). Analyzing ripening date for the 86 cultivars under study during the same period (2002-2008), average RD values ranged from DOY 292 (for 2002 and 2003) to DOY 313 (2004) with mean average DOY value of 301 (Table 3). When considering the cultivars (Table S1 [suppl.]) the average RD values varied from DOY 272 (‘Mavreya’) to DOY 330 (‘Gerboui’).

The significant interaction cultivar by year was confirmed by the differences in FFD between the earliest and latest olive cultivars by year. This ranged from 8 days (from DOY 113 to DOY 121, in 2008) to 17 days (from DOY 111 to DOY 129, in 2004) (Table 3). Analyzing by cultivar, the average range of FFD in the analyzed period was equal to 7.1 days, ranging from 4 days (‘Picual’, ‘Majhol 152’, ‘Escarabajuelo de Atarfe’ y ‘Escarabajuelo de Posadas’) to 13.5 days (‘Plementa Bjelica’) (Table S1 [suppl.]). Besides, the rank of cultivars according to their full flowering date was calculated for each year’s data. The correlation among the ranks obtained in the different years under study was very low (Spearman rank correlation between 0.34 and 0.69).

Cultivar was the factor with the highest contribution to total variance for RD. Accordingly, the Spearman correlations between the ranks of the cultivars in the different years under study were higher (between 0.56 and 0.72) than for FFD. The average range of RD by cultivar, in the period considered, was equal to 33 days, ranging from 10.5 days (‘Leccino’) to 63.5 days (‘Gordal de Velez Rubio’) (Table S1 [suppl.]). Differences in RD between the earliest and latest olive cultivars varied according to the year, with the largest range for 2005 (82 days, from DOY 268 to DOY 350) and the smallest for 2008 (55 days, from DOY 272 to DOY 327; Table 3).

Table 2. Variance components (%) for full flowering date (FFD) and ripening date (RD) for the cultivars evaluated in the 6 years under study. All factors (cultivar, year and their interaction) had a significant effect (p< 0.001).

Factor	FFD	RD
Cultivar	18.0	37.3
Year	41.8	19.8
Cultivar*Year	10.5	6.3
Error	29.7	36.6

Table 3. Average full flowering date (FFD) and ripening date (RD) by year, including the range of variation. DOY indicates de day of the year.

Year	FFD			RD		
	Avg. DOY	Max	Min	Avg. DOY	Max	Min
2002	120.0	128.0	114.0	292.4	334.3	256.5
2003	121.6	125.0	111.0	291.5	325.0	254.5
2004	120.8	128.5	111.3	313.1	353.3	270.3
2005	124.0	129.0	118.5	308.8	349.5	268.0
2006	121.2	125.0	116.0	299.2	342.0	271.5
2008	117.6	121.0	113.0	301.0	326.5	271.5
Avg.	120.9			301.0		

