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Programa de Doctorado en Ingeniería Agraria, Alimentaria, Forestal y del Desarrollo Rural Sostenible

TESIS DOCTORAL

VARIABILIDAD GENÉTICA DE LA ADAPTACIÓN DEL OLIVO A TEMPERATURAS INVERNALES ALTAS: CASO DE LA ISLA DE TENERIFE

GENETIC VARIABILITY OF THE ADAPTATION OF OLIVE TO HIGH WINTER TEMPERATURES: CASE OF THE ISLAND OF TENERIFE

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TITULO: *VARIABILIDAD GENÉTICA DE LA ADAPTACIÓN DEL OLIVO A TEMPERATURAS INVERNALES ALTAS: CASO DE LA ISLA DE TENERIFE*

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TÍTULO DE LA TESIS:

Variabilidad genética de la adaptación del olivo a temperaturas invernales altas: caso de la isla de Tenerife.

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El Dr. Raúl de la Rosa Navarro, Investigador Científico, Instituto de Agricultura Sostenible IAS-CSIC y el Dr. Lorenzo León Moreno, Investigador Titular, Coordinador del Área de Mejora Vegetal y Biotecnología del Instituto de Investigación y Formación Agraria, Pesquera, Alimentaria, y de la Producción Ecológica (IFAPA) Centro "Alameda del Obispo" y el Dr. Domingo Ríos Mesa, Profesor Asociado de Producción Vegetal de la Universidad de La Laguna (ULL) como Director y Co-Directores de la Tesis Doctoral titulada "Variabilidad genética de la adaptación del olivo a temperaturas invernales altas: caso de la isla de Tenerife", realizada por María Guacimara Medina Alonso, informan que los resultados obtenidos en las investigaciones que comprenden este trabajo, aportan interesantes y novedosos datos sobre el comportamiento de la fenología de floración del olivo y sobre las características en cuanto a la composición fenólica del aceite de oliva, en condiciones de inviernos cálidos. Estos resultados han contribuido a reducir la incertidumbre a la que se enfrenta el olivar en un contexto de cambio climático.
Esta investigación se ha desarrollado en colaboración con el Servicio Técnico de Agricultura y Desarrollo Rural del Cabildo Insular de Tenerife y el Área de Mejora Vegetal y Biotecnología del IFAPA Centro "Alameda del Obispo". Esta Tesis Doctoral ha sido finalizada con éxito, y como resultado se han publicado artículos científicos y de divulgación, así como aportaciones a congresos.
Todos los objetivos planteados inicialmente se han cumplido satisfactoriamente, por lo que, se informa favorablemente y se autoriza la exposición y defensa de esta Tesis Doctoral.

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DOCTORANDA/O

MARIA GUACIMARA MEDINA ALONSO

TÍTULO DE LA TESIS:

Variabilidad genética de la adaptación del olivo a temperaturas invernales altas: caso de la isla de Tenerife.

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RESUMEN

El cambio climático es una de las grandes preocupaciones de nuestro tiempo. La agricultura será sin duda uno de los sectores más afectados debido al incremento de temperaturas y menor disponibilidad de agua. En particular, en el caso de la fruticultura, la ausencia de frío invernal pondrá en riesgo la floración y la fructificación y, en consecuencia, la supervivencia de estas especies, incluyendo al olivo. El aumento de temperaturas también podrá modificar sustancialmente la cantidad y calidad del aceite de oliva virgen extra que se produzca.

Hasta el momento, los requerimientos de frío del olivo habían sido poco estudiados puesto que eran ampliamente cubiertos en la cuenca del Mediterráneo. Sin embargo, el nuevo escenario de cambio climático y la expansión del olivo a zonas más allá del clima mediterráneo donde tradicionalmente se ha cultivado han obligado a desarrollar estudios que permitan conocer los requerimientos de frío del olivo. También en algunos trabajos se han simulado las condiciones futuras de baja acumulación de frío durante el invierno, pero en muy pocas ocasiones estos estudios han sido desarrollados en condiciones de campo reales. Igualmente existen pocos daos sobre la variación de la composición del aceite en un entorno de cambio climático, la mayoría de ellos referidos a los efectos de la composición acídica, siendo casi nula la información sobre la influencia del cambio climático en la composición fenólica.

Por tanto, el principal objetivo de esta tesis consistió en evaluar el comportamiento de la fenología de floración y de la composición fenólica de los frutos en las condiciones del clima sub-tropical de Tenerife, caracterizado por la ausencia de frío invernal. Estos resultados fueron comparados con los obtenidos en condiciones del clima mediterráneo en Andalucía.

En relación con la fenología de floración, se evaluaron las variedades ‘Arbequina’ y ‘Picual’ en tres zonas diferentes de Tenerife con diferencias climáticas asociadas a su diferente altitud. Al mismo tiempo, este estudio fue llevado a cabo en tres localizaciones distintas de Andalucía también con marcadas diferencias climáticas entre ellas. Como resultado se observó que las condiciones climáticas en Tenerife inducían a una floración más temprana y con un período de floración más largo que en Andalucía. Además, la floración en las condiciones subtropicales fue muy asincrónica, de modo que se registró un gran número de estadios fenológicos diferentes al mismo tiempo en un mismo árbol. Esta situación suele traducirse en impactos negativos en la producción y en la calidad del aceite de oliva obtenido.

Con estos primeros resultados, se abordó el segundo objetivo de la tesis. Este consistió en la evaluación de la variabilidad genética, ambiental y su interacción en la fenología de floración de un conjunto de variedades en el clima subtropical de Tenerife y en el clima mediterráneo de Córdoba. Para desarrollar este objetivo, se evaluaron ensayos comparativos de las variedades ‘Arbequina’, ‘Picual, ‘Koroneiki’, ‘Hojiblanca’, ‘Coratina’, ‘Picholine Marocaine’ y ‘Martina’, localizados en ambos climas. También se evaluó la interacción entre el genotipo y el ambiente en la fenología de floración. En el Capítulo III de esta tesis, se exponen los resultados obtenidos de este trabajo. El principal factor responsable de la variabilidad en fenología de floración fue el ambiente. Una vez más, la floración en Tenerife fue más temprana y larga que en Córdoba. No se encontraron diferencias significativas entre variedades, a pesar de que provenían de zonas de origen muy diferentes (España, Italia, Marruecos y Grecia). La interacción genotipo x ambiente tampoco fue significativa para el período de floración. La asincronía observada en todos los genotipos estudiados fue, nuevamente, una característica destacable de la floración

en las condiciones del clima subtropical; observándose incluso dos momentos de plena floración en muchas variedades.

Cabe destacar que a lo largo de los tres años que duró este estudio en Tenerife, todas las variedades presentaron floración, a pesar de las cálidas temperaturas registradas durante el invierno. Por lo tanto, estos resultados muestran la necesidad de incluir la longitud del período de floración como un nuevo parámetro en los modelos de simulación del calentamiento global. Además, un número mayor de genotipos deberían ser evaluados con el fin de encontrar variedades mejor adaptadas a los inviernos cálidos.

Finalmente, el último objetivo de la tesis fue estudiar el efecto del clima en el contenido y los componentes fenólicos de frutos de 'Arbequina'. Para ello se evaluó el comportamiento de esta variedad en seis localidades: cuatro de ellas situadas en condiciones de clima mediterráneo en Andalucía (Antequera y Baena en secano, Córdoba y Úbeda en regadío) y las otras dos en las condiciones subtropicales de Tenerife (Los Tomillos y El Viso, ambas en regadío). La disponibilidad de agua marcó las principales diferencias en el contenido en fenoles y en sus componentes, con diferencias significativas entre las cuatro localizaciones de regadío (dos de Tenerife y dos de Andalucía) y las dos de secano (en Andalucía). Entre localidades bajo sistema de regadío, se registró un mayor conenido fenólico en Andalucía que en Tenerife, probablemente porque las altas temperaturas estivales del clima mediterráneo produjeron un mayor estrés en los árboles de dichas localidades. También la fecha de recolección mostró una influencia significativa tanto en el contenido fenólico total como en el de algunos de sus componentes. Este ensayo se detalla en el Capítulo IV de la presente tesis.

SUMMARY

Climate change is one of the great concerns of our time. Agriculture will be one of the most affected sectors, undoubtedly, due to the increase in temperatures and the less availability of water. In particular, in the case of temperate fruit trees, the lack of winter cold will endanger their flowering and fruit production and, consequently, the survival of these species, including olive tree. The increase in temperatures could also modify the quantity and quality of extra virgin olive oil produced substantially.

Until now, chilling requirements of olive had been little studied because they were widely fulfilled in the Mediterranean basin. However, the new climatic situation and the expansion of olive outside the Mediterranean climate where it has been traditionally cultivated have forced to develop studies which led to know olive chilling requirements. Moreover, in some studies, future conditions of low chilling accumulation in winter have been simulated, but a very few times, these studies have been developed in real field conditions. Likewise, there are a few data about the variation of oil in a climatic change scenario, the most of them are referred to the effects on acid composition, and there is almost no information about the influence of climate change in phenolic composition.

Therefore, the main goal of this thesis was to evaluate the behaviour of olive flowering phenology and the phenolic composition of the fruits under the Sub-tropical climate conditions in Tenerife, which is characterized by the lack of cold winter. These results were compared with those obtained under Mediterranean climate conditions in Andalusia.

With reference to flowering phenology, 'Arbequina' and 'Picual' cultivars were evaluated in three areas in Tenerife with climatic differences related to their different altitude. At the same time, this study was carried out in three different locations in Andalusia with strong climatic differences among them, too. As a result, it was observed that climatic conditions in Tenerife promoted an earlier flowering and a longer flowering period than in Andalusia. Moreover, flowering in Sub-tropical conditions was very asynchronous, so a great number of phenological stages were reported at the same time and in the same tree. This situation usually causes negative impacts on production and on the quality of the olive oil obtained.

With these first results, the second goal of the thesis was approached. This one consisted in the assessment of the influence of the genetic variability, environmental and its interaction in flowering phenology of a set of cultivars in the Sub-tropical climate of Tenerife and in the Mediterranean climate of Cordoba. To achieve this goal, comparative studies of the cultivars 'Arbequina', 'Picual, 'Koroneiki', 'Hojiblanca', 'Coratina', 'Picholine Marocaine' and 'Martina' were developed, located in both climates. The interaction between genotype and environment on flowering was also evaluated. In Chapter III, the results of this study are exposed. The main factor responsible for the variability of the flowering phenology was the environment. Once more, flowering in Tenerife was earlier and longer than in Cordoba. No significant differences among cultivars were found, even though these cultivars came from very contrasting areas of origin (Spain, Italy, Morocco and Greece). The interaction genotype x environment was not significant for flowering period either. Asynchronous observed in all the studied cultivars was again a remarkable character of flowering in the Sub-tropical climate; two full bloom dates were even reported for many of these cultivars.

It should be noted that through of the three years that the study last in Tenerife, all cultivars flowered, in spite of the warm temperatures registered in winter. Therefore, these results showed the need of including the length of the flowering period as a new parameter in the global warming simulation models. A wider range of genotypes should be also evaluated to find better adapted to milder winters cultivars.

Finally, the last goal of the thesis was to study the effect of the climate in the content and phenolic components of 'Arbequina' fruits. The behaviour fo this cultivar in six locations was evaluated: four of them situated in Mediterranean climate conditions in Andalusia (Antequera and Baena in dry farming, Córdoba and Úbeda irrigated) and the two others in Sub- Tropical conditions in Tenerife (Los Tomillos and El Viso, both irrigated). Water availability made the differences in content in phenols and their components, with significant differences between the four irrigated fields (two in Tenerife and two in Andalusia) and two dry farming (in Andalusia). Among irrigated fields, a higher phenolic content was registered in Andalusia than in Tenerife, probably because the high summer temperatures of Mediterranean climate produced greater stress in the trees of these locations. Harvest date also showed a significant influence both in the total phenolic content and in some of their components. This study is detailed in Chapter IV of this thesis.

CAPÍTULO I. INTRODUCCIÓN GENERAL Y OBJETIVOS

I.I. INTRODUCCIÓN GENERAL

1. El cultivo del olivo en Canarias

La Isla de Tenerife es parte del Archipiélago Canario, frente a la costa atlántica de África, localizado a 28º de latitud norte, 4º por encima del Trópico de Cáncer. Se sitúa entre el clima mediterráneo y el clima tropical, teniendo particularidades de ambos regímenes, por lo que su clima se define como clima subtropical árido. La isla se caracteriza por una gran biodiversidad debido a la existencia de una amplia variedad de microclimas locales (Antequera *et al.*, 2021).

Este clima subtropical presenta ciertas particularidades como la presencia de la Corriente Fría de Canarias, que hace que las temperaturas sean menos extremas que las de la cercana costa del desierto del Sáhara. También juega un importante papel la influencia de los vientos alisios procedentes del nordeste y que se cargan de humedad en su paso sobre el Atlántico. Además, la accidentada orografía de la isla con grandes pendientes hace que se diferencien claramente dos zonas o vertientes. Una vertiente norte y noroeste con clima húmedo, debido a que las nubes de los alisios quedan atrapadas en esta zona formando el llamado "mar de nubes". Y otra vertiente al sur, sureste y suroeste, caracterizada por tener un clima más seco y cálido debido a la ausencia de la humedad de los alisios. Así, en la Isla se pueden encontrar zonas que no llegan a los 100 mm anuales de precipitación mientras que, en otras zonas, las lluvias superan los 1.000 mm al año (Antequera *et al.*, 2021). La distribución de estas lluvias se parece a las del clima mediterráneo, con una marcada estación seca en el verano. Sin embargo, en cuanto a las temperaturas, la zona de costa puede calificarse como clima tropical o subtropical. Por ello, en esta zona de cota más baja, los cultivos principales son el plátano, el aguacate, el mango y la papaya, frutales claramente tropicales.

A todo esto, hay que sumar el gradiente térmico producido por la altitud, que hace que, desde la costa hasta las zonas altas, la temperatura descienda aproximadamente 1ºC por cada 100 metros de altura. Por ello, los regímenes de pluviometría y temperatura son muy variados y la Isla posee gran cantidad de meso y microclimas (Machín-Barroso et. al., 2018).

Además de sus peculiaridades descritas, el clima de Tenerife está sufriendo en los últimos años, como cualquier otra región terrestre, modificaciones por el calentamiento global debido a los gases de efecto invernadero. En la Isla, este cambio climático se ha traducido en un evidente aumento de la temperatura (Antequera *et al.*, 2021). Sin embargo, la modificación más destacable es el aumento de las precipitaciones en verano, lo que indica una tropicalización del clima (Dorta - Antequera *et al.*, 2018). A esto hay que añadir, la mayor frecuencia de la aparición de fenómenos de lluvias torrenciales, tormentas y ciclones tropicales que afectan durante los últimos años a la zona Atlántica donde se sitúan las Islas (Antequera *et al.*, 2021).

Actualmente, el cultivo del olivo en la isla de Tenerife ocupa, principalmente, terrenos situados en la vertiente sur de la isla y entre el nivel del mar y los 500 msnm. Esta zona corresponde a una zona de clima subtropical seco característico, con un régimen pluviométrico inferior a 200 mm/año y con temperaturas suaves a lo largo del año y con poca amplitud térmica (Tabla 1.1). En esta zona, los cultivos predominantes han sido, tradicionalmente, los tropicales como plátanos, aguacates, papaya y hortalizas (en las cotas más bajas) y papas y vid (en medianías).

Históricamente, en Canarias el único representante de la especie de *Olea europaea* L. es la subespecie denominada *guanchica* que además es endémica de estas islas. La llegada de esta sub-especie a Canarias pudo deberse a su dispersión a través de los pájaros, al tratarse la aceituna de un fruto con alto contenido en materia grasa, lo que ha podido ser fundamental para la dispersión en largas distancias desde su origen. Se aprecian diferencias entre ejemplares de diferentes islas, lo que pone de manifiesto un marcado aislamiento genético (Hess *et al.*, 2000). Los árboles de esta subespecie eran usados ya por los aborígenes canarios para la elaboración de herramientas, así como para pasto para el ganado en islas con escasos recursos hídricos como Fuerteventura (Hess *et al.*, 2000).

