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Flowering Phenology of Olive Cultivars in Two Climate Zones with Contrasting Temperatures (Subtropical and Mediterranean)

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Abstract: The large amount of olive cultivars conserved in germplasm banks can be used to overcome some of the challenges faced by the olive growing industry, including climate warming. One effect of climate warming in olive is the difficulty to fulfill the chilling requirements for flowering due to mild winter temperatures. In the present work, we evaluate seven olive cultivars for their adaptation to high winter temperatures by comparing their flowering phenology in the standard Mediterranean climate of Cordoba, Southern Iberian Peninsula, with the subtropical climate of Tenerife, Canary Islands. Flowering phenology in Tenerife was significantly earlier and longer than in Cordoba. However, genotype seems to have little influence on the effects of the lack of winter chilling temperatures, as in Tenerife. This was found even though the cultivars studied had a high genetic distance between them. In fact, all the cultivars tested in Tenerife flowered during the three-year study but showed asynchronous flowering bud burst. 'Arbequina' showed an earlier day of full flowering compared with the rest of the cultivars. The results observed here could be of interest to refine the phenological simulation models, including the length of the flowering period. More genetic variability should be evaluated in warm winter conditions to look for adaptation to climate warming.

Keywords: *Olea europaea* L.; genetic variability; climate warming; chilling requirements

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1. Introduction

Olive germplasm includes more than 2000 different cultivars, most of them very ancient and restricted to their area of origin [1], usually in the Mediterranean area. This wide diversity is hosted in many germplasm banks, whose evaluation has shown high variability for many agronomic traits [2,3]. Among them, the World Olive Germplasm Bank of Córdoba, Spain is one of the largest olive repositories that has shown great genetic variability for most of the important agronomic traits [4]. These repositories are essential to look for genetic variability for fighting against the challenges that threaten olive cultivation, such as diseases [5] or climate warming [6].

One of the main effects of climate warming on olive growing could be attributable to the increase in winter temperatures, which may affect flowering [7]. In fact, air temperature has been reported to be the main environmental factor driving the flowering phenology in olives [8] and other fruit crops [9]. Many models have been developed to predict how climate change could modify the areas suitable for olive growing [10–12]. However, most of these models were based on data taken on the Mediterranean area, where winter temperatures currently fulfil the olive chilling needs for normal flowering [8,13]. Normally, those models included data on single or few cultivars. When analyzing the variability for flowering phenology of several cultivars, little genetic variability was observed [14], even when those evaluations were performed in germplasm banks [13,15]. Again, these evaluations have been carried out under Mediterranean conditions fulfilling the winter chilling requirements of the olive.

To overcome this geographical limitation, some works have been carried out using field observations in conditions with lower winter chilling than the current Mediterranean climate. In this sense, an artificial increase of the air temperature [16] has promoted an earlier and longer flowering period in the cultivar 'Picual' with respect to the natural conditions in Cordoba, Southern Iberian Peninsula. The subtropical climate of Tenerife, Canary Islands, which has higher winter temperatures, has been used as a natural simulation of the increase of winter temperatures predicted in the Mediterranean climate by the climate warming models [17]. In these subtropical conditions, the same early and long flowering period was observed, but also an asynchronous burst of the flowering buds. Those experiments were performed only in 'Picual' and 'Arbequina'. Therefore, it would be of interest to evaluate, in warm winter conditions, the behavior of other cultivars coming from different origins and having a diverse genetic base.

For that matter, in the present study, we evaluate the genetic variability to meet chill and heat requirements for flowering phenology in a set of cultivars, coming from four different olive-growing countries, in the subtropical climate of Tenerife with high winter temperatures by comparing them with the phenology of the same cultivars grown in Cordoba, Southern Iberian Peninsula, a typical Mediterranean growing area. The interaction between cultivar and contrasting environmental effects is also evaluated.

2. Materials and Methods

2.1. Plant Material and Location

This study was carried out in two field trials located in areas with very different climatic conditions. One was in Araya, in the south-east of Tenerife, Canary Islands, at 450 m.a.s.l., with a subtropical climate, and the other in Cordoba, in the south of the Iberian Peninsula, at 94 m.a.s.l., characterized by a Mediterranean climate (Figure 1). Both orchards were maintained with the same olive-growing management aimed at maximizing productivity. The Tenerife field was irrigated with 2500 m^3/year, and the Cordoba field was irrigated with 1500 m^3/year. Those differences are based on a higher average rainfall in Cordoba than in Tenerife. In both sites, air temperature was recorded at 1 m height between canopies in each field by weather stations (Pessl Instrument iMetos) and was used to calculate the following parameters: Tmax: daily maximum temperature; Tmin: daily minimum temperature; Tave: daily average temperature (Tmax + Tmin/2); DTR: diurnal temperature range (Tmax-Tmin). Daily solar radiation data of the two locations was downloaded from the open access repository "POWER Project's Hourly 2.0.0 version" on 21 June 2023.