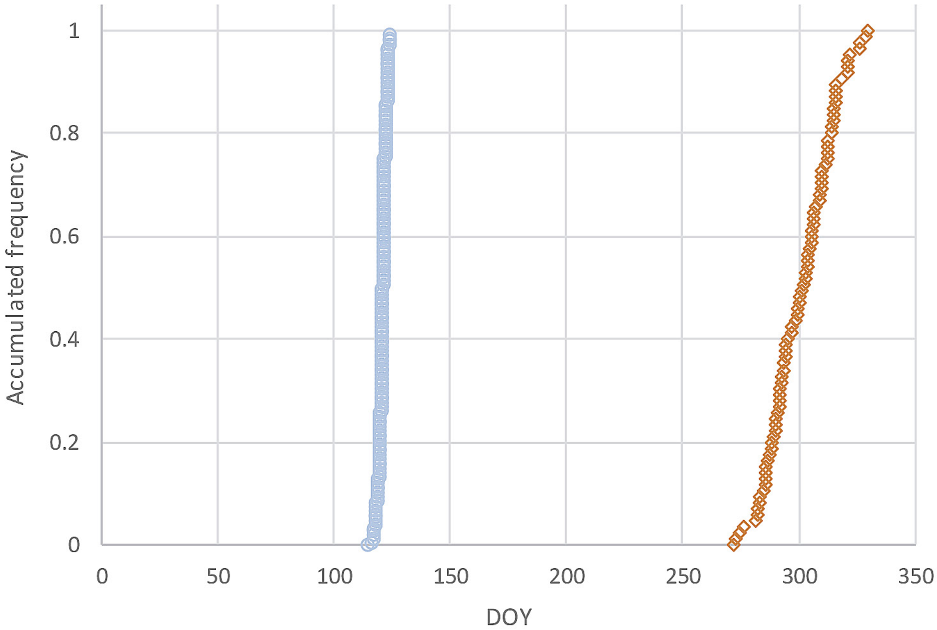

Figure 3. Inter-genotype full flowering date (FFD; in cyan) and ripening date (RD; in orange) variability for 148 and 86 genotypes, respectively. DOY means Day of Year

Correlations between FFD and RD were calculated at tree level by year. Pearson coefficients obtained were very low, ranging between 0.007 and 0.140, indicating the lack of relationship between those parameters. In addition, the comparison of FFD and RD for each cultivar led to the identification of cultivars with valuable phenology in terms of adaptation to climate change (*i.e.* early flowering date) or/and harvest (*i.e.* late harvest date) (Fig. 4). 'Maurino', 'Bolvino', 'Majhol1059', 'Kalamon', 'Dokkar' and 'Leccino' showed later flowering and early ripening. In the opposite site, 'Arbequina', 'Verdial de Badajoz', 'Bouteillan' and 'Gerboui' showed early flowering and late ripening (Fig. 4).