Tabla I.1.- *Datos climáticos de algunas zonas de Tenerife y Córdoba, donde se localizan los ensayos de esta tesis. Datos obtenidos de la red de estaciones Agroclimáticas del Servicio Técnico de Agricultura y Desarrollo Rural del Cabildo Insular de Tenerife y la Red de Información Agroclimática de Andalucía.*

Tabla 1 Municipio	Estación	Ubicación	Altitud (msnm)	Año	T ºC media anual	T ºC verano[1]	T ºC invierno[2]	H media anual (%)	Amplitud térmica anual (ºC)	P total anual (mm)
Candelaria	Araya	Tenerife	525	2018	17,6	23,3	13,4	67,7	9,9	309,0
				2019	18,3	23,6	16,4	63,7	7,2	197,8
				2020	19,1	24,7	17,9	63,4	6,8	245,0
				2021	18,4	23,4	14,6	65,8	8,8	314,8
Arico	Canales Altas	Tenerife	630	2018	16,2	21,4	12,5	72,1	8,8	69,4
				2019	16,8	22,0	12,8	67,9	9,1	175,0
				2020	17,8	22,8	14,0	66,0	8,7	207,8
				2021	17,0	21,9	13,5	70,0	8,3	274,0
Arico	El Viso	Tenerife	410	2018	16,9	21,0	13,5	78,5	7,5	126,9
				2019	17,5	21,3	14,0	76,0	7,3	77,0
				2020	18,2	22,1	14,7	78,1	7,4	162,8
				2021	17,5	21,5	14,5	77,2	7,0	104,4
Arico	Los Tomillos	Tenerife	200	2018	19,1	22,8	15,7	68,3	7,0	312,2
				2019	19,6	23,2	16,4	65,0	6,8	67,4
				2020	20,2	23,8	16,9	65,4	6,8	139,2
				2021	19,6	23,5	16,7	67,1	6,8	131,0
Córdoba	Córdoba	Andalucía	94	2018	17,1	29,3	8,4	69,8	20,8	802,4
				2019	17,7	28,0	7,2	62,7	20,8	378,2
				2020	18,3	27,9	8,8	66,2	19,1	481,1
				2021	18,1	28,7	7,9	64,3	20,8	441,2

1: Temperatura media del mes más cálido del año; 2: Temperatura media del mes más frío del año.

Ya en Edad Contemporánea, en Tenerife se han encontrado referencias a olivos desde el siglo XIX, citándose desde ese momento la necesidad de conocer los límites en altura combinado con la latitud para el cultivo óptimo del olivo (Hidalgo Tablada, 1870). Estas plantaciones antiguas se realizaron en su mayoría con la variedad 'Verdial de Huevar' procedente de Huelva. Sólo en fechas recientes el cultivo del olivo en Tenerife ha tenido una expansión importante que lo sitúa al nivel de otros frutales de la isla (Tabla 1.2), pasando de 54,3 hectáreas en 2015 a 111,4 hectáreas en 2021 (Fuente: ISTAC. Consulta online).

Esta introducción del cultivo del olivo como explotaciones regulares intensivas para la producción de aceite de oliva virgen extra se ha producido sin realizar, previamente, estudios que permitieran conocer cuáles eran las variedades más apropiadas para la zona o sin evaluar la aclimatación de este frutal a las condiciones climáticas de Tenerife. De esta forma, podría darse el caso, como ya se ha citado en otras introducciones del cultivo fuera de su área mediterránea tradicional, de llegar a poner en riesgo la posibilidad del cultivo. De hecho, en las condiciones climatológicas de inviernos cálidos de la Isla, se han observado anomalías como asincronía en la floración, e incluso, varias floraciones en el año; falta de cuajado o madurez irregular de los frutos, lo que conlleva un aumento de los tratamientos fitosanitarios y, en resumen, pérdida de rentabilidad en el cultivo (Mérida-García, 2018)-

Tabla I.2.- *Superficie por cultivo en la isla de Tenerife.*

Especie	Superficie (m2)	
	Año 2021	**Año 2015**
Futales de pepita	141,8	137,8
Frutales de hueso	101,4	85,4
Plátano	4.001,9	4.027,9
Aguacate	956,1	473,4
Otros tropicales	373,1	379,1
Viña	2.756,6	4.687,2
Cítricos	348,9	364,1
Frutos secos	87,5	54,4
Frutos rojos	1,8	2,7
Olivo	**111,4**	**54,3**
Otros	84,9	15,0

Fuente: Instituto Canario de Estadísticas (Gobierno de Canarias).

2. Floración y necesidades de frío en frutales y en olivo

a. Frutales

Los frutales templados han desarrollado estrategias de adaptación al frío invernal que soportan en sus lugares de procedencia. Por eso, cuando las temperaturas descienden y el fotoperíodo se reduce durante los meses más fríos del año, este tipo de frutales detienen su crecimiento y modifican la fisiología celular, para evitar los daños que podrían producir las bajas temperaturas. Este período de reposo es conocido como dormancia (Rodríguez *et al.*, 2021).

La dormancia o latencia se define como “la ausencia de crecimiento visible en cualquier estructura que contiene un meristemo” (Rallo y Cuevas, 2017), como puede ser en la yema. La latencia puede ser debida a tres causas fundamentales: por la presencia de otras estructuras que inhiben el crecimiento de la yema o paradormancia; debido a la incapacidad de la propia yema para desarrollarse a pesar de que las condiciones ambientales sean las apropiadas para su crecimiento, llamada endodormancia o reposo; y debido a que las condiciones ambientales no sean favorables, denominada ecodormancia. En el proceso de floración, es la endodormancia la que interviene puesto que determina la necesidad de una acumulación de frío para iniciar su brotación (Rallo y Cuevas, 2017).

Por lo tanto, la acumulación de frío invernal es un factor necesario para la correcta floración de los frutales templados. Es un requerimiento fisiológico que permite la diferenciación de las yemas hacia flores, saliendo éstas de su estado de endodormancia una vez que han sido acumuladas una cierta cantidad de frío, entre otros factores, y crecer durante la primavera (Chhetri *et al.*, 2018; Rodríguez *et al.*, 2021). Este mecanismo trata de sincronizar la fenología de los árboles frutales con el clima local, garantizando así que los árboles no sufran daños por las bajas temperaturas, ya que se supone que después de alcanzar un cierto umbral (necesidades de frío) las temperaturas muy bajas son menos frecuentes (Rodríguez *et al.*, 2021).

Numerosos autores han descrito las variaciones en necesidades de frío entre especies e incluso, entre variedades, sólo por citar algunos ejemplos, Hauagge and Cummins (1991) en manzana o Zhuang *et al.* (2016) en albaricoque. En estos trabajos, la cantidad de frío necesaria para una especie frutal puede determinarse por alguno de los diferentes modelos que se han desarrollado: método de horas frío entre 0 y 7,2ºC (Weinbenger, 1950), método Utah (Richardson *et al.*, 1974) y modelo dinámico (Fishman *et al.*, 1987). Este último modelo es el más aplicado en climas tropicales y subtropicales (Erez, 2000; Chhetri *et al.*, 2018). Este modelo ha sido aplicado a la isla de Tenerife para definir áreas con diferente acumulación de "Chilling Portions", lo que permite decidir las zonas de mejor adaptación para las diferentes especies frutales en función de sus requerimientos de horas de frío invernal, representado en la Fig. 1.1 (Velázquez-Barrera *et al.*, 2021). Incluso para algunos cultivos como la nuez en California se ha desarrollado un modelo para validar los requerimientos de frío de cuatro variedades de esta especie (Luedeling *et al.*, 2009). En los últimos tiempos también se están usando modelos basados en regresiones multivariantes para determinar el efecto de las temperaturas, a lo largo de todo el otoño y el invierno, en la fenología de la floración (Abou Saaid *et al.*, 2022).

En el caso concreto de Tenerife, se han definido zonas bioclimáticas para algunos cultivos como la vid (Machín-Barroso *et al.*, 2018) en función de las necesidades de frío de las diferentes variedades de este cultivo.

Por otro lado, el cultivo de frutales templados se ha extendido rápidamente a otras zonas fuera de su lugar de origen, donde estos requerimientos de frío no son cubiertos por sus característicos inviernos de temperaturas suaves. Para mejorar la productividad de los cultivos en estas zonas, ha sido fundamental la selección de variedades y especies con bajos requerimientos frío. La falta de experiencias previas en relación con la interacción variedad – ambiente, ha llevado a que la elección de estas variedades se haya realizado basándose en el cálculo de la cantidad de frío mediante uso de modelos de acumulación de frío durante el invierno en una localidad en concreto (Melke, 2015).

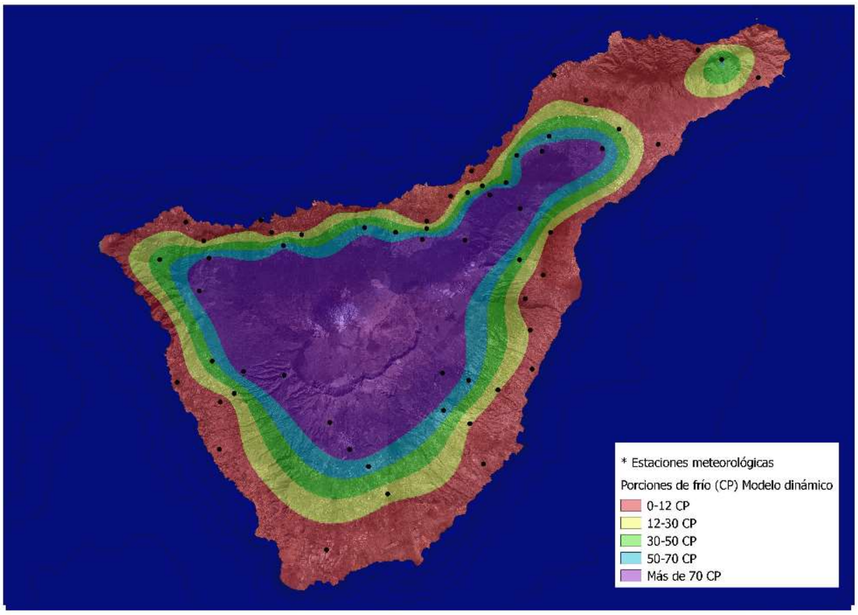

Fig. I.1.- *Representación del Modelo Dinámico de horas frío (Fishman* et al.*, 1987) en la Red de Estaciones Meteorológicas del Cabildo Insular de Tenerife (Velázquez-Barrera* et al.*, 2021).*

Cuando los frutales templados no consiguen acumular el frío necesario, con frecuencia, aparecen anomalías en la floración: floración errática y desincronizada (Flores, 2007; Fischer, 2013), falta de cuajado, reducción del tamaño de los frutos, deformaciones de los mismos (Torres *et al.*, 2017). También se ha descrito un incremento en los problemas con las plagas en algunos frutales como manzano (Soni Joshi, 2015; Chhetri *et al.*, 2018), reducción del tamaño de la flor y de la longitud del pecíolo en cereza (Soni Joshi, 2015) y falta de brotación en ciruelos (Universidad Autónoma Chapingo *et al.*, 2000). En algunos lugares como en Brasil, se ha extendido la práctica de la cosecha continua debido a que los frutales templados no entran nunca en reposo en condiciones de temperaturas cálidas todo el año (Fischer, 2013).

Es importante resaltar el hecho de que la mayoría de los modelos de acumulación de frío invernal se han desarrollado en frutales de hoja caduca. Sin embargo, existe poca bibliografía sobre frutales de hoja perenne, como es el caso del olivo.

b. Olivo

La reciente expansión del olivo a otras zonas del mundo fuera de la Cuenca Mediterránea ha llevado a que se describan anomalías o cambios en la fenología de floración, especialmente en aquellas zonas caracterizadas por tener inviernos cálidos. Al intentar establecer un modelo de predicción de la fecha de floración en función de la acumulación de frio invernal en olivo (De Melo-Abreu, 2004) se ha observado que cuando los olivos están expuestos a temperaturas cálidas en el período invernal, la intensidad de floración es menor, la floración se adelanta y es más larga. Esto implica una maduración irregular en un mismo árbol, dificultando así la recolección y/o aportando mayor inestabilidad a los aceites obtenidos. Además, en estas circunstancias, las fechas de floración varían entre variedades (Aybar *et al.*, 2015).

Hasta el momento, los estudios existentes han tratado de predecir la fecha de floración a través del empleo de modelos matemáticos en el momento actual por mediciones del contenido de polen en la atmósfera (Aguilera *et al.*, 2013), o mediante observaciones directas de la floración en lugares situados a diferente altitud (Aguilera y Ruiz Valenzuela, 2009). También existen algunos modelos de predicción de la floración del olivo en un escenario futuro de aumento de la temperatura media del aire (Gabaldón-Leal *et al.*, 2017) o simulando el calentamiento global mediante el empleo de estructuras tipo invernadero con atmósfera controlada (Benlloch-González *et al.*, 2018).

En este último caso, se observó que, al elevar las temperaturas en 4ºC, los frutos eran más pequeños que los que crecen en condiciones normales, con una menor relación pulpa/hueso, menor contenido en aceite y antocianos. Además, estos árboles presentaban un crecimiento vegetativo mayor (Benlloch - González *et al.*, 2019).

Además, en todos los casos, el incremento de la temperatura los meses antes de la floración ha tenido como consecuencia el adelanto en la floración y el alargamiento de la misma (Aguilera *et al.*, 2013; Benlloch-González *et al.*, 2018; Lopes *et al.*, 2019). Algunos autores hablan de un adelanto de la floración en dos días por cada ºC de aumento de la temperatura (Osborne *et al.*, 2000). En el caso de Gabaldón-Leal *et al.* (2017) predice que se producirá un adelanto en el inicio de la floración de 17 días como consecuencia del cambio climático a finales del siglo XXI.

Actualmente ya se ha determinado que el olivo ha visto un adelanto en su fecha habitual de floración en la zona del Mediterráneo a lo largo del siglo XX debido al incremento de la temperatura global media en un 0,6 +/-0,2ºC. Esto hace del olivo una especie frutal muy útil como bioindicador del cambio climático (Osborne *et al.*, 2000; Orlandi *et al.*, 2005; Aguilera *et al.*, 2013).

Por otro lado, el uso de estos modelos en zonas de inviernos cálidos puede ser una herramienta útil para determinar la elección de la variedad. Por ejemplo, en el caso del NW de Argentina, se observó que algunas variedades con altos requerimientos de frío como 'Frantoio' y 'Leccino' debían ser sustituidas por otras menos exigentes como es el caso de 'Arbequina' (Aybar *et al.*, 2015). Esta información daría más garantías de éxito en la implantación de nuevas explotaciones de olivo en zonas de clima subtropical y tropical.

En conclusión, en este contexto de incertidumbre, se plantea la forma de contrarrestar los efectos negativos en la fruticultura moderna para garantizar el abastecimiento y las producciones de las explotaciones. Las estrategias futuras de adaptación al cambio climático pasan por la selección de variedades mejoradas, la aplicación de inductores de la brotación, contrastados para otros frutales templados como ciruelo (Universidad Autónoma Chapingo *et al.*, 2000) o en viña (Almanza M. *et al.*, 2010) y el empleo de técnicas de manejo, especialmente, del riego (Castillo–Llanque *et al.*, 2014; González-Zafra, 2017; Cabezas-Luque, 2022). Incluso, Lorite *et al.* (2018) propone un modelo (AdaptaOlive) que permite establecer estrategias de adaptación del olivo a las condiciones climáticas futuras en zonas áridas y semiáridas del sur de España.

3. Contenido y composición de fenoles en olivo

Hoy en día, son indudables las propiedades saludables que tiene el aceite de oliva virgen extra, componente principal y diferenciador de la dieta Mediterránea. Entre todos sus componentes, uno de los más importantes son los polifenoles, que son un potente antioxidante natural, y que contribuyen decisivamente a algunas de las características organolépticas propias del aceite de oliva como son el picante y el amargo (Beltrán *et al.*, 2000; Bendini *et al.*, 2007; Beltrán *et al.*, 2007). Además, los polifenoles contribuyen a la estabilidad y conservación del aceite de oliva virgen extra (Beltrán *et al.*, 2000; Bendini *et al.*, 2007) y aportan una serie de efectos positivos en la salud como antiinflamatorio, para la prevención del Alzheimer (Omar *et al.*, 2017) y de algunos tipos de cáncer (Piroddi *et al.*, 2017). El contenido y la composición de fenoles tienen una influencia tanto genética como ambiental (Pérez *et al.*, 2018).