For each site, at least four trees of seven cultivars aged between 3 and 5 years were included in this study. Six cultivars were traditional and came originally from: southern ('Hojiblanca' and 'Picual') and northern ('Arbequina') Spain, southern Italy ('Coratina'), Crete, Greece ('Koroneiki'), and Morocco ('Picholine Marocaine'), and were propagated from the World Olive Germplasm Bank of Cordoba, Spain [1]. They were selected for their genetic distance and for being widely planted in their countries of origin [18]. The other cultivar, 'Martina', is a new cultivar from the Olive Breeding Program of Cordoba [19]. This new cultivar derived from the cross of 'Arbequina' and 'Picual'.

2.2. Flowering Phenology

Flowering load was scored according to a previously reported methodology [14] on a scale of 0 to 3. Only trees with a score of 2 or 3 were considered in the present study.

Flowering phenology was evaluated in three consecutive years, from 2019 to 2021. For this purpose, the international standardized BBCH numerical scale for olive [20] was used. The observations started with the first appearance of stage 53 and finished when stage 69was the most common one. One or two times per week the earliest, most common, and latest phenological stages were evaluated for each tree [14,21].

Figure 1. Location of the two olive trials under evaluation.

All these data were used to calculate three phenological parameters:

- Length of flowering period (FP): 61 days being the earliest stage to 68 days being the most common stage.
- Length of full bloom period (FBP): Number of days from first time stage 61 appears as most common to last time for stage 65 (full bloom, at least 50% of flowers open) appears as most common.
- Full bloom date (FBD): Average Julian date of the start and end of the FBP.

2.3. Statistical Analysis

Analysis of variance was used to test the significance of cultivar and the environment, and their interaction on FP, FBP and FBD. As the data obtained was unbalanced (no data for three cultivars in 2019), we used Type III sum of squares [22] to evaluate the influence of the factors on the three variables considered. Each location/year combination was considered as a different environment (2 locations and 3 years = 6 environments), as completed in previous studies [14]. Comparison of means was used to test for differences between these factors when significant.

3. Results

As expected, Tenerife and Cordoba had contrasting climatic characteristics (Figure 2). Air temperature in Tenerife was in general milder than in Cordoba. Autumn and winter were colder in Cordoba, with Tmin below 0 °C in late December and early January. In Tenerife, the Tmin winter temperatures were below 10 °C on only 8 days of the year, Tmax were above 20 °C on almost all days of the winter. Spring was hotter in Cordoba, with Tmax reaching 40 °C in May and June. In Tenerife, the highest Tmax recorded during the study were below 35 °C. Differences were also observed in the DTR, which in Cordoba was sometimes close to 25 °C, while in Tenerife rarely exceeded 15 °C. Precipitation was higher in Córdoba (396, 472, and 422 mm, respectively, in the three years under study) than in Tenerife (314, 223, and 302 mm, respectively) during the three years under study. However, these differences were compensated with a higher irrigation dose in Tenerife. Solar radiation values were higher in Tenerife than in Cordoba in the whole period considered.