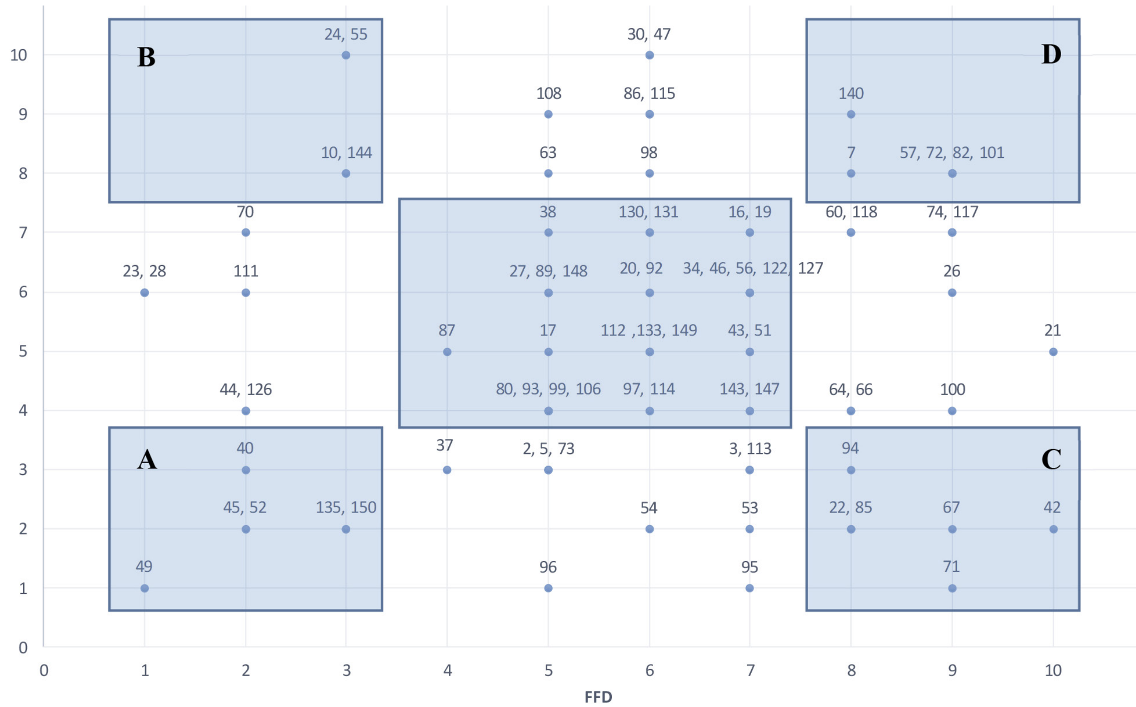

Figure 4. Comparison of beginning of full flowering (FFD) and ripening (RD) dates for each analyzed genotype. Codes for each cultivar are shown in Table S1 [suppl.]. A, B, C and D mark the cultivars with early FFD and early RD, early FFD and late RD, late FFD and early RD, and late FFD and late RD, respectively.

Finally, a spatial analysis of the origin of the cultivars was carried out. Thus, three regions within the Mediterranean basin were considered (Fig. 1) to evaluate differences in FFD and RD depending on the cultivar origin. Averaged FFD was very similar for the three regions, DOY 121, indicating thus a lack of clear pattern between phenological data and the origin of the cultivars under study. Thus, analyzing the cultivars with the latest FFD, diverse origins were found such as 'Ulliri i Kuq' (Albania), 'Blanqueta' (Spain), 'Dokkar' (Tunisia) and 'Itrana' (Italy) (Table 1 and Table S1 [suppl.]). Similarly, no-spatial pattern was detected for RD, although more differences between regions were found, with values ranging from averaged DOY 293 in cultivars from Central Mediterranean region to averaged DOY 307 from Western Mediterranean region.

Cultivar parameterization for assessing full flowering date

Chilling+Heat flowering model

Average chilling requirements (TU) was equal to 279 U, ranging from 256 U ('Blanqueta' and 'Fulla de Salze') to 313 U ('Vera'). Averaged heat requirements (TT) were equal to 313 U, ranging from 291 U ('Borriolenca', 'Canetera' and 'Vallesa'), to 349 U ('Dokkar') (Table 4 and Fig. 5a, and Table S4 [suppl.]).

The proposed parameterization described in Tables 4 and S4 [suppl] generated an averaged RMSE equal to 4.9 days, ranging from 3.6 days (for 'Leccino') to 6.4 days ('Chetoui' and 'Manzanilla de Abla'). The performance of the validation varied with the year, ranging from averaged RMSE equal to 5.1 days in 1995 to 9.1 days in 1994, with an averaged RMSE for the whole validation period of 7.1 days (Table 5). This pattern was similar for all the analyzed cultivars. Thus, for example, the differences between observed and simulated FFD for 'Picual' varied from 1 day in 1995 to 9.3 days in 1994 (data not shown).

Heat flowering model

Averaged heat requirements (TT parameter) were equal to 583 U, ranging from 506 (for 'Borriolenca' cultivar) to 624 U (for 'Ulliri i Kuq' cultivar) (Table 4 and Fig. 5b, and Table S4 [suppl.]). The TT values for each cultivar generated an averaged root mean square error (RMSE) equal to 5.2 days, ranging from 3.7 days (for 'Changlot Real', 'Carrasqueño de Alcaudete' and 'Negrillo Redondo' cultivars) to 6.9 days (for 'Joanenca' cultivar).

The validation provided RMSE values from 3.7 days (in 1994) to 7.3 days (in 1995), with an averaged RMSE equal to 5.2 days (Table 5). This pattern was very similar for all the analyzed cultivars. For example, for 'Picual' differences ranged from 1.3 days (in 1994) to 13.0 days (in 1995).