La diferente composición fenólica entre variedades ha quedado ampliamente demostrada en diferentes estudios de colecciones de variedades (Gómez-Rico *et al.*, 2008; Miho *et al.*, 2018) y se ha incluido como objetivo en programas de mejora (Pérez *et al.*, 2014).

Además, los polifenoles contenidos en el aceite de oliva dependen también del proceso de extracción en almazara y del momento de la recolección, variando su concentración a medida que avanza la maduración (Beltrán *et al.*, 2004; Gómez-Rico *et al.*, 2008). Dicha composición también se ve influida por las técnicas de manejo (Servili y Montedoro, 2002). Entre las prácticas agronómicas que influyen en esta composición, destaca el riego, observándose un mayor contenido en los polifenoles responsables del amargor en aquellos olivos sometidos a riegos deficitarios (Salas *et al.*, 1997).

También se ha encontrado que las condiciones ambientales, tanto las precipitaciones anuales (Beltrán *et al.*, 2004), como las temperaturas desde endurecimiento del hueso hasta la madurez (Bodoira *et al.*, 2016) juegan un papel importante en la composición fenólica del fruto y, por tanto, del aceite de oliva obtenido. Por ello, es importante conocer las características de los frutos formados en condiciones diferentes a las típicas mediterráneas.

4. Interacción genotipo x ambiente en olivo

El factor varietal o genotipo resulta determinante para algunos parámetros agronómicos o de calidad del aceite de oliva como se ha descrito anteriormente. Sin embargo, también existen algunas características que varían enormemente para la misma variedad en función del ambiente donde se produzca el crecimiento y desarrollo de la flor y el fruto. Así, el momento de floración y de sus diferentes etapas está altamente influenciado por el ambiente, mientras que, la variabilidad en la calidad de la flor tiene un componente genético más importante (Navas-López *et al.*, 2019). En ambos casos, la interacción genotipo x ambiente es significativa (Navas-López *et al.*, 2019). Asimismo, para un lugar determinado, se han descrito diferencias en cuanto a la fecha e intensidad de floración entre cultivares de olivo como en variedades portuguesas (Lopes *et al.*, 2019) o en otro estudio centrado en variedades griegas (Koubouris *et al.*, 2019). En este último caso, se observó incluso, la ausencia de floración en algunas variedades en una situación de baja acumulación de frío invernal. Ejemplos similares se dan para factores relacionados con la calidad del aceite de oliva, observándose diferente composición entre variedades que crecen en un mismo lugar, así como para la misma variedad cultivada en ambientes distintos (Baiano *et al.*, 2013).

Por lo tanto, es fundamental estudiar la interacción genotipo x ambiente con ensayos multi-ambiente que permitan el establecimiento de las variedades más adecuadas según el lugar de implantación (Navas-López *et al.*, 2020).

Cabe destacar que, hasta el momento, los programas de mejora y la investigación en olivo han estado orientadas a la búsqueda de selecciones más precoces y productivas (León *et al.*, 2007), a mejorar la calidad del aceite obtenido (Dabbou *et al.*, 2010), adaptadas a la mecanización para determinados sistemas de conducción (Rallo *et al.*, 2008) y selecciones con cierta resistencia o tolerancia a las principales enfermedades (Serrano *et al.*, 2021) pero existen pocas referencias a la obtención de nuevas variedades adaptadas a las nuevas condiciones climáticas provocadas por el calentamiento global. En el caso de otras especies frutales, la obtención de nuevas variedades ha sido mucho más desarrollada y el estudio de su comportamiento en situaciones con baja acumulación de frío, ha permitido ya la adaptación de su cultivo a muy variadas condiciones climáticas, como es el caso del manzano (Laurens, 1998), el almendro (Prudencio *et al.*, 2018), el melocotón (Maneethon *et al.*, 2007; Scariotto *et al.*, 2013) o los arándanos que se han establecido en regiones subtropicales y tropicales (Meza *et al.*, 2023).

Los programas de mejora han tenido poco desarrollo hasta el momento en la búsqueda de variedades adaptadas a temperaturas invernales altas. La citada interacción genotipo x ambiente puede ser un factor que dificulte esta labor y que hace que sea imprescindible el desarrollo de ensayos de variedades y programas de mejora multi-ambiente.

5. El cultivo del olivo frente al cambio climático

El cambio climático se refiere a los cambios a largo plazo de las temperaturas y los patrones climáticos. Las concentraciones de gases de efecto invernadero se encuentran en su nivel más elevado en 2 millones de años y siguen aumentando. Esto ha llevado a que la temperatura de la Tierra sea ahora 1,1 ºC más alta que a finales del siglo XIX y que la década de 2011 a 2020 haya sido la más cálida registrada hasta el momento. Además, hay que tener en cuenta que el aumento de temperatura no es el único elemento del cambio climático. La Tierra es un sistema en el que, un cambio en uno de los elementos lleva a variaciones en el resto. Por lo tanto, las consecuencias del cambio climático son también largos períodos de sequías, falta de agua, graves incendios, aumento del nivel del mar, inundaciones, el deshielo de los polos, aumento de fenómenos climáticos extremos y disminución de la biodiversidad (Naciones Unidas, Acción por el Clima, consulta online).

a. Floración

En este escenario de cambio climático, los frutales serán más vulnerables que las especies anuales, porque podrán ver comprometida su producción por la ausencia de floración en condiciones de baja acumulación de frío invernal (Luedeling *et al.*, 2009). Estas anomalías en la floración de los frutales templados han empezado a detectarse ya en zonas donde hasta el momento existía un reposo invernal claro y ahora, sin embargo, debido al aumento de temperatura causado por los gases de efecto invernadero, la acumulación de frío invernal es menor (García–Mozo *et al.*, 2010).

Para permitir la adaptación de los frutales frente el cambio climático, es necesario el desarrollo de variedades con bajas necesidades de frío y de estrategias que hagan frente a la insuficiente acumulación de frío durante el invierno. También hay que profundizar en el conocimiento de las temperaturas mínimas necesarias para la floración en las especies y variedades frutales (Luedeling *et al.*, 2009).

Algunos estudios en los que se han simulado las condiciones futuras de calentamiento global debido a la presencia de gases de efecto invernadero en la atmósfera, prevén que, en la zona del Mediterráneo, la acumulación de frío invernal en chilling portions (Fishman *et al.*, 1987) disminuya entre 30 y 40 puntos a mediados y finales del siglo XXI (Luedeling *et al.*, 2009; Fernandez *et al.*, 2023). Incluso, predice que muchas zonas cálidas como el norte de África pierdan la mayor parte del frío invernal (Luedeling *et al.*, 2009).

Por lo tanto, el olivo se verá afectado en gran medida por estos cambios ya que, la superficie dedicada al olivo más importante se encuentra en la Cuenca Mediterránea y esta área ha sido definida, en base al Índice Regional de Cambio Climático, como un "Punto Caliente" o región en la que los impactos producidos por el cambio climático en el medio ambiente o en cualquier otro sector de actividad, son altamente pronunciados (Giorgi, 2006).

En el escenario de cambio climático futuro, se prevé que las áreas donde tradicionalmente se ha desarrollado el cultivo del olivo varíen (Gabaldón-Leal *et al.*, 2017; Meza *et al.*, 2023). Esto traería graves consecuencias, no sólo económicas, si no también sociales para un sector tan importante en España como el olivo.

Ante esta situación, el clima subtropical canario representa un escenario natural adecuado para el estudio de la influencia de condiciones climáticas no mediterráneas en la fenología de floración, especialmente debido a la ausencia de bajas temperaturas invernales que son típicas del clima Mediterráneo (El Yaacoubi *et al.*, 2014). Incluso, el estudio de la fenología en el clima subtropical puede suponer un escenario natural que emule las características del clima Mediterráneo en un futuro próximo dada la situación de calentamiento global existente.

b. Composición fenólica

Como se ha apuntado anteriormente (apartado 3), y a pesar de que el contenido y la composición fenólica en el aceite de oliva virgen extra está altamente influida por el componente genético (Talhaoui *et al.*, 2016; Pérez *et al.*, 2018; Serrano *et al.*, 2020), apenas hay trabajos donde se haya estudiado en profundidad la interacción genotipo x ambiente (Pérez *et al.*, 2018).

Trabajos realizados para otros componentes del aceite han demostrado como las variaciones ambientales llegan a comprometer la calidad del producto obtenido. Así por ejmplo ha ocurrido con 'Arbequina' en algunas zonas de Australia, donde el contenido en ácido oleico del aceite producido es tan bajo que no puede obtener la clasificación de aceite de oliva virgen extra de acuerdo con los parámetros oficiales establecidos (Mailer *et al.*, 2010). Algo similar se cita en estudios realizados en Argentina, se encontraron valores de ácido oleico para 'Arbequina' sustancialmente más bajos que los registrados para esta misma variedad cuando crece en la Península Ibérica (Cornejo, 2018). En este sentido, son varias las investigaciones llevadas a cabo con el fin de comprobar que los aceites de oliva producidos en las nuevas zonas de cultivo cumplen con la normativa COI para ser considerados como aceites de oliva por su grado de acidez libre, índice de peróxidos, coeficientes K_{232} y K_{270}, composición en ácidos grasos, estabilidad oxidativa y contenido total de sustancias fenólicas (Bruzzone *et al.*, 2015; Torres *et al.*, 2017).

Por lo tanto, cabe esperar que los frutos que se forman y desarrollan en climas fuera de la Cuenca del Mediterráneo tengan un contenido en polifenoles claramente diferenciado (Bodoira *et al.*, 2016). Así, se ha demostrado que aceites de oliva procedentes de la misma variedad, pero recolectados en zonas diferentes, cuentan con una composición fenólica diferente, como en los estudios con la variedad 'Chemlali' en Túnez (Gargouri *et al.*, 2013) o con 'Baladi' en el Líbano (El Riachy *et al.*, 2018). Estos estudios han sido realizados en condiciones de clima Mediterráneo. Sin embargo, no existen datos de la influencia de estos factores y su interacción en condiciones de ausencia de frío invernal como es el caso del clima subtropical canario, donde todo el ciclo fenológico del olivo sufre modificaciones (Mérida García, 2018).

Por otra parte, en un contexto de cambio climático, la escasez de precipitaciones condicionará el estado hídrico de las aceitunas durante la síntesis del aceite de oliva y este estrés hídrico se ha relacionado con un aumento en el contenido total de polifenoles (Patumi *et al.*, 1999; Gómez-Rico *et al.*, 2006; Cirilli *et al.*, 2017). Por lo tanto, este sería un efecto positivo, especialmente interesante para aquellas variedades con bajo contenido genotípico en fenoles. Aunque debe apuntarse que la falta de agua también pueda implicar una disminución en la productividad media de las explotaciones de olivo (Pastor *et al.*, 1999).

Otra característica asociada al cambio climático se relaciona con el adelanto de la recolección (Benlloch-González *et al.*, 2019). Este hecho, sumado a que las recolecciones cada vez son más tempranas para obtener aceites de oliva virgen extra con mejores parámetros organolépticos (Jiménez Herrera *et al.*, 2012), puede llevar a recolecciones con altas temperaturas (Plasquy, 2022) y, con ello, una temperatura inicial en la almazara inadecuada para la obtención de aceites de oliva virgen extra de calidad y/o a un mayor gasto energético para el enfriamiento de la aceituna en el proceso previo a la preparación de la pasta (Plasquy, 2022).

I.II. OBJETIVOS

Por todo lo expuesto, Tenerife se postula como un escenario natural ideal para el estudio del impacto de los inviernos suaves sobre la fenología del olivo y sobre la composición del aceite aquí obtenido en las variedades comerciales más extendidas.

Los resultados obtenidos en esas condiciones pueden servir de base para conocer las consecuencias del calentamiento global y del cambio climático en la Cuenca Mediterránea donde el olivo es el cultivo más representativo social y económicamente.

Objetivo 1. Estudio de la fenología de floración en plantas de olivo en relación con las temperaturas invernales en distintos pisos bioclimáticos de la isla de Tenerife y su comparación con la floración en plantas de olivo en distintas localidades de Andalucía (Capítulo II).

Objetivo 2. Evaluación de la influencia de la interacción genotipo x ambiente en la evolución de la fenología de floración en un grupo de variedades de olivo comerciales en dos zonas con condiciones climáticas muy distintas: Tenerife y Andalucía (Capítulo III).

Objetivo 3. Estudio del contenido fenólico y de la composición del aceite de oliva en diferentes variedades a lo largo del período de madurez en condiciones de altas temperaturas invernales y su comparación con las mismas variedades producidas en condiciones de clima mediterráneo (Capítulo IV).

I.III. REFERENCIAS BIBLIOGRÁFICAS

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CAPÍTULO II. Differences on flowering phenology under Mediterranean and Subtropical environments for two representative olive cultivars

II.I. Abstract

Olive flowering phenology is highly affected by climatic conditions. Phenological models have been developed to forecast flowering date on olive mainly based on temperature. These models have used flowering datasets collected from trees growing under Mediterranean climatic conditions. In most of the cases, in those conditions, chilling requirements are rapidly fulfilled. In other cases, artificial modifications of the climatic conditions has been practiced by using growth chambers. In the present work, we compare the flowering phenology of 'Picual' and 'Arbequina' olive cultivars in Mediterranean conditions of Andalucía, Southern Spain, with those in Tenerife, Canary Islands with Sub-Tropical climate. The climatic conditions of Tenerife respect to Andalucía promoted an earlier flowering date but, more importantly, a much longer flowering period. This is mainly produced by an asynchronous flowering bud burst that will generate negative impacts on yield and quality. Quite likely, those differences on flowering phenology between Andalucía and Tenerife climatic conditions are mainly caused by the lack of winter chilling in Tenerife locations. Based on those results, we propose that future works studying the effect of lack of winter chilling on olive should include the length of the flowering period as a parameter to be modeled. Besides, studies on natural climatic conditions with warm winters, as the one here reported, are needed to really assess the effect of winter chilling on olive.

Keywords: Asynchrony, Climate change, Flowering period, Forecasting, Winter chilling.

II.II. Introduction

Flowering is a critical reproductive stage for the final yield in many plant species (Díez-Palet *et al.*, 2019), being highly influenced by climatic conditions (Rodríguez *et al.*, 2019). This included olive (Hartmann and Porlingis, 1957; Navas-Lopez *et al.*, 2019), an evergreen fruit species typical from Mediterranean climate. For that reason, climatic models have been developed to forecast the flowering date in olive, mainly based on air temperature (De Melo-Abreu *et al.*, 2004; Gabaldón-Leal *et al.*, 2017). Those studies concluded that, in the absence of characteristic climatic conditions for olive growing, specially lack of winter chilling, flowering will not occur (Aybar *et al.*, 2015). This is important taken into account the expansion of olive growing outside the Mediterranean climate, as the case of Argentina (Torres *et al.*, 2017) or Australia (Kailis and Sweeney, 2002). Those models are also used to forecast the influence the projections of temperature in- crease provided by the phenological models (Lorite *et al.*, 2018).

The development of phenological models requires high quality experimental data with observations collected in a wide range of climatic conditions. However, most of the studies regarding olive flowering phenology have been performed using data gathered in Mediterranean climatic conditions (De Melo-Abreu *et al.*, 2004) or artificial environments (Benlloch-González *et al.*, 2018; Gabaldón-Leal *et al.*, 2017). But few reports have studied flowering phenology using non-Mediterranean natural environments data (Aybar *et al.*, 2015).

In this sense, Tenerife, in the Canary Islands, is one of the non- Mediterranean locations where olive has been grown from some time now (Medina *et al.*, 2018). It is situated 4° above the Tropic of Cancer in the Atlantic Ocean and close to the African coast. The sub-Tropical climate of this island represents a convenient natural scenario to study the influence of natural non-Mediterranean climatic conditions on olive flowering phenology, particularly the lack of the low winter temperatures that are typical of the Mediterranean climate (El Yaacoubi *et al.*, 2014).