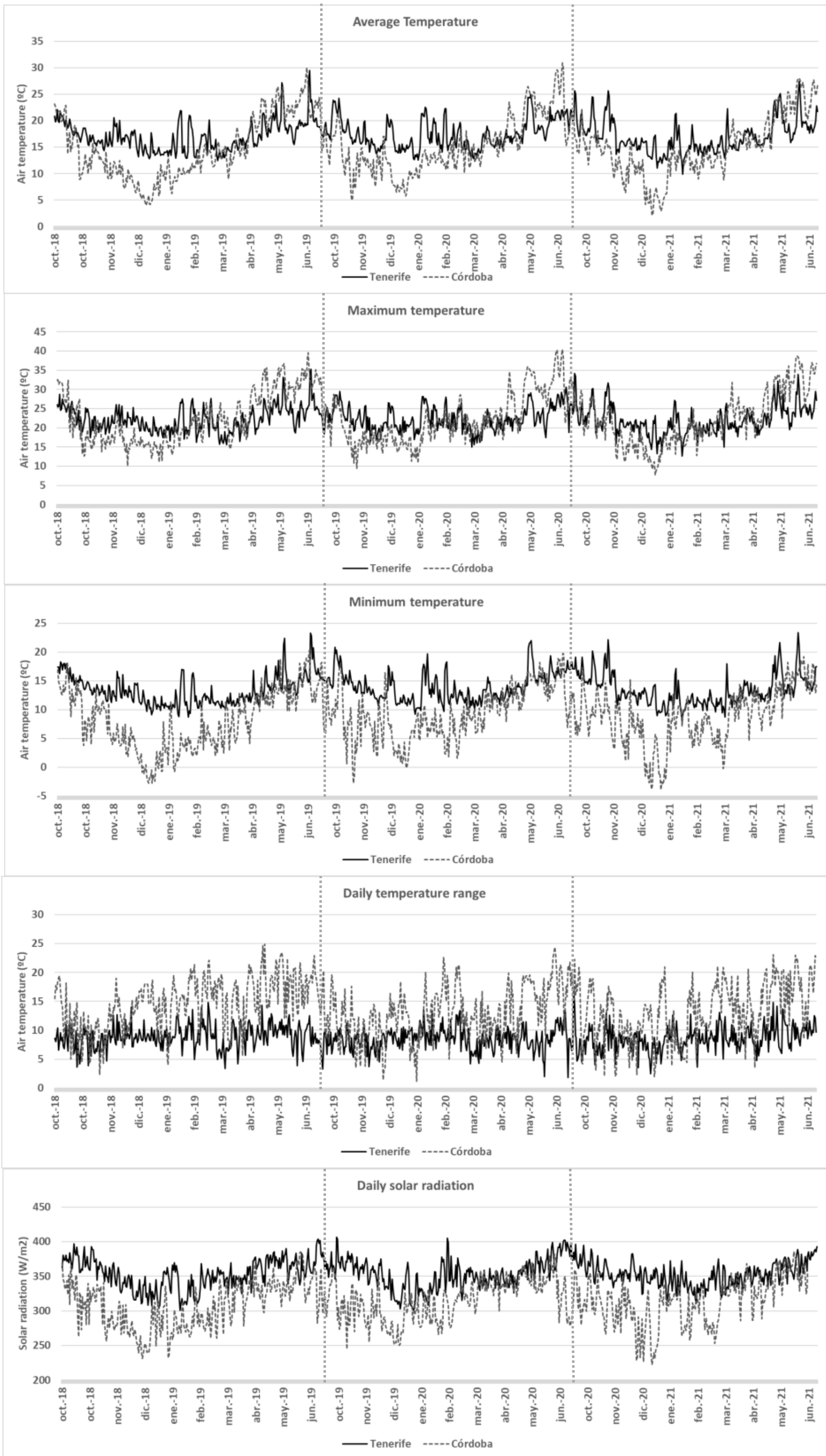

Figure 2. Weather data of Tenerife and Andalucía locations from October to June in the three seasons considered (2018–2019, 2019–2020, and 2020–2021). Daily temperature (mean, maximum, average, and range) and solar radiation are included.

All these climatic differences between Cordoba and Tenerife promote significant differences in flowering phenology. Indeed, the analysis of variance for the flowering phenology parameters (Table 1) showed that environment (defining each year–location combination as a different environment) was the main and significant contributor to the variability of both FP and FBD. For both FP and FBD, the cultivar x environment interaction was also significant. For FBP, only environment was significant, but with a little amount of the total variability. However, in the latter case, a high error term of the percentage of the variance was observed, indicating a high variance among olive trees of each cultivar and environment.

Table 1. Percentage of sums of squares of cultivar and the environment, and their interaction for the three flowering parameters included in the study: flowering period (FP in days), full flowering period (FBP in days), and full bloom time (FBD in Julian date). Values in bold indicate significant influence of the factor at $p < 0.01$.