Table 5. Averaged and annual root mean square error (RMSE, days) generated for each model (Flowering Chilling+Heat (C+H), Flowering Heat (H) and Ripening) in the validation process

Year	Flowering C+H	Flowering H	Ripening
1994	9.1	3.7	19.5
1995	5.1	7.3	22.9
1999	7.0	4.5	30.2
Avg.	7.1	5.2	24.2

Table 4. Optimum parameterization (chilling and heat requirements, TU and TT, respectively) for Flowering Chilling+Heat (C+H), Flowering Heat (H) and Ripening models, and root mean square error (RMSE) for representative cultivars sorted by full flowering date.

Flowering group	Representative cultivar	Flowering C+H			Flowering H		Ripening	
		TU	TT	RMSE (days)	TT	RMSE (days)	TT	RMSE (days)
F1	Borriolenca	288	291	5.6	506	5.0	5663	25.7
F2	Empeltre	304	319	5.0	553	5.0	5506	16.8
F3	Arbequina	290	324	4.6	568	5.8	5835	21.0
F4	Manzanilla Cacereña	284	324	4.9	570	4.4	5611	16.9
F5	Nevado Azul	290	332	5.2	579	4.8	5512	16.8
F6	Picholine Marocaine	274	334	4.7	584	5.2	5933	18.1
F7	Frantoio	271	334	4.7	589	5.1	5644	18.9
F8	Bolvino	271	334	4.8	599	4.8	5389	15.2
F9	Picual	270	343	5.4	611	5.0	5823	15.4
F10	Blanqueta	256	344	5.5	615	5.0	5624	19.8