Therefore, the objective of the present work is to compare olive flowering phenology in three locations of Andalucía, Southern Iberian Peninsula, having typical Mediterranean climate, with other three lo- cations in Tenerife having Sub-Tropical climatic conditions. Cultivars 'Picual' and 'Arbequina', two of the most widely planted in the world, were used for this comparison. Differences on flowering behavior among locations was discussed under the hypothesis of differences on winter chilling.

II.III. Materials and methods

1. Plant material and location

This study was carried out in commercial orchards of six different locations with a wide range of weather conditions. Three of them, namely Canales Altas, El Viso and Los Tomillos, are in the Southeast of Tenerife, Canary Islands, with a Sub-tropical climate (Fig. 2.1) at 630, 410 and 200 m.a.s.l. respectively. The other three are in Andalucía, Southern Iberian Peninsula; Úbeda and Baena located in typical olive growing areas with a Mediterranean climate, and Gibraleón, with milder temperatures from November to February. They are located at 748, 405 and 26 m.a.s.l. respectively. All orchards were grown with typical olive growing management aimed at maximizing productivity. Tenerife locations were irrigated with 3.000 m3/year, Úbeda and Gibraleón with 1.500 m3/year and Baena was maintained in dry farming. Air temperature was recorded in each field by weather stations (Pessl Instrument iMetos).

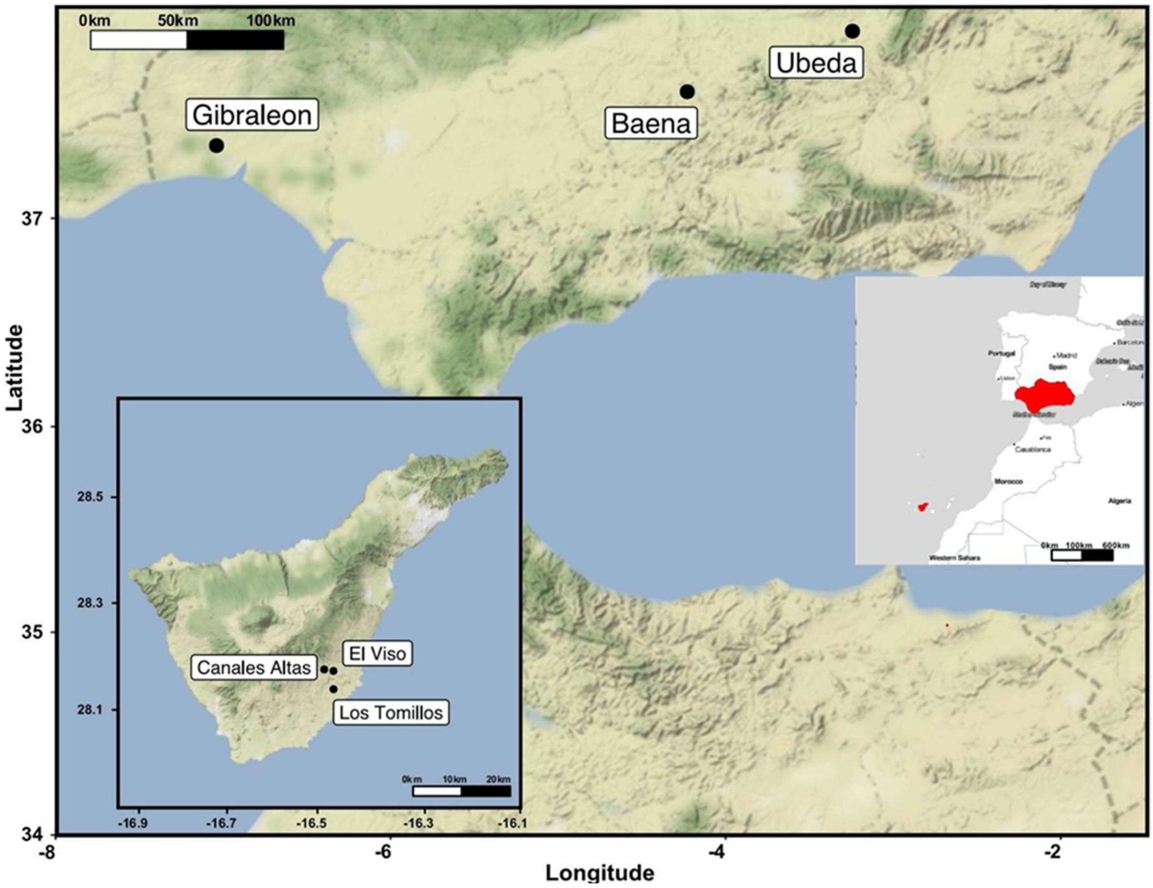

Fig. II.1.- *Location of phenological sampling sites in Tenerife (Canary Islands) and in Andalucia (Southern Iberian Peninsula).*

2. Flowering phenology

For each location and year, four trees of 'Arbequina' and 'Picual' cultivars, aged from 7 to 9 years and with significant amount flowering were chosen for the study to avoid the potential effect of yield on phenology. Amount of flowering was evaluated following a previously reported methodology (Navas-Lopez *et al.*, 2019) in a 0–3 scale (0 = no flowering, 1 = less than 33 % of the canopy having flowers, 2 = from 33 to 66 % of the canopy flowered and 3 = more than 66 % of the canopy flowered). Phenology was evaluated in three consecutive years from 2016 to 2018 except 'Picual' at El Viso in 2016 and Los Tomillos in 2017 and 2018. The international standardized BBCH numerical scale for olive (Sanz-Cortés *et al.*, 2002) was used. Observations started when stage 53 appeared (inflorescence buds open and lower cluster development are visible) and ended when stage 69 was the most common in the tree (end of flowering, fruit set, non-fertilized ovaries fallen). Twice a week, the earliest, most common and latest stages were recorded for each tree and location after visually inspection of the whole canopy, as standard protocol on olive (Navas-Lopez et a., 2019; Vuletin-Selak *et al.*, 2013).

All of these data were used to calculate three phenological para- meters as previously reported (Navas-Lopez *et al.*, 2019):

- Length of flowering period (FP): Number of days from the first time for stage 61 (first flower open) to appear as earliest stage, until the first time for stage 68 (majority of petals fallen) to appear as most common stage.
- Length of full bloom period (FBP): Number of days from the first time for stage 61 to appear as most common, until last time for stage 65 (full bloom, at least 50 % of flowers open) as most common.
- Full bloom date (FB): Average of the starting and ending date of the FBP, expressed as Julian date.

3. Statistical analysis

Analysis of variance was performed to evaluate the relative influence of location, year and their interaction on the variability of phenological parameters by each of the two cultivars studied. It was not possible to perform a joint analysis of both cultivars as there was not a randomize design for them in each location. Comparison of means was used to test differences among locations, years and location-year, when significant. The analysis was performed with R software (R development Core Team, 2016).

For assessing the chill accumulation throughout the crop season, chill portions (CP) were calculated using the Dynamic Model (Fishman *et al.*, 1987), based on hourly temperature datasets, beginning 1st October of each season (Pope *et al.*, 2014; Jarvis-Shean *et al.*, 2015) until summer.

Thermal time or growing degree days (GDD) considered the accumulated daily mean temperatures higher than a base temperature. Following De Melo-Abreu *et al.* (2004) the base temperature was set at 9.1 °C. Due to the early flowering dates observed in some locations, the thermal time accumulation was calculated from 1st October of the year before flowering.

II.IV. Results

Air temperatures in Tenerife (Canales Altas, El Viso and Los Tomillos) were milder than in Andalucía (Úbeda, Baena and Gibraleón), i.e., higher temperatures in autumn and winter and lower in spring (Fig. 2.2). In fact, the daily temperature range was much narrow in Tenerife than in Andalucía. Gibraleón was the location in Andalucía with milder winter temperatures, while Canales Altas was the location in Tenerife with lower temperatures and higher range of variation. The winter of 2016 was the mildest of the studied years while 2018 was the coolest. And the spring of 2017 was the hottest of the three recorded. Annual rainfall in Tenerife was lower than in Andalucía in every location and year (Supplementary data).

According to differences on air temperature, chill portions (CP) accumulation in Andalucía, was much higher than in Tenerife (Fig. 2.3 and 2.4). Two Andalucía locations, Baena and Úbeda, showed an almost identical CP pattern in the three seasons considered (Fig. 2.3). Gibraleón, the other Andalucía location, showed a lower CP accumulation. From the three Tenerife locations, Canales Altas showed a CP accumulation similar to Gibraleón but somewhat delayed, especially in 2015–2016 and 2016–2017 winters. In Los Tomillos and El Viso CP accumulation was very low. On the contrary, growing degree days (GDD) accumulation was much higher in the three Tenerife locations than in the Andalucía ones (Fig. 2.4). Again, Canales Altas showed the closest pattern to Gibraleón, especially in 2016–2017 and 2017–2018.

Total variance of the two flowering period parameters studied showed similar distribution for both 'Arbequina' and 'Picual' (Table 2.1). For FP, location and year*location were the main contributors to the total variance while for FPB year*location has greater effect that the individual factors. On the contrary, different distribution of variance components between cultivars was observed for FB. The main effect was location in the case of 'Arbequina' and year in the case of 'Picual'. All the effects were significant except year for FBP in 'Arbequina'. Error term generally allocated low percentage of the total variance, thus indicating low variance among trees of each elementary plot. The only exception was FPB in Arbequina.

Among the six locations considered, the three in Tenerife showed the longest FP, with Canales Altas having significant higher values than the other two (Tables 2.2 and 2.3). For the three Andalucía locations, Gibraleón showed higher FP than the other two but only for 'Arbequina'. FBP showed very low differences among locations, with Tenerife having slightly higher values than Andalucía, again only for 'Arbequina'. In relation of full bloom date (FB), three groups with significant differences were found: Úbeda and Baena; Canales Altas and Gibraleón; Los Tomillos and El Viso, with late, intermediate and early date, respectively.

Among the three years studied, 2016 showed the highest values for FB, being 2017 and 2018 very similar. The same happened to FPB but only in 'Picual'. FB variation across years was similar in 'Arbequina' and 'Picual', with the lowest values in 2017.

It is also remarkable the significant effect of the location by year interaction (Table 2.3). In fact, the FP of Canales Altas 2016 was much higher than the rest of the year by location combinations, for both 'Arbequina' and 'Picual'. The rest of the FP values were higher in 'Arbequina' than in 'Picual'. High values were also found for FP in 2018 for the three locations in Tenerife. FBP was very high in Canales Altas 2016, specially for 'Picual', being the highest in Los Tomillos 2018 and Gibraleón 2016 for 'Arbequina'. FP and FBP in Gibraleón in 2016 was greater than in the rest of locations and years in Andalucía. The highest values of FB were found in Úbeda and Baena 2016 and 2018, being much lower in 2017.

In general, the flowering started and ended later in Andalucía locations than in Tenerife ones (Figs. 2.5 and 2.6). Among Andalucía locations, Gibraleón was the earliest one followed by Baena and Úbeda. The behavior of 'Arbequina' and 'Picual' was very similar in Andalucía. Only in Gibraleón 2016 the flowering period was much longer in 'Arbequina' than in 'Picual'. In Tenerife, the flowering phenology observed was much more extended than in Andalucía, as previously stated; and higher differences between 'Picual' and 'Arbequina' were found. The very long flowering period found in Canales Altas 2016 included two full flowering periods, in the same trees, at different dates. This also happened in Los Tomillos 2018 in 'Arbequina'. In 'Picual', FP started later than in 'Arbequina' and flowering periods were shorter.

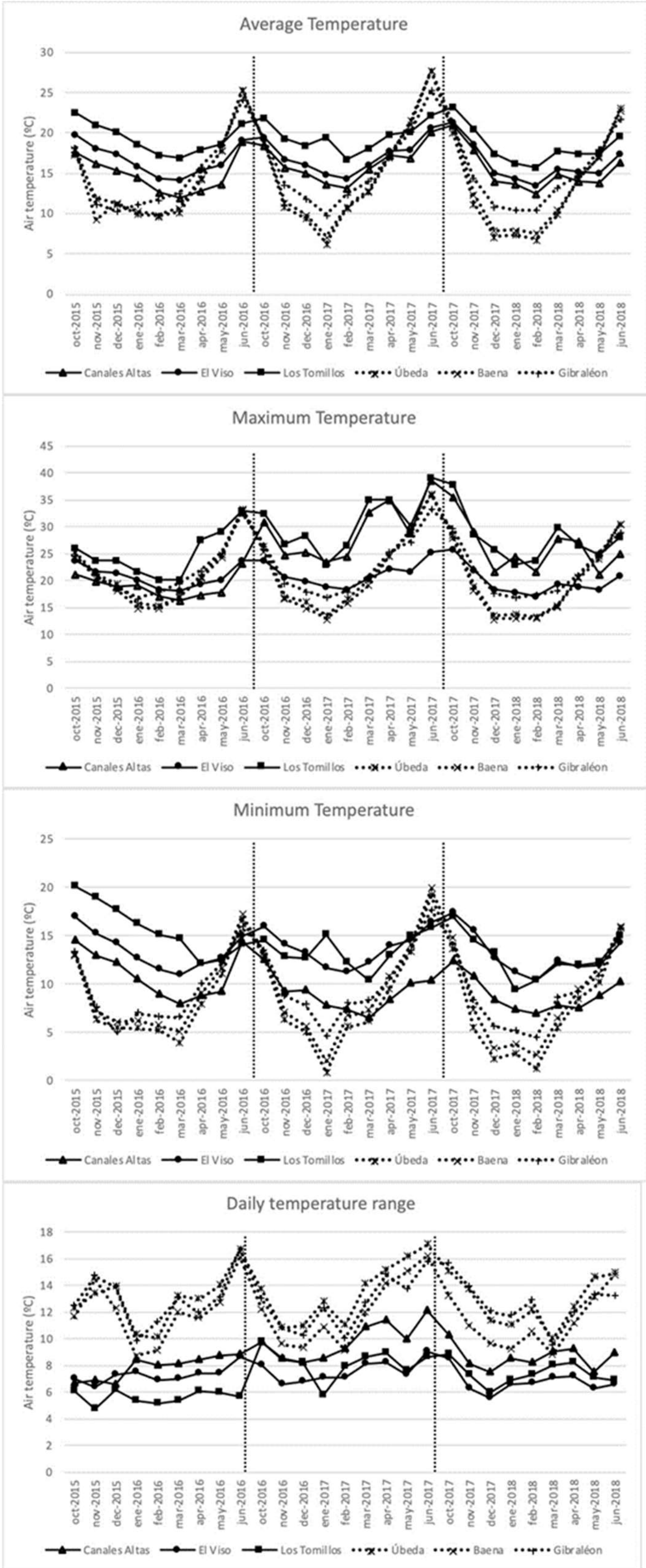

Fig. II.2.- *Wheather data of the three Andalucía (Úbeda, Baena and Gibraleón) and Tenerife (Canales Altas, El Viso and Los Tomillos) locations from October to June in the three seasons considered (2015–2016, 2016–2017 and 2017–2018). Daily mean of air temperature (mean, maximum, average and range) are included.*

The variation of the average most advanced, common and delayed phenology stage across the flowering season was also very different in Andalucía and Tenerife (Figs. 2.7 and 2.8). In Andalucía 2016, Úbeda showed very small differences between those three parameters, being a bit higher in Gibraleón, especially for ‘Arbequina’. On the contrary, for locations in Tenerife, differences among those three parameters were very wide. Again, ‘Arbequina’ showed some higher differences than ‘Picual’ especially in Los Tomillos (Figs. 2.7 and 2.8). Similar pattern of variation was found in 2017 and 2018 (data not shown).

It is remarkable that the most common phenology stage in Tenerife was not always increasing with time at should be expected. For example, in ‘Arbequina’, Canales Altas 2016, the most common phenology stage in day 41 was 69 while in day 83 was 53 (Fig. 2.8). This decrease was caused by the blooming of new flowering buds that were still dormant at the first date. This is what caused the above-mentioned occurrence of two full flowering periods in the same tree and year. In general, the erratic behavior of the flowering phenology in Tenerife was caused by the asynchronous bud blooming observed in both ‘Arbequina’ and ‘Picual’ (Fig. 2.9). In fact, in some flowering branches, up to 9 different phenology stages were found (data not shown). Besides, those different stages were randomly distributed thorough the branch without following a common pattern.