	FP	FBP	FBD
Cultivar	2.5	1.1	**8.0**
Environment	**52.5**	**8.3**	**54.4**
Cultivar × Environment	**11.8**	12.1	**16.1**
Error	33.2	78.6	21.6

FP in TF-19 (Tenerife in 2019) was significantly longer than in the other environments, reaching 55 days (Table 2), five times higher than in CO-21 (Cordoba in 2021). The other two Tenerife environments (TF-20 and TF-21) also had higher FP than those of Cordoba (CO-19, CO-20, and CO-21). Considering the individual FP values per cultivar and environment, TF-19 was the environment with the highest differences between cultivars. 'Arbequina', 'Koroneiki', and 'Martina' showed significantly higher FP values in TF-19 than in TF-20 and TP-21 (Table 2). In contrast, no significant differences among cultivars were observed in the three Cordoba environments (CO-19, CO-20, and CO-21) and in TF-21. Furthermore, 'Arbequina' had by far the longest FP in TF-19, with 79 days, while 'Picual' had the shortest in CO-21 with 8 days.

Table 2. Comparison of the means of length of the flowering period (FP, in days) by cultivar, environment and their interaction. Each environment was considered as a combination of a year (2019, 2020, and 2021) and location (Cordoba-CO and Tenerife-TF). Different letters indicate significant differences ($p < 0.01$) among means within each source of variation.

	CO-19		CO-20		CO-21		TF-19		TF-20		TF-21		Average	
Arbequina	25.0	fgh	16.7	gh	13.2	gh	79.4	a	32.3	efg	34.2	efg	33.5	n.s.
Coratina			12.0	gh	12.0	gh	54.0	bcde	42.0	cdefg	37.3	defg	31.5	n.s.
Hojiblanca			15.0	gh	10.0	gh	38.3	defg	54.3	bcd	36.0	defg	30.7	n.s.
Koroneiki	20.0	gh	15.6	gh	10.2	gh	62.6	bc	26.7	fg	35.6	defg	28.5	n.s.
Martina	9.0	gh	13.5	gh	16.2	gh	66.1	b	33.3	efg	36.7	defg	29.1	n.s.
Picholine			13.3	gh	10.0	gh	42.3	cdefg	23.0	fgh	38.7	defg	25.5	n.s.
Picual	16.0	gh	15.2	gh	8.0	h	44.8	cdef	52.5	cde	28.5	fg	27.5	n.s.
Average	**17.5**	**c**	**14.5**	**c**	**11.4**	**c**	**55.4**	**a**	**37.7**	**b**	**35.3**	**b**		

Regarding the length of the FBP, only small differences were found between environments. These differences were mainly due to the low values in CO-20 and CO-21 (Table 3).

Table 3. Comparison of the means of length of the full flowering period (FBP in days) by cultivar, environment, and their interaction. Each environment was considered as a combination of a year (2019, 2020, and 2021) and location (Cordoba-CO and Tenerife-TF). Different letters indicate significant differences ($p < 0.01$) among means within each source of variation.

	CO-19		CO-20		CO-21		TF-19		TF-20		TF-21		Average	
Arbequina	14.8	n.s.	4.5	n.s.	7.8	n.s.	8.9	n.s.	6.9	n.s.	10.0	n.s.	8.8	n.s.
Coratina			5.7	n.s.	8.0	n.s.	27.0	n.s.	9.5	n.s.	9.0	n.s.	11.8	n.s.
Hojiblanca			8.0	n.s.	6.2	n.s.	5.7	n.s.	10.0	n.s.	9.3	n.s.	7.8	n.s.
Koroneiki	8.0	n.s.	6.6	n.s.	5.6	n.s.	13.2	n.s.	10.0	n.s.	6.8	n.s.	8.4	n.s.
Martina	3.0	n.s.	3.8	n.s.	9.2	n.s.	10.3	n.s.	8.8	n.s.	10.3	n.s.	7.6	n.s.
Picholine			6.0	n.s.	7.0	n.s.	18.0	n.s.	9.5	n.s.	6.7	n.s.	9.4	n.s.
Picual	8.0	n.s.	6.5	n.s.	4.5	n.s.	9.7	n.s.	11.5	n.s.	6.5	n.s.	7.8	n.s.
Average	**8.4**	**ab**	**5.9**	**C**	**6.9**	**bc**	**13.3**	**a**	**9.5**	**ab**	**8.4**	**ab**		

Average FBD was earliest in TF-20, followed by TF-21 (Table 4), while no differences in FBD were observed in the other environments. In TF-19 and TF-20, significant differences in FBD were observed between cultivars. 'Arbequina' in TF-19 showed an earlier FBD compared to the rest of the cultivars in this environment. Very early FBD was observed in TF-20 for 'Arbequina', 'Koroneiki', 'Martina', and 'Picholine Marocaine' on approximately day 60 (1 March). In the other environments, the behavior of all cultivars was very homogeneous, as in the case of FP. Therefore, only slight significant differences of cultivars across the environments were found.