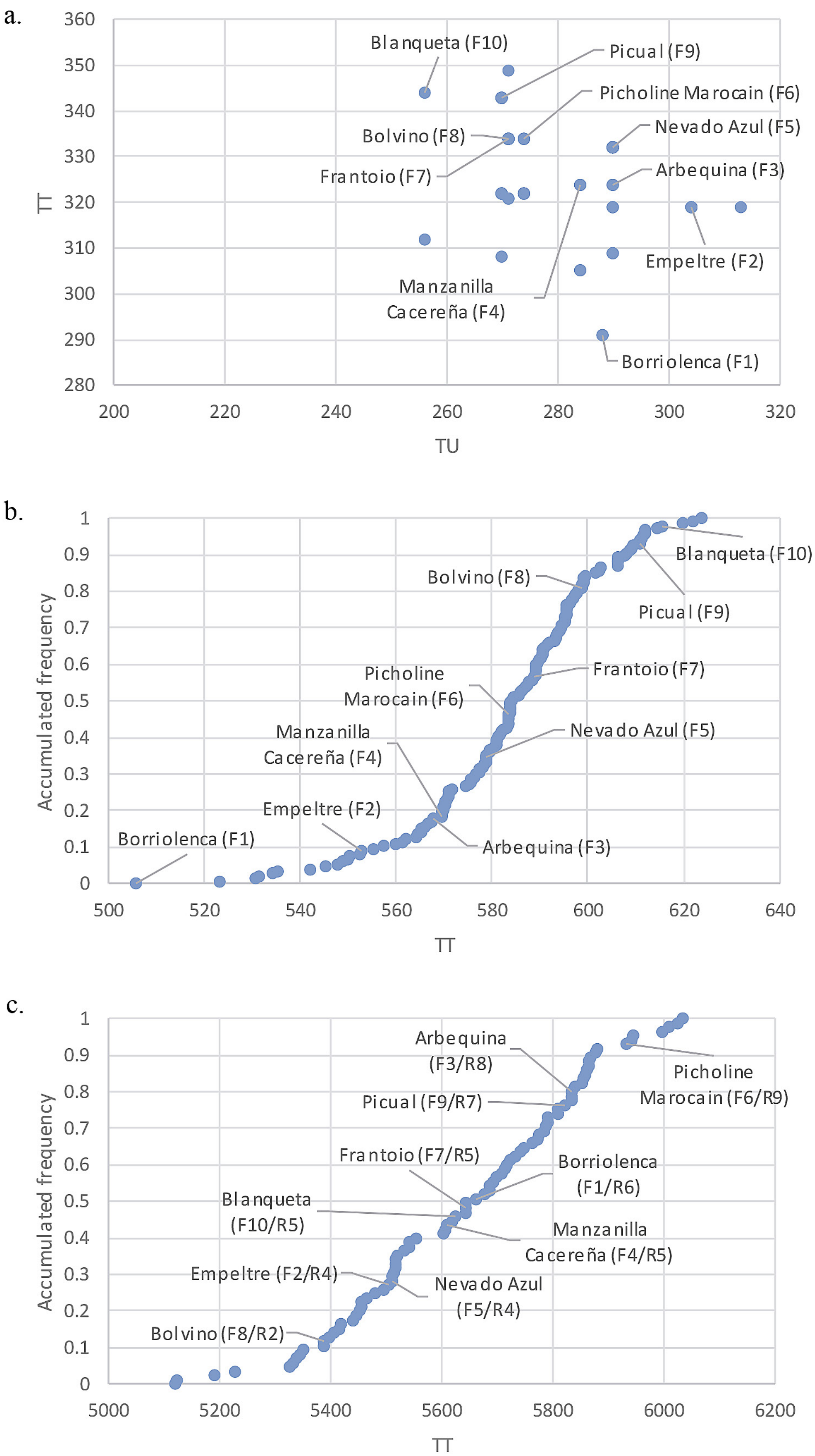

Figure 5. Parameterization of the 150 genotypes for Flowering Chilling+Heat (C+H) (a), Flowering Heat (H) (b) and Ripening (c) models.

Cultivar parameterization for assessing ripening date

Average TT was equal to 5638 U, ranging from 5120 U (for 'Figueretes') to 6034 U (for 'Gerboui') (Fig. 5c). The TT values for each cultivar (Tables 4 and S4 [suppl.]) generated an averaged root mean square error (RMSE) for ripening date assessment equal to 16.2 days, ranging from 5.8 days to 25.7 days (for 'Leccino' and 'Borriolenca', respectively).

Validation results provided differences depending on the year. Thus, RMSE ranged from 19.5 (in 1994) to 30.2 days (in 1999), with averaged RMSE values of 24.2 days (Table 5). Similar behavior was found analyzing each cultivar. For instance, for 'Picholine Marocaine' the differences ranged from 7.3 days (in 1994) to 30 days (in 1995).

Discussion

Adaptation measures to climate change are required under Mediterranean environments as climate projections describe a clear increase in the temperatures (Giorgi & Lionello, 2008), that will aggravate negative impacts related to water and heat stress on the crops. However, the identification of adaptation measures to climate change for tree crops is not an easy task due to the limited flexibility in agricultural practices compared with those for annual crops, such as modifications in sowing date or crop cycle selection (Gabaldón *et al.*, 2015; Ruiz-Ramos *et al.*, 2018). Thus, for new olive orchards the identification of cultivars with early flowering date (Gabaldón *et al.*, 2017) or/and with low chilling requirements (De Melo-Abreu *et al.*, 2004; Torres *et al.*, 2017) emerge as some of the most promising adaptation measures. This may be possibly related to the fact that heat and water stress impacts during the critical period of flowering and irrigation requirements (considering controlled deficit irrigation strategies) will be reduced (Gabaldón *et al.*, 2017; Lorite *et al.*, 2018). Equally, issues related to ripening could be considered; cultivars with early ripening could be impacted by damages in the olive trunk generated by the mechanized harvest process as sap flow reduction only is done under cold temperatures. Moreover, high temperatures during harvesting and fruit processing in mills may also represents some disadvantages regarding oil quality (Torres *et al.*, 2017). Then, cultivars with late ripening would be desirable in a future climate warming scenario.