Tabla II.1.- *Percentage of variance of location, year and their interaction for the flowering period (FP in days) and full flowering period (FBP in days) and full bloom time (FB in Day of the Year) in ‘Arbequina’ and ‘Picual’. Values in bold indicate significant differences for this source of variation at $p < 0.01$.*

		‘Arbequina’				‘Picual’		
	df	FP	FBP	FB	df	FP	FBP	FB
Year	2	5.65	2.41	18.55	2	3.88	10.10	53.34
Location	5	54.04	3.05	47.16	5	46.41	16.93	28.86
Year*Location	7	33.40	42.67	28.74	7	48.26	55.84	17.31
Error	42	6.91	51.87	5.54	40	1.45	17.14	0.49

Tabla II.2.- *Comparison of means of length of flowering period (FP, in days) and length of full flowering period (FBP, in days) in ‘Arbequina’ by location, year and their interaction. Different letters indicate significant differences ($p < 0.01$) among means within each of those three groups of data*

	FP								FBP							
	2016		2017		2018		AVERAGE		2016		2017		2018		AVERAGE	
Úbeda	12.5	f	8.5	f	11.0	f	10.7	d	5.5	cde	2.0	e	4.8	de	4.1	b
Baena	16.5	f	14.7	f	10.7	f	14.0	d	9.5	cde	6.3	cde	5.8	cde	7.2	ab
Gibraleón	53.3	cd	18.0	f	14.2	f	28.1	c	31.3	ab	5.0	cde	5.8	cde	14.1	ab
Canales Altas	130.0	a	52.5	cd	55.7	cd	79.4	a	20.0	bc	15.0	cd	18.0	bc	17.7	a
Los Tomillos	67.0	bc	36.2	e	64.8	bc	56.0	b	6.5	cde	10.0	cde	37.0	a	17.8	a
El Viso	46.0	de	47.7	de	73.0	b	55.6	b	6.0	cde	10.5	cde	18.8	bc	11.8	ab
Average	54.3	a	30.7	b	38.2	b			12.3		8.4		15.0			

Tabla II.3.- *Comparison of means of length of full bloom date (FB, in Day of Year) in 'Arbequina' by location, year and their interaction. Different letters indicate significant differences (p < 0.01) among means within each of those three groups of data*

	FB							
	2016		2017		2018		AVERAGE	
Úbeda	144.3	ab	127.0	cd	150.1	a	140.44	a
Baena	141.5	ab	120.6	de	147.1	a	136.41	a
Gibraleón	111.3	ef	121.8	cde	131.1	c	122.42	b
Canales Altas	134.0	bc	86.3	ij	129.8	cd	116.67	b
Los Tomillos	123.8	cd	77.0	j	89.0	hi	96.58	c
El Viso	106.0	fg	98.3	gh	90.9	hi	98.37	c
Average	127.5	a	103.6	b	122.9	a		

Tabla II.4.- *Comparison of means of length of flowering period (FP, in days) and length of full flowering period (FBP, in days) in 'Picual' by location, year and their interaction. Different letters indicate significant differences (p < 0.01) among means within each of those three groups of data.*

	FP								FBP							
	2016		2017		2018		AVERAGE		2016		2017		2018		AVERAGE	
Úbeda	10.0	f	6.0	f	8.3	f	7.7	c	4.0	cd	2.0	d	3.5	d	3.0	b
Baena	14.3	ef	20.3	de	10.0	ef	14.8	c	7.8	cd	8.0	cd	4.3	cd	6.7	b
Gibraleón	27.5	cd	14.3	ef	13.5	ef	16.8	c	17.5	b	6.7	cd	3.0	d	7.4	b
Canales Altas	142.5	a	27.8	cd	60.0	b	76.8	a	39.5	a	12.0	bc	7.0	cd	19.5	a
Los Tomillos	29.0	cd	NA		NA		29.0	b	6.0	cd	NA		NA		6.0	b
El Viso	NA		37.5	c	37.8	c	37.6	b	NA		6.8	cd	12.3	bc	9.5	b
Average	51.1	a	21.5	b	25.9	b			16.0	a	7.1	b	6.0	b		

Tabla II.5.- *Comparison of means of full bloom date (FB, in Day of Year) in 'Picual' by location, year and their interaction. Different letters indicate significant differences (p < 0.01) among means within each of those three groups of data.*

	FB						
	2016		2017		2018		AVERAGE
Úbeda	145.0	bc	126.0	e	151.6	a	140.0
Baena	143.1	c	121.3	f	147.8	ab	137.4
Gibraleón	126.3	e	120.6	f	131.5	d	126.7
Canales Altas	143.3	c	113.8	g	135.3	d	130.7
Los Tomillos	141.0	c	NA		NA		141.0
El Viso	NA		108.1	h	114.1	g	111.1
Average	140.8	a	117.8	c	136.0	b	

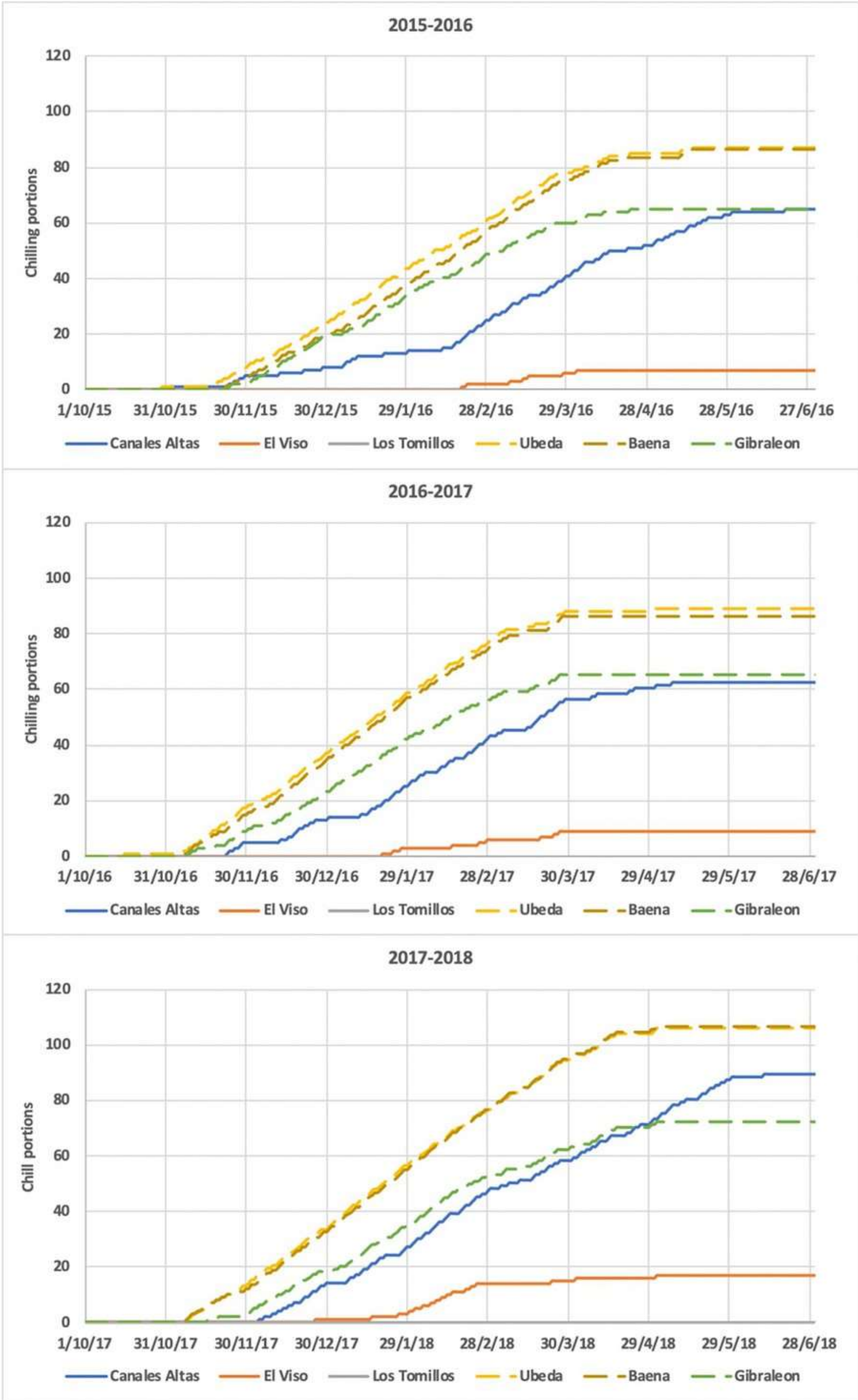

Fig. II.3.- *Variation of the chill portions accumulation with time in the three Andalucía (Úbeda, Baena and Gibraleón) and Tenerife (Canales Altas, El Viso and Los Tomillos) locations in 2016, 2017 and 2018.*

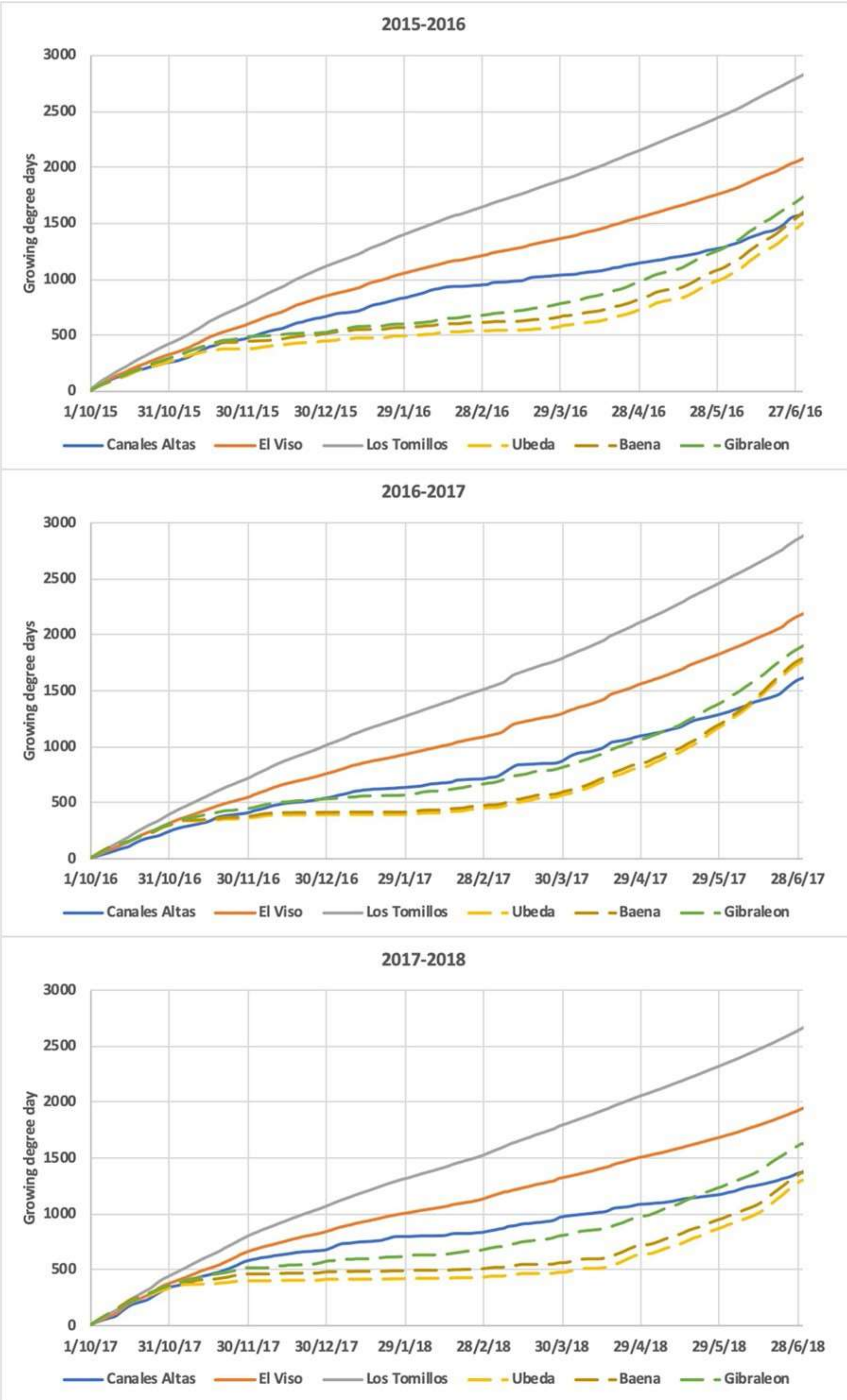

Fig. II.4.- *Variation of the growing degree days accumulation with time in the three Andalucía (Úbeda, Baena and Gibraleón) and Tenerife (Canales Altas, El Viso and Los Tomillos) locations in 2016, 2017 and 2018.*

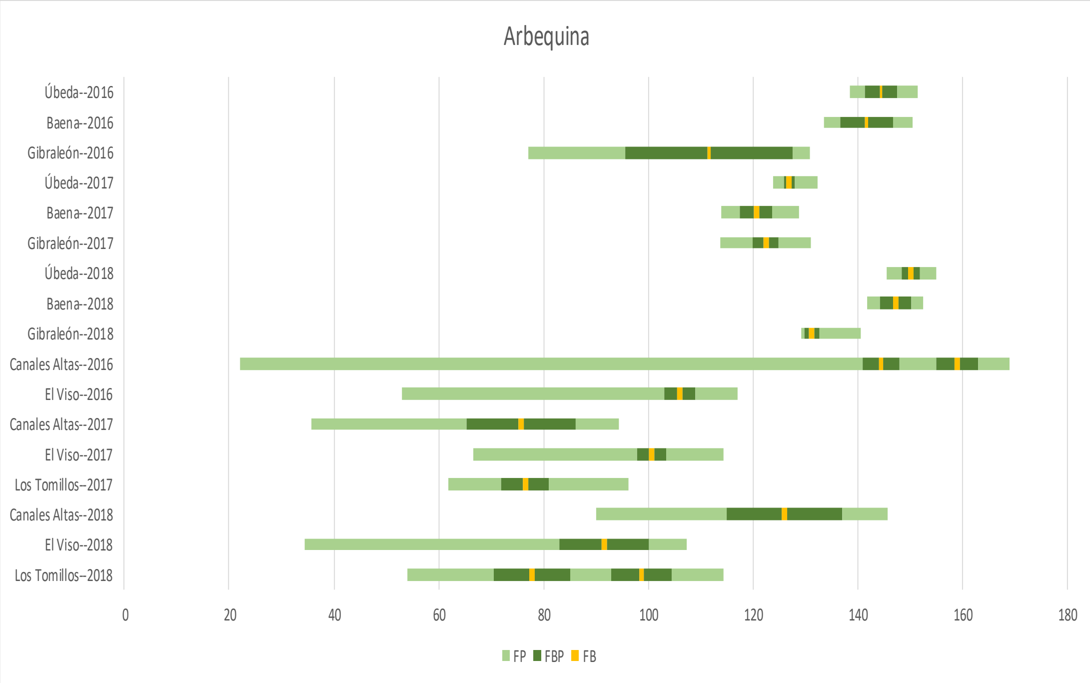

Fig. II.5.- Means of flowering period in days (FP in light green), full flowering period in days (FBP, in dark green) and full bloom time in Day of the Year (FB in yellow) in 'Arbequina' in the three Andalucía (Úbeda, Baena and Gibraleón) and Tenerife (Canales Altas, El Viso and Los Tomillos) locations in 2016, 2017 and 2018.