Table 4. Comparison of the means of length of the full bloom date (FBD in Julian date) by cultivar and environment, and their interaction. Each environment was considered as a combination of a year (2019, 2020, and 2021) and location (Cordoba-CO and Tenerife-TF). Different letters indicate significant differences ($p < 0.01$) among means within each source of variation.

	CO-19		CO-20		CO-21		TF-19		TF-20		TF-21		Average	
Arbequina	111.4	cdef	109.9	cdefg	110.1	cdefg	89.4	h	62.0	i	94.5	gh	96.2	c
Coratina			113.2	cdef	110.0	cdefg	127.5	abc	96.8	efgh	96.5	fgh	108.8	a
Hojiblanca			115.7	bcde	110.9	cdef	119.5	bcd	88.7	h	97.3	efgh	106.4	a
Koroneiki	118.0	bcde	116.1	bcde	111.2	cdef	135.8	a	60.5	i	95.2	fgh	106.1	ab
Martina	125.5	abcd	110.4	cdefg	107.8	defg	114.5	cde	61.1	i	95.4	fgh	102.4	bc
Picholine			114.7	bcde	110.5	cdefg	133.0	ab	59.3	i	94.7	fgh	102.4	bc
Picual	118.0	bcde	117.1	bcde	112.1	cdef	117.7	bcde	97.3	efgh	95.5	fgh	109.6	a
Average	**118.2**	**c**	**113.9**	**c**	**110.4**	**c**	**119.6**	**c**	**75.1**	**a**	**95.6**	**b**		

In general, flowering in Cordoba's environments started and ended 60 and 30 days later than in TF-20 and TF-21, respectively (Figure 3). While in TF-19, flowering duration was very variable between cultivars but, in general occurred at a similar date to that in Cordoba's environments.

It is remarkable that the differences between the most advanced and the most delayed phenology stages for a given date were much greater in Tenerife than in Cordoba for all cultivars (Figure 4). Consequently, two very distant phenological stages were observed simultaneously in a tree in all cultivars in Tenerife's environments compared to those in Cordoba. Moreover, in Tenerife, stage 53 (Inflorescence buds open, flower cluster development starts) was the most delayed stage over a long time for all the cultivars tested, and the variation of the phenological stages with time was not always ascending, as happened in Cordoba. These differences in phenology are due to the asynchronous bud blooming in Tenerife's environments, where new flowers appear on the trees over a very long period.