In the search of cultivars with early flowering date and late ripening, optimal phenology adapted to future climate scenarios, the use of germplasm collection´s diversity could be an interesting option. Thus, analyzing the dataset obtained from WOGB-IFAPA, cultivars such as 'Arbequina', originally from Northeastern of Spain (Fernández i Martí *et al.*, 2015), showed an early flowering date and late ripening, and confirm the recommendations carried out in previous studies (Gabaldón *et al.*, 2017). However, at WOGB-IFAPA, under the weather conditions of continental Southern Spain, a low variability of full flowering dates was observed between the 148 cultivars under study. Thus, for the analyzed period, limited differences in flowering date between the earliest and the latest cultivar (12 days in average) and a significant interaction between cultivar and year was observed. Similar overlapping of flowering dates was found previously by Barranco *et al.* (2005) in Córdoba (Spain), by Trentacoste & Puertas (2011) in Mendoza (Argentina) as well as by Vuletin-Selak *et al.* (2018) in Split (South-West Croatia). The limited variability observed in full flowering date between cultivars from the WOGB-IFAPA may be related to the meteorological conditions of Córdoba (cold winters and warm springs). Besides, the overlapping in flowering date may represent an adaptation of the cultivars to ensure cross pollination as most of them are self-incompatible (Díaz *et al.*, 2007). In addition, these results and the lack of a clear pattern between phenology and the origin of the cultivars may indicate that flowering phenology has not been a selection criterion in olive growing regions.

The lack of genetic variability detected in this study seriously hampered the possibility to include early flowering as a selection character on breeding programs and the development of adaptation strategies to climate change. It contrasted with the significant genetic variability found for other plant, fruit and oil quality traits on olive cultivars (Ben Sadok *et al.*, 2013; León *et al.*, 2016; De la Rosa *et al.*, 2016). As consequence of this lack of variability, no-recommendations related with earliness of flowering can be provided as all the genotypes showed similar phenological characteristics, especially for the most-common grown olive cultivars (average full flowering date was DOY 123, DOY 120 and DOY 121 for 'Picual', 'Arbequina' and 'Frantoio', respectively). Considering fewer common cultivars as 'Borriolenca' (DOY 115), 'Canetera' (DOY 116) or 'Vallesa' (DOY 117), some variability could be found, although other agronomic traits and their behavior under different environmental conditions (Navas *et al.*, 2018) should be considered in order to recommend those cultivars. Flowering time is greatly influenced by environment. Flowering normally occurs in a time when high air temperatures and water stress are frequent. Therefore, to breed for earliness of flowering seems very convenient as a way of escaping from stresses that could affect normal fecundation and initial fruit set. Considering that chilling requirements and flowering date are clearly related, the generation of early flowering cultivars implies indirect selection for low chilling requirements.

In this study, two traits were considered to characterize the phenological diversity of a World Olive Germplasm Bank; FFD and RD. However, other components such as alternate bearing or fruit set have a critical relevance. Although effects of alternate bearing on olive yield have been described (Lavee, 2007; Rojo *et al.*, 2015), the agronomical practices carried out in the WOGB such as the early harvest and the irrigation management (Lodolini *et al.,* 2016), have reduced the incidence of alternate bearing on olive yield and phenology. However, the limited available data avoided the consideration of alternate bearing or fruit set in the modelling process carried out in the current study. Future studies combining all these traits are required.

The variability in flowering date could be significantly increased by including wild genetic resources (Klepo *et al.*, 2014). Thus, Gabaldón *et al.* (2017) found significant variability in flowering date when wild genotypes from Canary Islands were considered. In that case, however, variability was increased only in terms of delayed flowering, *i.e.* the opposite to the desirable early flowering for climate change adaptation. In any case, the consideration of different wild genetic resources in breeding programs for increase variability could be a reasonable strategy (Lavee & Zohary, 2011; León *et al.*, 2018).