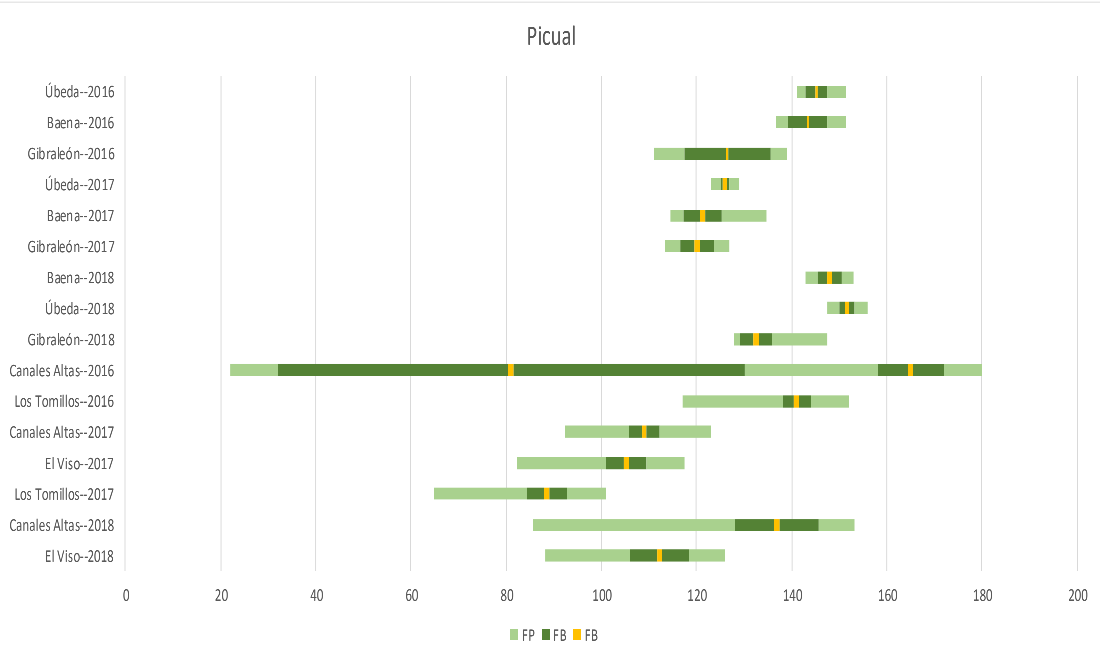

Fig. II.6.- Means of flowering period in days (FP in light green), full flowering period in days (FBP, in dark green) and full bloom date in Day of the Year (FB in yellow) in 'Picual' in the three Andalucía (Úbeda, Baena and Gibraleón) and Tenerife (Canales Altas, El Viso and Los Tomillos) locations in 2016, 2017 and 2018.

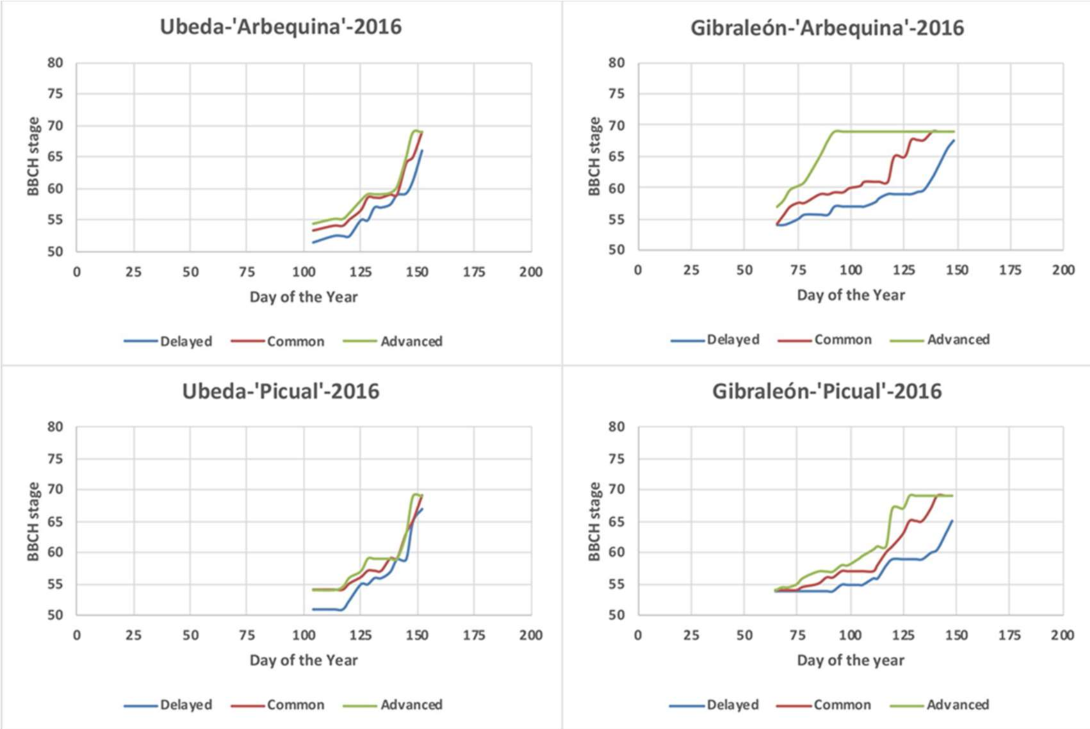

***Fig. II.7.-** Variation of the average most delayed, common and advanced flowering stage (BBCH scale) in 'Arbequina' and 'Picual' across the flowering period (Julian day) in two Andalusian locations, Úbeda and Gibraleón in 2016.*

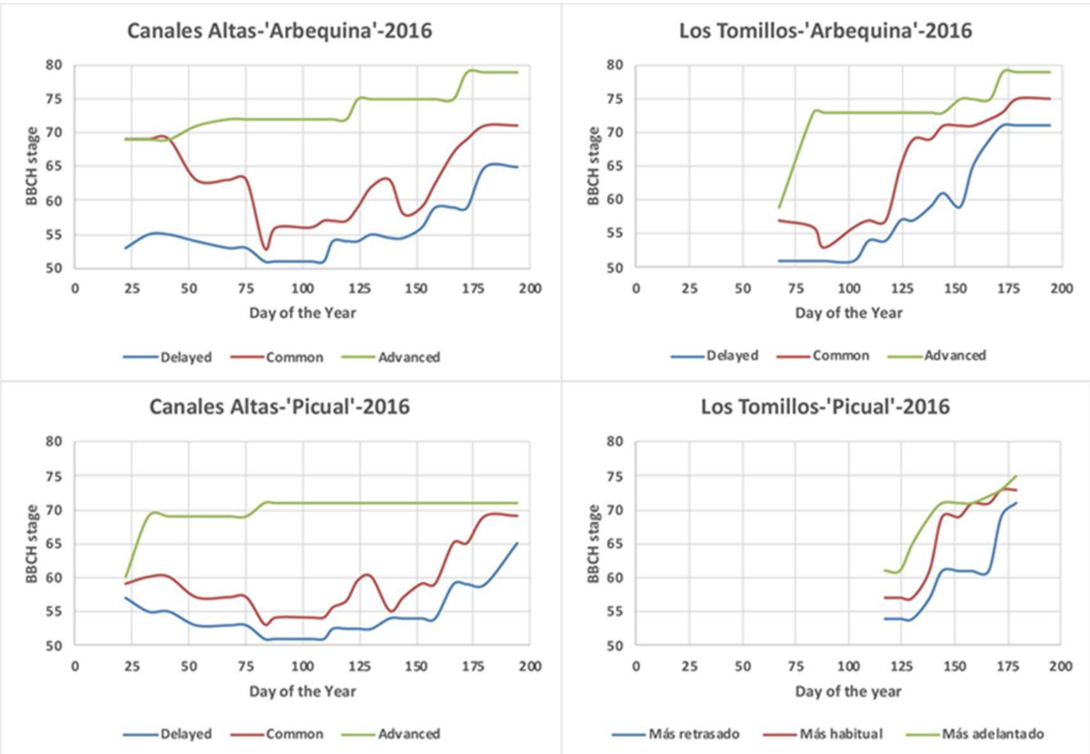

***Fig. II.8.-** Variation of the average most delayed, common and advanced flowering stage (BBCH scale) in 'Arbequina' and 'Picual' across the flowering period (Julian day) in the two Tenerife locations, Canales Altas and Los Tomillos in 2016.*

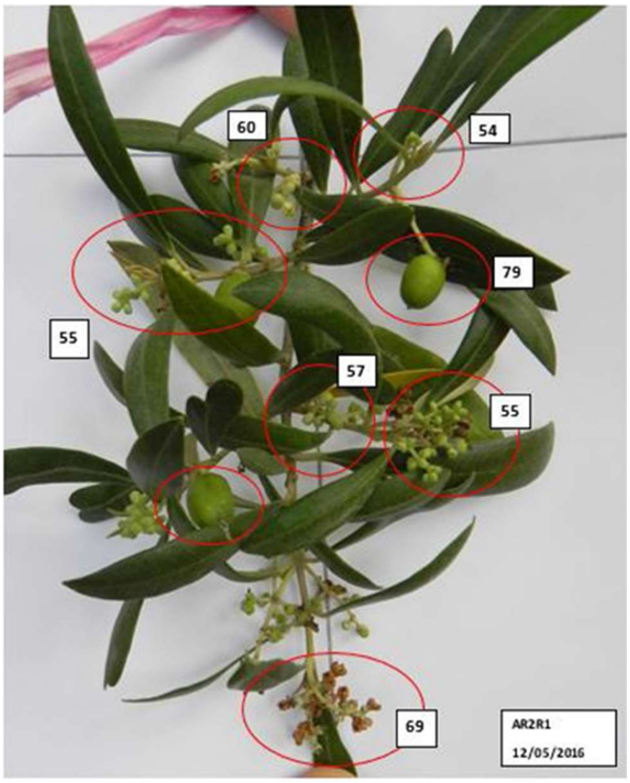

Fig. II.9.- *Flowering branch of 'Arbequina' in May 12, 2016. Six different phenological stages (54, 55, 57, 60, 69 ad 79) are observed in a random assortment.*

II.V. Discussion

This study has identified that environmental conditions have a high influence on olive flowering phenology, not only on the date for full flowering as previously reported (Garcia-Mozo et al., 2009), but also on the length of the flowering and full flowering period. The significant year by location interaction indicates that the site-specific environmental conditions of each year and location are determinant for the behavior of the flowering.

The flowering phenology observed in Andalucía is in accordance with previous report in the same locations (Navas-Lopez *et al.*, 2019); with very similar flowering phenology of Úbeda and Baena, located in the typical olive growing area in the South of the Iberian Peninsula. However, Gibraleón, with milder winters, showed earlier full flowering dates and also slightly longer length of flowering period than Úbeda and Baena. We have also calculated the difference between the most delayed and most advanced phenology stage by each cultivar, location and date. Those differences were higher in Gibraleón respect to the other two Andalucía locations.

However, the greatest differences on flowering phenology were found when comparing locations in Andalucía vs. Tenerife. Full flowering date occurs much earlier in Tenerife than in Andalucía. Besides, flowering period is much more prolonged in Tenerife; even, two full flowering periods were found in some years and locations. On the reviewed bibliography no such a long flowering period was previously reported. This is mainly caused by the lack of synchronization on flowering bud burst. In fact, up to nine different phenology stages were observed in single branches in some Tenerife locations.

As this erratic behavior occurs in both cultivars studied, it could be mainly caused by the differences in the environmental conditions be- tween Andalucía and Tenerife. Tenerife, with Subtropical climate, is characterized by higher minimum temperatures in autumn and winter and by a lower daily temperature range, and Andalucia, with Mediterranean climate, had lower autumn and winter temperatures, especially in Baena and Úbeda. Therefore, we can hypothesize that the lack of enough winter chilling in Tenerife caused a lack of synchronization on the flower bud burst. This hypothesis agrees with previous studies assigning to low winter temperatures a fundamental role on the occurrence of flowering (Hartmann and Porlingis, 1957; Ramos *et al.*, 2018). Among the years studied, 2016 showed milder winter temperatures. Accordingly, the flowering period was longer that year respect to 2017 and 2018.

Due to the high relevance of flowering stage on the impact of heat and water stress, studies have been attempted to develop models to forecast flowering date in olive (De Melo-Abreu *et al.*, 2004; Garcia- Mozo *et al.*, 2009). On the basis of those studies, the effect of future climate warming in olive growing areas has also been modeled (Lorite *et al.*, 2018). Those previous studies used flowering phenology datasets of Mediterranean climates or different devices to artificially modify the climatic conditions as greenhouses or growth chambers (Gabaldón-Leal *et al.*, 2017).

The fact that most of the studies on the chilling requirements on olive has been done in areas where those requirements are rapidly fulfilled has led to the unclear identification of the chill requirements and the temperature range where olive as species is accumulating chilling hours. Some studies indicated that chilling accumulation occurs with temperatures around 7 °C (De Melo-Abreu *et al.*, 2004) up to 12−15 °C (Orlandi *et al.*, 2002; Ramos *et al.*, 2018). The use of phenology data from contrasting climatic conditions, as the Subtropical here reported, has been proposed to improve the forecasting model performance (Gabaldón-Leal *et al.*, 2017). In our study, locations in Tenerife showed minimum winter temperatures rarely lower than those values above mentioned, but flowering was recorded, which underlines the difficulties to established clear thresholds for chilling accumulation.

Flowering phenology was previously evaluated in some non- Mediterranean climates as the case of Argentina locations, some of them with no enough chilling (Aybar *et al.*, 2015); but only flowering date was recorded and the only observed effect of lack of winter chilling was a low flowering intensity. In fact, most of the previous studies have in common that the only significant effect identified of the lack of winter chilling is the absence of flowering. Then, other flowering parameters as the length of the flowering period of olive has been rarely considered. In other fruit crops, some additional effects of warm winter have been described as erratic floral bud-break in pistachio (Elloumi *et al.*, 2013), low rate of effective fructification in apple (Petri and Leite, 2004) and increases in length of bloom period in cherry (Lakatos *et al.*, 2014).

Our study suggests that, when olive is grown on natural climates with apparently not enough chilling temperatures, as the Subtropical, the first effect on flowering phenology is not the lack of flowering but the lack of synchronization of flowering bud burst. This has been common to the two olive cultivars here studied, ‘Picual’ and ‘Arbequina’. Besides, blooming in each bud seem to be independent and it could not be stablished a relationship between the position in the shoot with the time of blooming. This led on the above-mentioned excessive enlargement of the flowering period observed in Tenerife, including two full flowering periods in some cases. A "second flowering" was mentioned in Hawaii (USA) olives trees of cultivar 'Koroneiki' under warm winter conditions (Miyasaka and Hamasaki, 2016). This lack of synchronization on flowering phenology could led on lack of synchronization of fruit ripening, with a negative impact in both final yield and oil quality (Bustan *et al.*, 2014) and, therefore, should be avoided.

Among the climatic factors here considered, the main differences are a higher daily temperature range and a lower winter minimum temperature in Andalucía respect to Tenerife. Rainfall was also higher in Andalucía, but this was compensated by a higher irrigation supply in Tenerife. A slightly longer flowering period and earlier flowering date was observed in 'Picual' trees when grown in 4 °C artificially warmed environments with open top chambers respect to typical Mediterranean climate of Córdoba, Southern Spain (Benlloch-González *et al.*, 2018). On the contrary, negative correlation between temperature and the duration of the length of the flowering period was previously observed (Rojo and Pérez-badia, 2015; Vuletin Selak *et al.*, 2013). But those studies only used data from Mediterranean conditions were chilling requirements were sufficiently fulfilled. The accumulation curves of chilling portions (CP) and growing degree days (GDD) was very different in the Tenerife locations respect to Úbeda and Baena, located in typical olive growing areas with a Mediterranean climate. However, it is striking that in Canales Altas, where two flowering periods have been observed in 2016 in both 'Arbequina' and 'Picual', a CP and GDD pattern similar to Gibraleón in Andalucía was observed. In 2016, the CP accumulation in Canales Altas is significant from February 2016, a time when flowering has already started. Similarly, in Los Tomillos 2018, two flowering periods were also found for 'Arbequina'. Interestingly, the 2017–2018 season is the one when a higher CP was accumulated in Los Tomillos. Again, this CP accumulation occurs only when flowering has already started in this location. It is also interesting that no CP has been accumulated in Los Tomillos at flowering in any of the three years under study and, however, flowering has been observed. Finally, the earliness of flowering in Tenerife locations respect to Andalucía ones could be due to a higher GDD accumulation in the former ones. In any case, we need to gather much more phenology and climate data to really stablish a correlation between the different temperature regimes and the occurrence of asynchronous flowering.