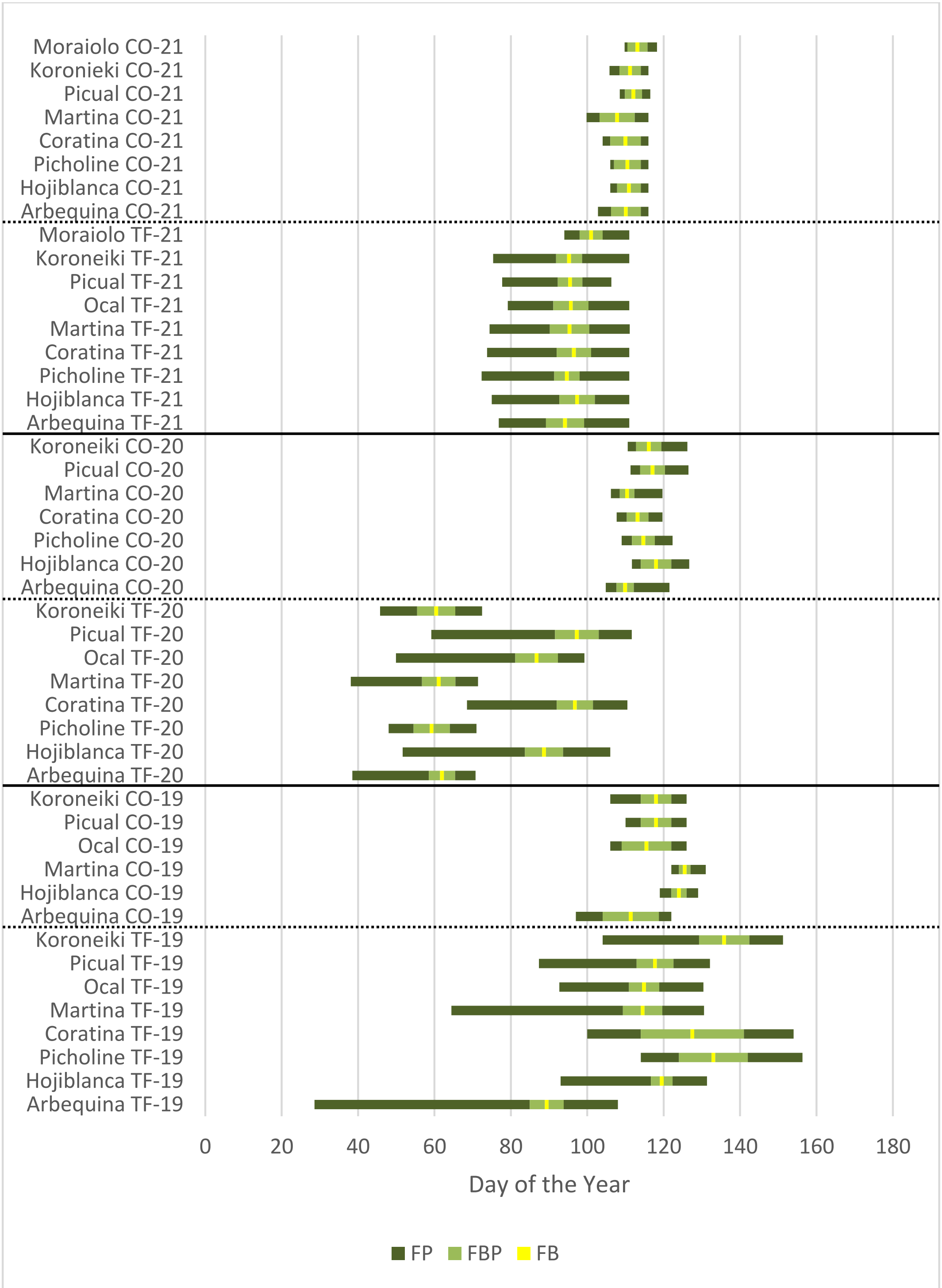

Figure 3. Flowering period in days (FP in dark green), full bloom period in days (FBP, in light green), and full bloom date in Julian day (FBD in yellow) in seven cultivars in the Tenerife and Andalucía locations in the three seasons considered (2018–2019, 2019–2020, and 2020–2021).