Phenological models for olive flowering parameters usually have been based on experimental data under moderate cold winter conditions (Gabaldón *et al.*, 2017). Thus, previous studies carried out in locations such as Córdoba (Spain) or Mendoza (Argentina) have provided specific cultivar-parameterization of phenological models (De Melo-Abreu *et al.*, 2004; Trentacoste & Puertas, 2011). However, some cautions must be considered as the low winter temperatures could generate uncertainties in the identification of chilling requirements due to in excess fulfillment of these requirements for all the cultivars, homogenizing the end of endodormancy stage for all the cultivars, independently of the chilling requirements of each cultivar. For example, average temperature in winter time in Córdoba during the analyzed period was 10.3ºC, generating enough accumulation of chilling-hour to fulfil well above the chilling requirements for all the analyzed cultivars, even for those with the highest chilling requirements. Thus, following the methodology and parameterization provided by De Melo-Abreu *et al.* (2004) the accumulation of chilling units in WOGB-IFAPA were high and variable depending on the year, with annual chilling units for the period 2001-2008 varying between 635 U (for 2004/05) and 1188 U (for 2001/02). Then, experimental fields located in regions with warmer winter temperatures than the identified under Mediterranean weather conditions (Aybar *et al.*, 2015) would provide more confidence in the evaluation and characterization of olive chilling requirements. Validation results for the flowering models obtained in this study confirm the uncertainty for assessing olive phenology. The validation of Chilling+Heat flowering model provided unsatisfactory results mainly caused by uncertainties in the estimation of chilling requirements parameter (TU). Under weather conditions with moderate winter temperatures, the consideration of phenological models uniquely based on heat requirements could be a reasonable alternative (Fornaciari *et al.*, 1998; Galán *et al.*, 2005; Perez-Lopez *et al.*, 2008; Gabaldón *et al.*, 2017). This approach avoids the simulation/parameterization of the end of endodormancy, cause of high uncertainty by the difficulty in the identification of this phenological stage (Chuine *et al.*, 2016), and overcomes the huge variability in the calibration parameters related with basal temperatures and chilling requirements thresholds (De Melo-Abreu *et al.*, 2004; Orlandi *et al.*, 2006; Aguilera *et al.*, 2014). Thus, when the Heat flowering model was considered for the same years, and then TU parameter was not considered, more satisfactory results were obtained although these are not satisfactory either. These results coincide with those provided by Gabaldón *et al.* (2017) testing both approaches under controlled weather conditions. As consequence of those uncertainties, when the parameterization obtained in the WOGB-IFAPA was compared with studies carried out in other locations, significant differences were found, indicating that the local weather conditions impacted on the calibration process. Thus, the parameterization of chilling requirements (TU) showed significant differences (De Melo-Abreu *et al.*, 2004; Aybar *et al.*, 2015), even detecting some contradictory results for same cultivars as ‘Frantoio’ (Barranco *et al.*, 2005; Aybar *et al.*, 2015). However, when uniquely accumulated temperature was evaluated (*i.e.* Heat flowering model), differences were lower (Trentacoste & Puertas, 2011).

A correct assessment of dormancy release is critical for a correct crop model performance. Unlike other crops as apricot (Andreini *et al.*, 2014) or almond (Pope *et al.,* 2014), olive shows a low variability in chilling requirements. However, despite the high relevance of olive in the Mediterranean agriculture, still high uncertainties about the quantification of chilling requirements and thermal accumulation for olive remains

(Aybar *et al.*, 2015; Gabaldón *et al.*, 2017) with a very limited number of studies analyzing this component (De Melo-Abreu *et al.*, 2004; Aybar *et al.*, 2015). Thus, even some authors as Fornaciari *et al.* (1998) and El Yaacoubi *et al.* (2014) suggest that the olive flowering is highly dependent on heat accumulation, with limited and uncertain chilling requirements. These uncertainties also impacted on the low performance of the model identified in this study.