Considering that different climate change scenarios forecast an increase in winter temperatures in Mediterranean climate where olive is grown, measures to prevent the lack of synchronization of olive flowering should be developed. One possible strategy could be the breeding and selection of new cultivars with low winter chilling requirements, taking advantage of potential genetic variability for winter chilling requirements. In the present study, the lack of flowering bloom synchronization seems to be a bit more intense in 'Arbequina' than in 'Picual'. However, more experiments with a larger set of cultivars planted in areas with high winter temperatures are needed to really look for genetic variability on winter chilling requirements, as previously suggested (Belaj *et al.*, 2020). For the identification of cultural practices to mitigate the lack of chilling temperatures, it has been suggested that water stress may play a role in the flowering of olive similar to that of low winter temperatures (Connor and Fereres, 2005). For that reason, cultural practices related to irrigation withholding has been proposed in other fruit trees to substitute winter chilling (Atkinson *et al.*, 2013) as well as in olive (Castillo-Llanque *et al.*, 2014).

Besides, the development of advanced phenology models adapted to the assessment of the impact of climate change on olive will require to add the length of the flowering period as a critical factor affected by climatic conditions. As other authors have reported before (Benlloch-González *et al.*, 2018; Fadón and Rodrigo, 2018) flowering is a complex process influenced by many factors, apart from air temperature as, for example, photoperiod (Garcia-Mozo *et al.*, 2009). Therefore, inclusion of more factors than air temperature is recommendable in future models.

Equally, more investigation should be done to determine differences found between growth chamber and field experiments to determine chilling requirements on olive. In growth chamber experiments, differences between shoots with chilling and no chilling accumulation was on the percentage of bud burst (Ramos *et al.*, 2018). However, in field experiments as the one here reported and a previous one (Benlloch-González *et al.*, 2018), the effect of lack of chilling was the advancement of phenology and enlargement of the flowering period. In fact, well long ago, it was proposed that winter chilling has an effect on bud burst rather than in flower induction (Rallo and Martin, 1991).

II.VI. Conclusions

Based on the results here presented, we propose that future works studying the effect of lack of winter chilling on olive should include the length of flowering period as a parameter to be modeled. Then, to achieve accurate phenological models, the experimentation under warm winters and the consideration of new modelling approaches will be necessary to really determine the effect of winter chilling on different olive cultivars.

II.VII. Funding

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CAPÍTULO III. Flowering phenology of olive cultivars in two climates zones with contrasting temperatures (Subtropical and Mediterranean)

III.I. Abstract

The large amount of olive cultivars conserved in Germplasm Banks can be used to overcome some of the challenges faced by olive growing, including climate warming. One effect of climate warming in olive is the difficulty to fulfill the chilling requirements for flowering due to high 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 lack of winter chilling temperatures as in Tenerife. This even though the cultivars studied have a high genetic distance between them. In fact, all the cultivars tested in Tenerife flowered the three years under study but showing asynchronous flowering bud burst. Only 'Arbequina' showed an earlier day of full flowering than the rest of cultivars. The results observed here could be of interest to refine the phenological simulation models and including of 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.

III.II. Introduction

Olive germplasm includes more than 2,000 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 environmental factor driving the flowering phenology in olive [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]. And 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 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 cultivar 'Picual' respect to the natural conditions in Cordoba, Southern Iberian Peninsula. I Besides, the Subtropical climate of Tenerife, Canary Islands, with high winter temperature, 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 this warm winter conditions, the behavior of other cultivars coming from different origin and having a diverse genetic base.

For that matter, in the present study, we evaluate the genetic variability for flowering phenology of 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 be evaluated.

III.III. Materials and methods

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 3.1). Both orchards were maintained with the same olive growing management aimed at maximizing productivity. The Tenerife field was irrigated with 2,500 m^3/year, and the Cordoba field was irrigated with 1,500 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.

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 come 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 one, 'Martina', is a new cultivar from the Olive Breeding Program of Cordoba [19]. This new cultivar derived from the cross of 'Arbequina' and 'Picual'.

Fig. III.1.- *Location of the two olive trials under evaluation.*

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 comprise from the appearance of stage 53 until 69 was the most common. One or two times per week and, the earliest, most common and latest phenological stages were evaluated for each tree [14,21].

All these data were used to calculate three phenological parameters:

- Length of flowering period (FP): days from 61 being the earliest stage to the 68 being the most common stage.
- Length of full bloom period (FBP): days from the first appearance of stage 61 to last time that 65 is the most common stage.
- Full bloom date (FBD): Average Julian date of the start and end of the FBP.

3. Statistical analysis

Analysis of variance was used to test the significance of cultivar, environment and their interaction on FP, FBP and FBD. As the data obtained was unbalanced (no data for three cultivars in 2019), we use 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 done in previous studies [14]. Comparison of means was used to test for differences between these factors when significant.

III.IV. Results

As expected, Tenerife and Cordoba had contrasting climatic characteristics (Figure 3.2). Air temperature in Tenerife was in general, milder than in Cordoba. Autumn and winter were colder in Cordoba, with Tmin below 0ºC in late December and early January. While in Tenerife, the Tmin winter temperatures were below 10 ºC on only 8 days of the year, Tmax were above 20 ºC on almost all days of the winter. Besides, spring was hotter in Cordoba, with Tmax reaching 40ºC in May and June. In Tenerife, the highest Tmax recorded during the study were below 35ºC. Differences were also observed in the DTR, which in Cordoba was sometimes close to 25ºC, while in Tenerife rarely exceeded 15ºC. Precipitation was higher in Córdoba (396, 472 and 422 mm in the three years under study) than in Tenerife (314, 223 and 302 mm) during the three years under study. But those differences were compensated with a higher irrigation dose in Tenerife. Solar radiation values were higher in Tenerife than in Cordoba in all the period considered.

All these climatic differences between Cordoba and Tenerife promotes significant differences in flowering phenology. Indeed, the analysis of variance for the flowering phenology parameters (Table 3.1) 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 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.

Tabla III.1.- *Percentage of sums of squares of cultivar, 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

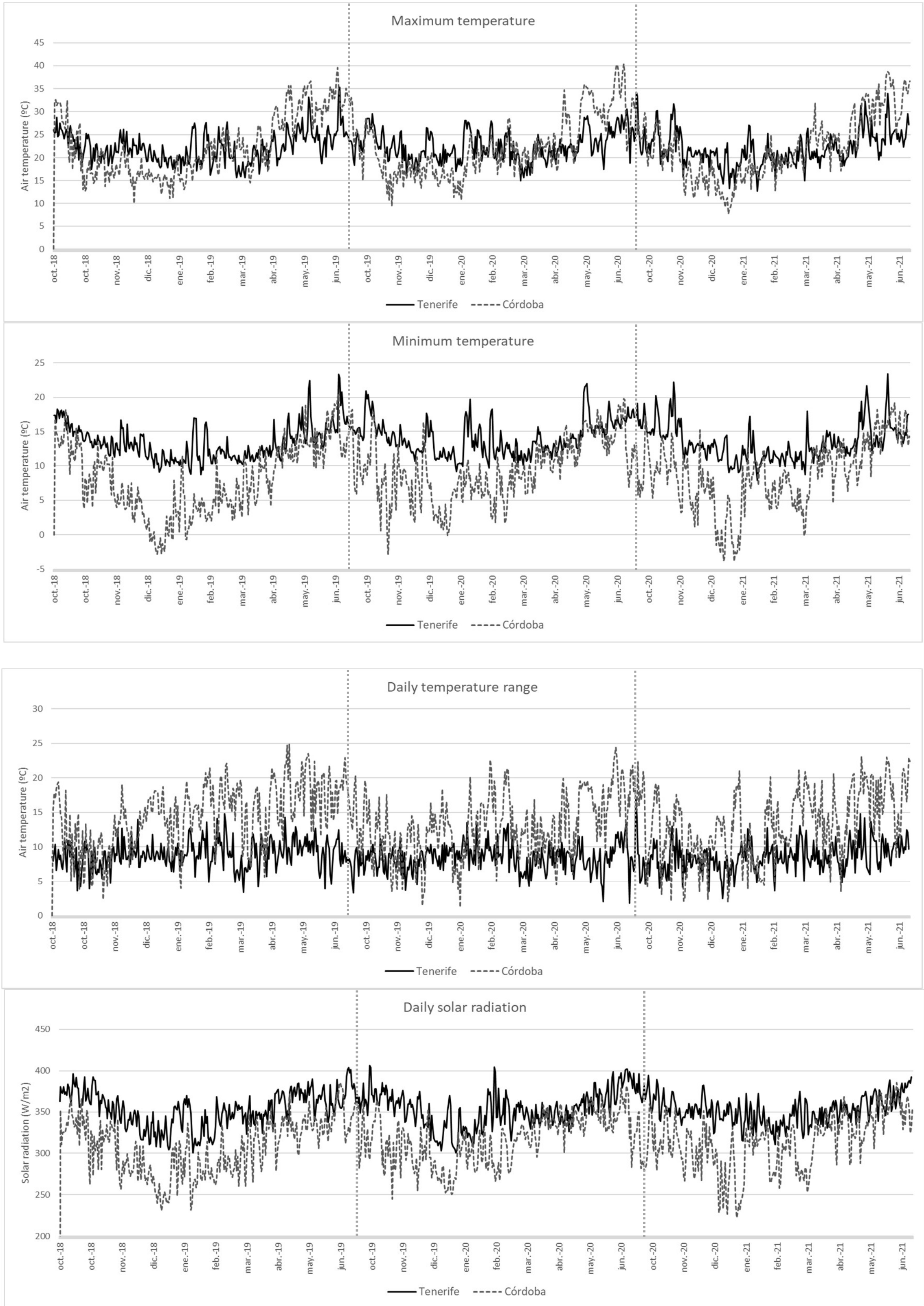

Fig. III.2.- Weather data of Tenerife and Andalucía locations from October to June in the three seasons considered (2018-2019, 2019-2020 and 2020-2021). Daily temperature (mean, maximum, average and range) and solar radiation are included.

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. Besides, 'Arbequina', 'Koroneiki' and 'Martina' showed significant 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.

Tabla III.2.- *Comparison of means of length of 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 3.3).

Tabla III.3.- *Comparison of means of length of 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 3.4), 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 cultivars in this environment. Very early FBD was observed in TF-20 for 'Arbequina', 'Koroneiki', 'Martina' and 'Picholine Marocaine' around day 60 (1st 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 environments were found.

Tabla III.4.- *Comparison of means of length 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 environments started and ended 60 and 30 days later than in TF-20 and TF-21, respectively (Figure 3.3). While in TF-19, flowering duration was very variable between cultivars but, in generally occurred at a similar date to that in Cordoba 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 3.4). Consequently, two very distant phenological stages were observed simultaneously in a tree in all cultivars in Tenerife environments compared to those in Cordoba. Moreover, in Tenerife, stage 53 (Inflorescence buds open, flower cluster development starts) was the most delayed stage during 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 the Tenerife environments, where new flowers appear on the trees over a very long period.

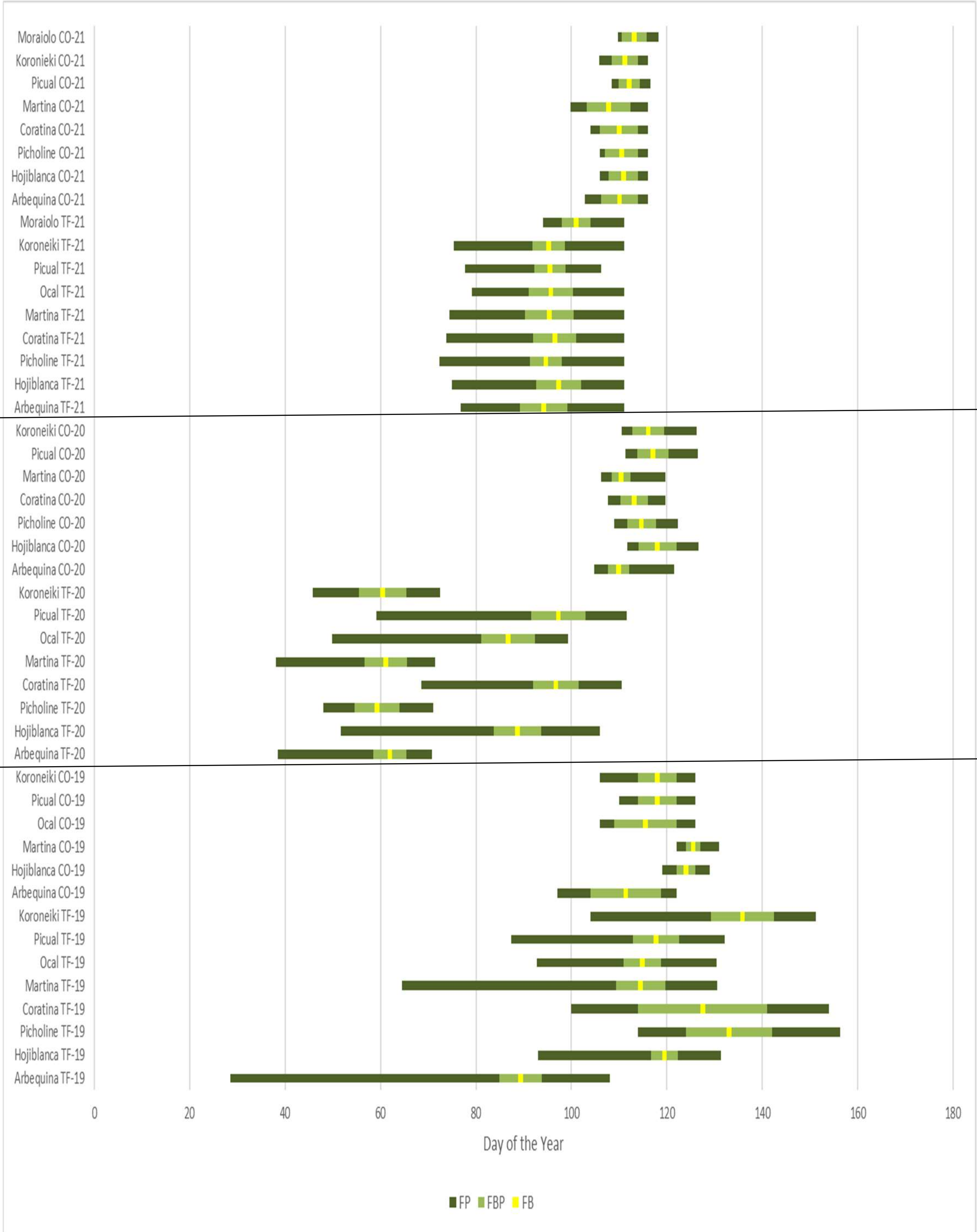

Fig. III.3.- *Flowering period in days (FP in dark green), full bloom period in days (FBP, in light green) and full bloom date in Julian day (FBD in yellow) in seven cultivars in the Tenerife and Andalucía locations in the three seasons considered (2018-2019, 2019-2020 and 2020-2021).*