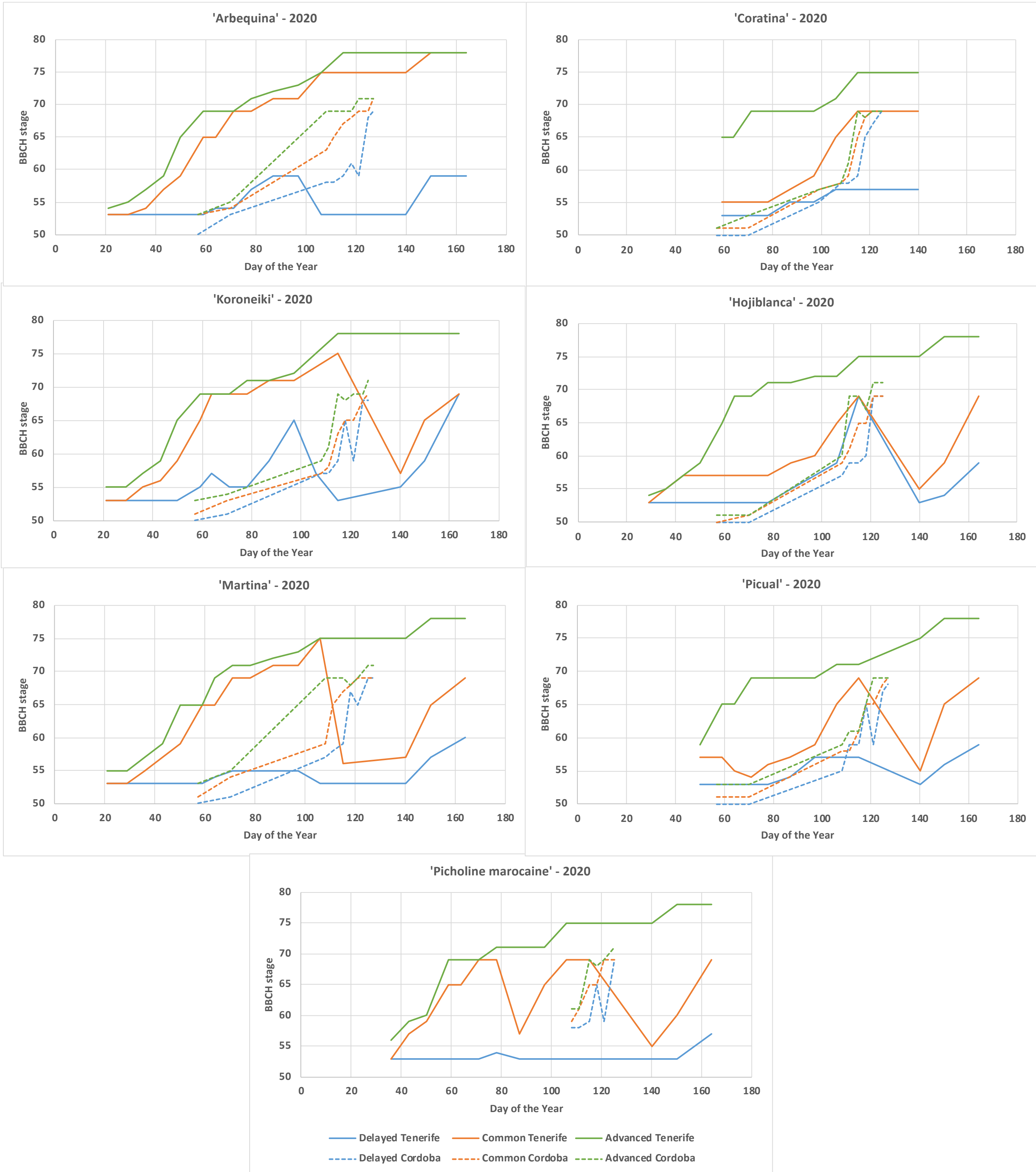

Figure 4. Variation of the average most delayed, common, and advanced flowering stage (BBCH scale) in the seven studied cultivars along the flowering period (Julian day) in Tenerife and Cordoba in 2020.

4. Discussion

In this study, we evaluate the relative influence of genotype, environment, and their interaction on the olive flowering phenology. The genotype consisted of seven cultivars coming traditionally from growing areas with very different climatic conditions. Environment included two locations, one with a Mediterranean climate (Cordoba), typical of olive growing, and the other with a subtropical climate (Tenerife), with winter temperatures higher than those considered suitable for olive growing [8]. These warm winter temperatures have been reported as a good natural scenario to simulate the effect of climate warming in the Mediterranean [17]. The environment factor was the combination of these two locations and three years (2019 to 2021) in which climatic conditions were variable.

To our knowledge, this is the first time that a comparative trial of olive cultivars has been evaluated in subtropical conditions, such as the one in Tenerife. Flowering was observed in all of Tenerife's environments and in all the cultivars tested. This happened despite the fact that the optimal chilling accumulation temperature previously established at 7–12 °C for olive crops [23–25] was rarely reached in winter in all three of Tenerife's environments.

The analysis of variance of the flowering phenology parameters showed that the environment was the main factor responsible for the variability of FP and FBD. This factor was also significant for the FBP. A high environmental influence has been reported for flowering phenology in Mediterranean conditions [14] and in diverse climatic conditions, such as in Argentina [26]. In this work, the high environmental influence on flowering phenology is mainly due to the lack of winter chilling in all three of Tenerife's environments (TF-19, TF-20, and TF-21) as mentioned above. The most remarkable effect of this is an asynchronous flower bud burst that was also previously observed for 'Arbequina' and 'Picual' in the subtropical climate [17]. This asynchrony in the flowering could be the cause of the high error variance for the FBP as observed in Tenerife. It also caused a much greater difference between the most delayed and the most advanced phenological stage, for a given date, in Tenerife than in Cordoba; consequently, the flowering period was much longer in the former location. A longer flowering period has also been observed in experiments with artificial temperature increases in Mediterranean conditions [16] and with warmer winters [27]. The higher solar radiation measured in Tenerife, when compared with Cordoba, might also have an influence on these differences, but more specific experiments should be performed to clarify this fact.

Both the asynchrony and the increase in FP might have a negative impact on the profitability of olive cultivation in warm areas. It also causes an asynchronous olive ripening, with important consequences for the quality of the olive oil obtained. In the case of Tenerife, this long-flowering period is exposed to a large number of extreme climatic phenomena, such as hot sub-Saharan air masses, which could cause a massive drop in flowers and a deficit in fruit set or pistil abortion [28]. In addition, flowering period occurred earlier in Tenerife than in Cordoba. This fact is consistent with the observations registered under natural conditions [15], using an artificial increase of air temperature during winter [16] and in flowering phenology models [13,29].