Chilling requirements for most olive cultivars evaluated in this study were small and very similar between them. Thus, more than 95% of the cultivars analyzed, included well-known cultivars such as 'Arbequina', 'Hojiblanca', 'Picual' or 'Frantoio', had chilling requirements ranging from 270 to 290 TU; a narrow range that can be achieved in less than 2 cold days under the weather conditions of Córdoba. This high uniformity in TU and TT values is coherent with the low differences identified in the field observations (average observed FFD was equal to DOY 120, DOY 121 and DOY 123 for 'Arbequina', 'Frantoio' and 'Picual', respectively), very similar to those described by Barranco *et al.* (2005) and within the range of the observation error. However, these values differ from those previously described (De Melo-Abreu *et al.*, 2004; Aybar *et al.*, 2015). Between the causes of these divergences are the different weather conditions of the experimental fields. Thus, cold conditions of WOGB-IFAPA minimize the importance of small differences in chilling requirements, as those do not generate differences in full flowering date. Thus, under the weather conditions of the WOGB-IFAPA, the Heat flowering approach provided more satisfactory performance than Chilling+Heat flowering approach, due to the uncertainty associated to the calibration of chilling requirements per cultivar, required in this latter approach. In addition, some studies have questioned the assessment of chilling requirements in simulation models for assessing flowering date when considering uniquely observations of budbreak dates (Chuine *et al.*, 2016), recommending for a correct procedure, the measurement of endodormancy break dates. Similarly, Pope *et al.* (2015) warning about the consideration of budbreak-based chilling requirements approaches for yield assessment for crops as pistachios and walnuts. For these reasons, the small differences in chilling requirements between cultivars found in our study as results of a simulation process cannot be considered relevant.

As result of the non-optimal results obtained in the validation of simulation models for FFD and RD assessment compared with previous studies such as De Melo-Abreu *et al.* (2004), alternative approaches are required. The evaluation of another approaches for FFD and RD assessment as the combination of Dynamic model (Fishman *et al.*, 1987) and ASYMCUR approach (Anderson *et al.*, 1986; Pope *et al.*, 2014) or the consideration of maximum or minimum temperature instead of the traditional average temperature (Andreini *et al.*, 2014) are valuable alternatives for future research. However, the low variability of the observations will be a critical fact that impact on the model efficiency when indexes such as the Nash-Sutcliffe model efficiency was considered.

Regarding ripening date, the evaluation of WOGB-IFAPA allowed to identify early ripening cultivars such as 'Mavreya' (29^{th} September; DOY 272), 'Figueretes' (DOY 273), 'Leccino' (DOY 275) and 'Menya' (DOY 277). The effect of cultivar for this character seems to be much higher than for the case of the flowering time. However, modelling for assessing ripening date equally provided a non-optimal performance. Thus, the unique consideration of temperatures does not explain correctly the process. Other components such as crop load (Trentacoste & Puertas, 2011) or water status must be included in the phenological models for assessing ripening date. Moreover, it should be noted that ripening phenology (measured as a pigmentation index of the fruit) represents uniquely an indirect measurement of the actual agronomic traits of interest such as oil content and quality and, therefore, the development of specific models based on direct measurements of these traits should be considered in future works (De la Rosa *et al.*, 2013).

Under future weather conditions the detected high homogeneity in flowering date between cultivars will be increased (Gabaldón *et al.*, 2017) but also the uncertainties associated to climate projections, chilling hour requirements parameterization and crop behavior. This implies that recommendations to stakeholders must be carried out with caution. To solve the detected limitations, future studies must be focused on the integration of additional olive germplasm banks under warmer weather conditions for assessing chilling requirements, and to promote the experimentation and adapted modelling under future weather conditions such as under elevated CO_2 or impact of heat/water stress under flowering and ripening.

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