The significant differences observed among all three of Tenerife's environments are difficult to explain, as the air temperatures in the three-year study were relatively similar. Maybe other factors apart from air temperature or solar radiation are involved, for example, soil temperature.

In contrast to the strong environmental effect, the evaluation performed seems to suggest that genotype has little influence on the flowering phenology. This is despite the fact that the cultivars studied come from distant areas and have a high genetic distance between them [1]. Only some differences were observed in the FBD, which was earlier in 'Arbequina', curiously the only cultivar evaluated from the northern part of the Mediterranean olive-growing area.

The interaction between genotype and environment was significant for FP and for FBD. In particular, no significant differences between cultivars were found in the Mediterranean climate of Cordoba, where olive winter temperatures are low enough to fulfill the chilling requirements of all the cultivars [14]. However, in Tenerife significant differences among cultivars were observed. It is noteworthy that there is no specific pattern of variation for cultivars for both FP and FBD along all three of Tenerife's environments which were tested. For example, 'Koroneiki' had the longest FP in TF-19, and one of the lowest in TF-20. In other words, the flowering behavior of the cultivars in the warmer winters of Tenerife seems to be erratic and with little consistent genetic influence. Previous work has reported that different cultivars have different winter chilling requirements or chilling portions for flowering, including some of those used in the present study such as 'Arbequina', 'Hojiblanca', and 'Picual' [7,8]. However, in this work, where cultivars were placed in a natural environment with a warm winter, such as in Tenerife, no consistent significant differences were observed among these cultivars for flowering phenology or winter chilling needs. Previously, differences in terms of flowering intensity due to the lack of sufficient winter chilling were reported among cultivars in Argentina [23], but the length of the flowering period was not reported. In the only previous report of cultivar evaluation for FP in multiple Mediterranean environments, no significant differences among cultivars were observed [14]. Growth chamber experiments at constant temperatures have been also proposed to test the winter chilling needs of olive cultivars [25,30]. In those works, whenever not enough chilling was accumulated, flower bud burst was not observed. However, a lack of synchronization of flower bud burst was never reported. In other crops such as walnut, it has been reported that chilling requirements in constant temperature conditions are not representative of the chilling requirements in natural orchard conditions [31].

The lack of genetic variability observed here indicates that more cultivars need to be tested to identify genetic variability for adaptation to the warmer winters predicted by climate models [6]. Unfortunately, little genetic influence on the FBD has been observed when evaluated in typical Mediterranean climates such as Cordoba [15] and Morocco [13], suggesting that differences in winter chilling requirements may also be difficult to find. Additionally, current breeding programs have been focused on other characteristics such as disease resistance [32] or adaptation to new growing systems [33], but low chilling requirements has not yet been reported as a selection trait. Perhaps the use of native wild olives from the Canary Islands, such as *Olea europaea* subsp. *guanchica* [7,18], could be a long-term strategy to introduce warm winter adaptation genes into cultivated material. In other fruit crops, breeding programs have identified new genotypes adapted to climates with warm winter temperatures [34].

Future work should also consider the effect of the lack of winter chilling on flower quality. Indeed, lack of winter chilling seems to reduce the number of inflorescences, increase flower abortion [23,35], and to deform floral buds [36].

All these studies, carried out under non-Mediterranean weather conditions, will contribute to reducing the uncertainty in phenology assessment [37,38] in the context of climate change, complementing previous studies carried out under colder winter weather conditions [7,8].

5. Conclusions

The seven olive cultivars evaluated in this study originated from very different climatic conditions, from Greece to Morocco. However, the lack of winter chilling in the subtropical climate of Tenerife promoted a desynchronized flower bud burst in all of them. Such climatic conditions produced an erratic flowering pattern across the three years studied. This indicates that they might have an undesirable behavior in a future climate-warming scenario in the Mediterranean olive-growing area. Therefore, more genetic variability needs to be explored to find cultivars adapted to warm winters. The fact that the cultivars used in this study have a very different origin might indicate that the cultivated germplasm might have poor adaptation to these conditions and that it might be necessary to explore a wild germplasm, such as the one native of the Canary Islands, namely, *Olea europaea* subsp. *Guanchica*. The results observed in this study stress the need to include the length of the flowering period as a new parameter in the climate-warming simulation models currently under development for olive growth. Finally, the results obtained highlight the usefulness of phenological evaluation as a reliable indicator of climate warming.

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