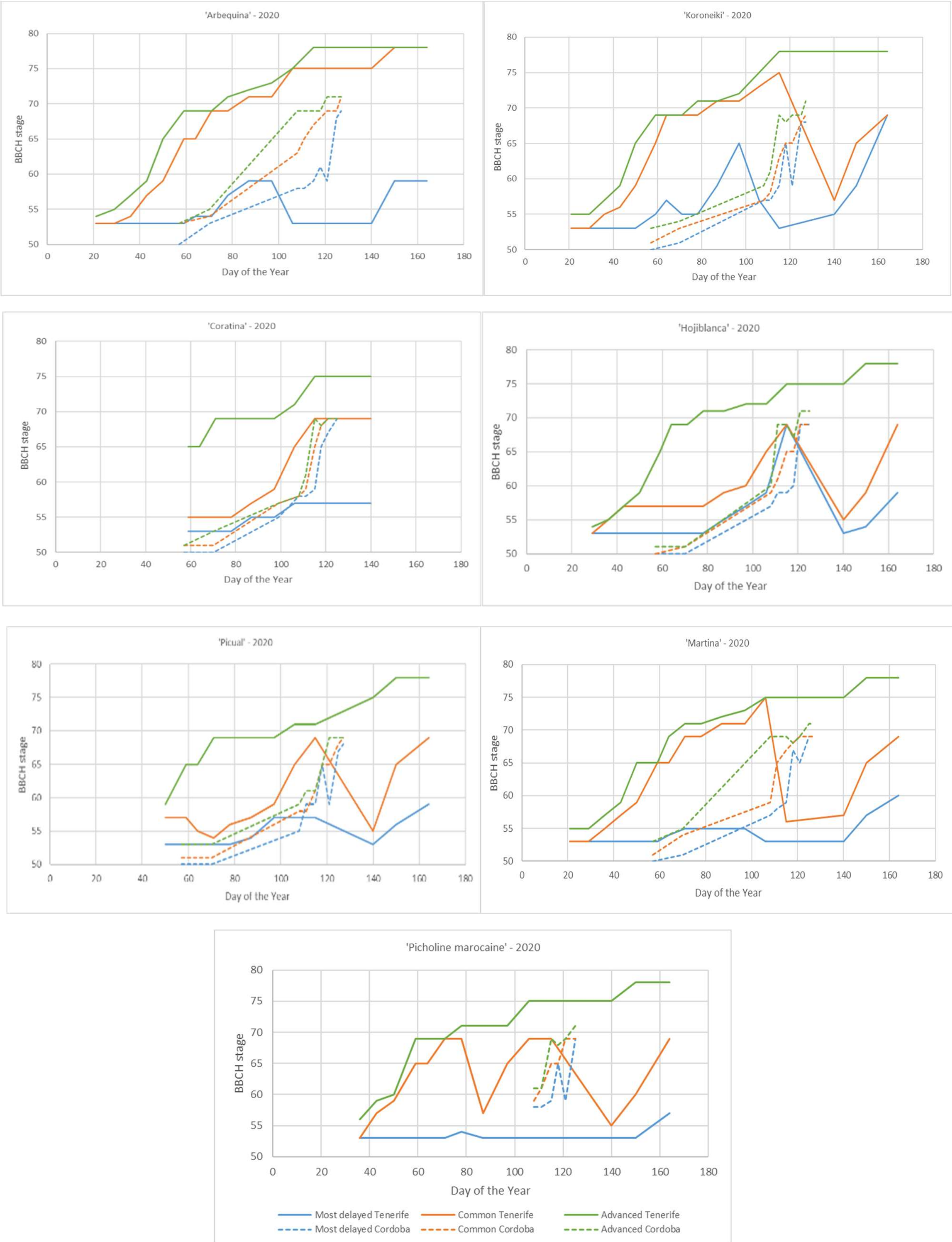

Fig. III.4.- *Variation of the average most delayed, common and advanced flowering stage (BBCH scale) in the seven studied cultivars along the flowering period (Julian day) in Tenerife and Cordoba in 2020.*

III.V. 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 from traditionally in 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 as the one in Tenerife. Flowering was observed in all the Tenerife environments and in all the cultivars tested. This despite the fact that the optimal chilling accumulation temperature previously established at 7-12 ºC for olive crop [23–25] was rarely reached in winter in the three Tenerife environments.

The analysis of variance of the flowering phenology parameters showed that the environment was the main factor responsible for the variability 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 the three Tenerife environments (TF-19, TF-20 and TF-21) above mentioned. The most remarkable effect of this is an asynchronous flower bud burst that was also previously observed for 'Arbequina' and 'Picual' in Subtropical climate [17]. This asynchrony in the flowering could be the cause of the high error variance for the FBP 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; and 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 respect to Cordoba might also have influence on these differences, but more specific experiment 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, 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 artificial increase of air temperature during winter [16] and in flowering phenology models [13,29].

The significant differences observed among the three Tenerife environments are difficult to explain, as the air temperatures in the three years under study were relatively similar.

Maybe other factors apart from air temperature or solar radiation are involved, as for example, soil temperature.

In contrast to the strong environmental effect, the evaluation here 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 cultivars [14]. However, in Tenerife significant differences among cultivars were observed. And it is noteworthy that there is no specific pattern of variation for cultivars for both FP and FBD along the three Tenerife environments 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 as 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 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, few 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. Also, current breeding programs has been focused in other characters 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, 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 lack of winter chilling on flower quality. Indeed, lack of winter chilling seems to reduce the number of inflorescences and 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].

III.VI. Conclusions

The seven olive cultivars here evaluated were originated in very different climatic conditions, from Greece to Morocco. However, the lack of winter chilling of the Subtropical climate of Tenerife promoted a desynchronize flower bud burst in all of them. Besides those climatic conditions produced an erratic flowering pattern across years. This indicates that all 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 here used have a very different origin might indicate that the cultivated germplasm might have little adaptation to these conditions and that it might be needed to explore wild germplasm as the one native of Canary Islands namely, *Olea europaea* subsp. *Guanchica.* Besides, the results observed here stress the need of including the length of the flowering period as a new parameter in the climate warming simulation models currently under development for olive. Finally, the results obtained here highlight the usefulness of phenological evaluation as a reliable indicator of climate warming.

III.VII. Funding

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CAPÍTULO IV. Phenolic variability in fruit from the ‘Arbequina’ olive cultivar under Mediterranean and Subtropical climatic conditions

IV.I. Abstract

In the present work, we compared the phenol content and composition of fruit from the 'Arbequina' cultivar in four Mediterranean (in Andalucía, Southern Iberian Peninsula) and two Sub-Tropical (Canary Islands) locations throughout the harvest period. Two Mediterranean and two Sub-Tropical locations were maintained with drip irrigation, while the remaining two Mediterranean locations were in dry farming. Water availability and harvest date seemed to play more important roles than air temperature on the phenolic content and most of the studied components. The variability associated with location was a result of the high values observed in the two Mediterranean locations in dry farming, with respect to the other four maintained with drip irrigation. Few differences were found among the four drip-irrigated locations, despite the fact that two were Mediterranean and the other two Sub-Tropical. In addition, a sharp decrease was observed during the harvest period for phenolic content and most of the phenolic compounds.

Keywords: *Environment; Harvest date; Location; Maturity; Olive fruit*

IV.II. Introduction

Olive oil is one of the main components of the Mediterranean diet due to its nutraceutical properties (Piroddi *et al.,* 2017). Among olive oil constituents, phenols play a very important role in its healthy properties (Serreli and Deiana, 2019). For example, olive phenols reduce chronic inflammation and help in the prevention of some diseases, such as Alzheimer's (Omar *et al.,* 2017) or different types of cancer (Piroddi *et al.,* 2017).

The phenolic composition of olive oil depends, mainly on the phenolic composition of the fruit upon arriving to the mill factory, which is then modified by enzymatic processes occurring during oil extraction (Lukić *et al.,* 2017). This initial fruit phenolic composition could be affected by many factors including genotype, harvest date, tree environmental conditions and management practices (Servili and Montedoro, 2002).

The wide olive germplasm has shown a high genetic variability for both phenolic content and composition in the evaluation of cultivar collections (Miho *et al.,* 2018), breeding progenies (Pérez *et al.,* 2014) and comparative trials (Pérez *et al.,* 2018; El Riachy *et al.,* 2013).

Several studies have also attempted to evaluate the phenolic variability of single cultivars in different environments of their country of origin, such as 'Gemlik' (Ben Ghorbal *et al.,* 2018) in Turkey, 'Picholine Marrocaine' in Morocco (Bajoub *et al.,* 2015), 'Chemlali' (Bouaziz *et al.,* 2004) in Tunis and 'Baladi' in Lebanon (El Riachy *et al.,* 2018). Among the environmental variables, phenol composition is mainly influenced by both abiotic and biotic stresses. Among the first ones, water stress has been reported as one of the main factors influencing phenol content and composition (Dabbou *et al.,* 2015; Gómez-Rico *et al.,* 2006; Gucci *et al.,* 2019). In general, an increase in water stress implies an increase in phenolic content in olive oil (García *et al.*, 2020). However, some individual phenols, such as lignans, might show the opposite pattern (Ovar *et al.,* 2002). Biotic stresses such as olive fruit fly (Medjkouh *et al.,* 2018) and Verticillium Wilt (Landa *et al.,* 2019) can also increase oil phenolic content and composition.

The total phenolic content and composition normally decreases during the harvest season, as shown for different olive cultivars (Alowaiesh *et al.,* 2018; Bengana *et al.,* 2013; Bodoira *et al.,* 2015; Dag *et al.,* 2011). This includes 'Arbequina', the most widely planted cultivar in the world (Fernández-Escobar *et al.,* 2013), whose phenolic content has been evaluated in olive oil (Abenoza *et al.,* 2015; Morelló *et al.,* 2004) and fruit (Talhaoui *et al.,* 2015) through maturation in single locations in Spain. On the contrary, some phenols such as lignans and flavones have been reported to increase in oil through maturation (Bengana *et al.,* 2013) as well as verbascoside (Bodoira *et al.,* 2015) and hydroxytyrosol (Bouaziz *et al.,* 2004) in fruits. In other cases, irregular patterns of variation in fruit phenols through maturation have been reported (Ben Ghorbal *et al.,* 2018).

All the mentioned works on olive fruit phenols have been performed in Mediterranean climatic conditions. Few reports exist on olive phenols in other climates as is the case of Argentina (Bodoira *et al.,* 2015). However, olive growing is currently expanding worldwide, in many cases outside the Mediterranean climate. Besides, this expansion is mainly based on few cultivars specially adapted to the new trends of olive growing (Fernández-Escobar *et al.,* 2013), with 'Arbequina' being the clearest example. Therefore, it would be of great interest to compare the influence of very different climate conditions (Mediterranean vs. non-Mediterranean) on variation in an important component of olive oil such as phenolic compounds.

In this sense, Tenerife, in the Canary Islands, is one of the non-Mediterranean locations where olives now have some importance (Medina *et al.*, 2018). Its Sub-Tropical climate might be of interest as a natural scenario with climatic conditions similar to those of the typical Mediterranean olive growing area in the likely event of a climate warming (Medina *et al.*, 2020).

In the present work, we compared the variation in phenolic content and components of 'Arbequina' fruits through maturation in typical Mediterranean locations from Andalusia in Sothern Iberian Peninsula, with others from the Sub-Tropical climatic conditions in Tenerife, Canary Islands. That comparison was used to compare the test of location, harvest date and their interaction on phenolic content and composition.

IV.III. Materials and methods

1. Study sites and plant material

The trees of 'Arbequina' olive cultivar were sampled in four locations of Andalusia, Southern Iberian Peninsula from typical olive growing areas and Mediterranean climate: Antequera (37.17N, -4.64W), Baena (37.60N, -4.23W), La Rambla (37.62N, -4.82W) and Úbeda (37.89N, 3.24W); and two locations in Tenerife, Canary Islands, with Sub-Tropical climate: Los Tomillos (28.13N-16.49W) and El Viso (28.30N, -16,37W, Figure 4.1). The Mediterranean climate is characterized by colder winters and hotter summers than the Sub-Tropical one, which also has a low intraday temperature range. Antequera and Baena are managed in dry farming while the other four with drip irrigation (year amount of irrigation: La Rambla 250 mm, Úbeda 150 mm, Los Tomillos 372 mm and El Viso 312 mm). In the four Andalusia locations, trees were planted in 2008 at 7 x 6 m distance in a clay-loam soils. In the two Tenerife locations, trees were planted in 2010 at 5 x 5 m distance in a sandy-loam soil.

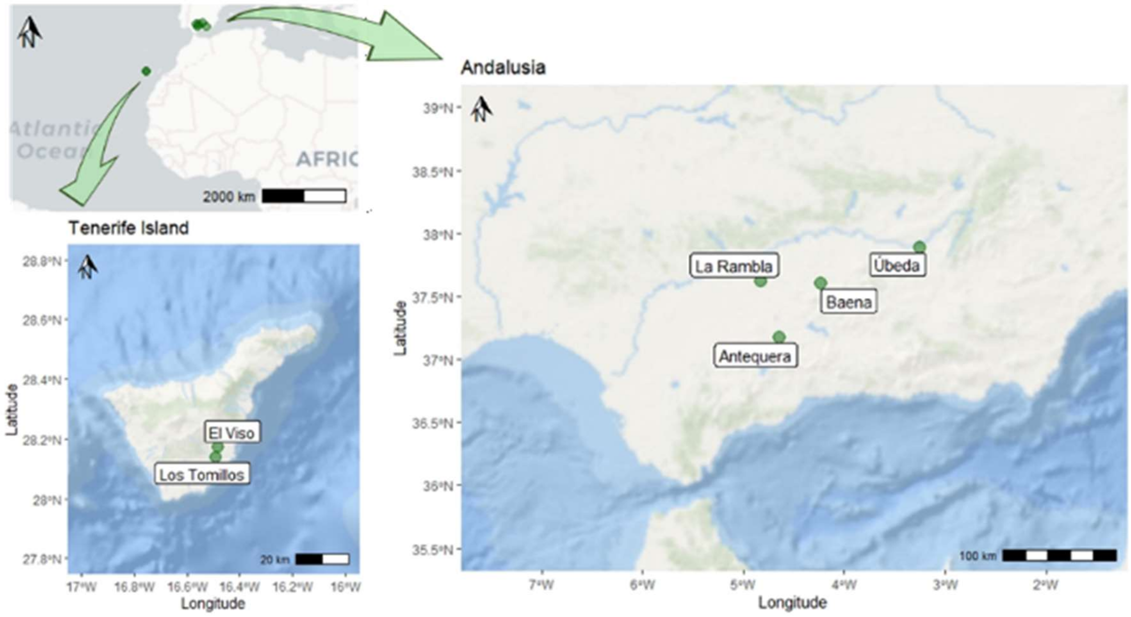

Fig. IV.1.- *Geografical location of the different tested orchards*

Fertilization was applied on the basis of foliar analysis in order to avoid any limitations on growth and yield.

Four sets of three trees per location with similar fruit load (2-3 in a 0-3 visual scale) were sampled on three dates of the olive maturation period in 2017. In the case of Andalusian locations, sampling was performed in mid-September (harvest 1), mid-October (harvest 2) and mid-November (harvest 3). In the case of Tenerife, as the oil accumulation occurs earlier (Figure 4.2), harvest dates were mid-August (harvest 1), mid-September (harvest 2) and mid-October (harvest 3).

In each harvest date, a sample of 1 kg fruit was randomly hand-picked for each set of trees and location (4 sets x 3 harvest dates x 6 locations) to evaluate phenolic content and composition as well as fruit traits.

2. Phenols analysis

Reagents for extraction and other measurements were supplied by Sigma-Aldrich (St. Louis, MO). Phenolic standards were purchased from Extrasynthese (Genay, France).

Fruit phenolic compounds were extracted from each sample according to a previously developed protocol (García-Rodríguez *et al.,* 2011). Longitudinal pieces of mesocarp were cut at least from twenty olives.

For each sample, fruits were finely chopped and used to prepare phenolic extracts. Representative fruits samples (1 g) were kept at 4 °C for 72 h in dimethyl sulphoxide (DMSO, 6 mL) containing siringic acid as internal standard. The extracts were filtered through a 0.45 µm mesh nylon and kept at -20 ºC until HPLC analysis.

The phenolic extracts were analyzed by HPLC on a Beckman Coulter liquid chromatography system equipped with a System Gold 168 detector, solvent module 126, autosampler module 508 and a Waters column heater module following a previously described methodology (Pérez *et al.,* 2014). A Superspher RP 18 column (4.6 mm i.d. x 250 mm, particle size 4 µm: Dr Maisch GmbH, Germany) at a flow rate of 1 mL/ min and a temperature of 35 ºC was used for all the analyses. A total of 9 phenolic compounds were quantified in the phenolic extracts: hydroxytyrosol-4-glucoside, tyrosol-1-glucoside, demethyloleuropein, verbascoside, luteolin-7-glucoside, rutin, oleuropein, comselogoside, and ligustroside.

Phenolic compounds were monitored at two different wavelengths of 280 nm and 335 nm and quantified taking into account the internal standard and specific response factors for each of them (García-Rodríguez *et al.,* 2011).

The tentative identification of compounds by their UVvis spectra was confirmed by HPLC/ESI-qTOF-HRMS. The liquid chromatography system was Dionex Ultimate 3000 RS U-HPLC liquid chromatography system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a similar Superspher RP 18 column but with 3 µm particle size. Formic acid 1% was used instead of phosphoric acid 0.5% for the mobile phase. A split post-column of 0.4 mL/ min was introduced directly onto the mass spectrometer electrospray ion source. The HPLC/ESI-qTOF operated for mass analysis using a micrOTOF-QII High Resolution Time-of-Flight mass spectrometer (UHRTOF) with qQ-TOF geometry (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) interface. Mass spectra were acquired in MS full scan mode and data were processed using Target Analysis 1.2 software (Bruker Daltonics, Bremen, Germany).

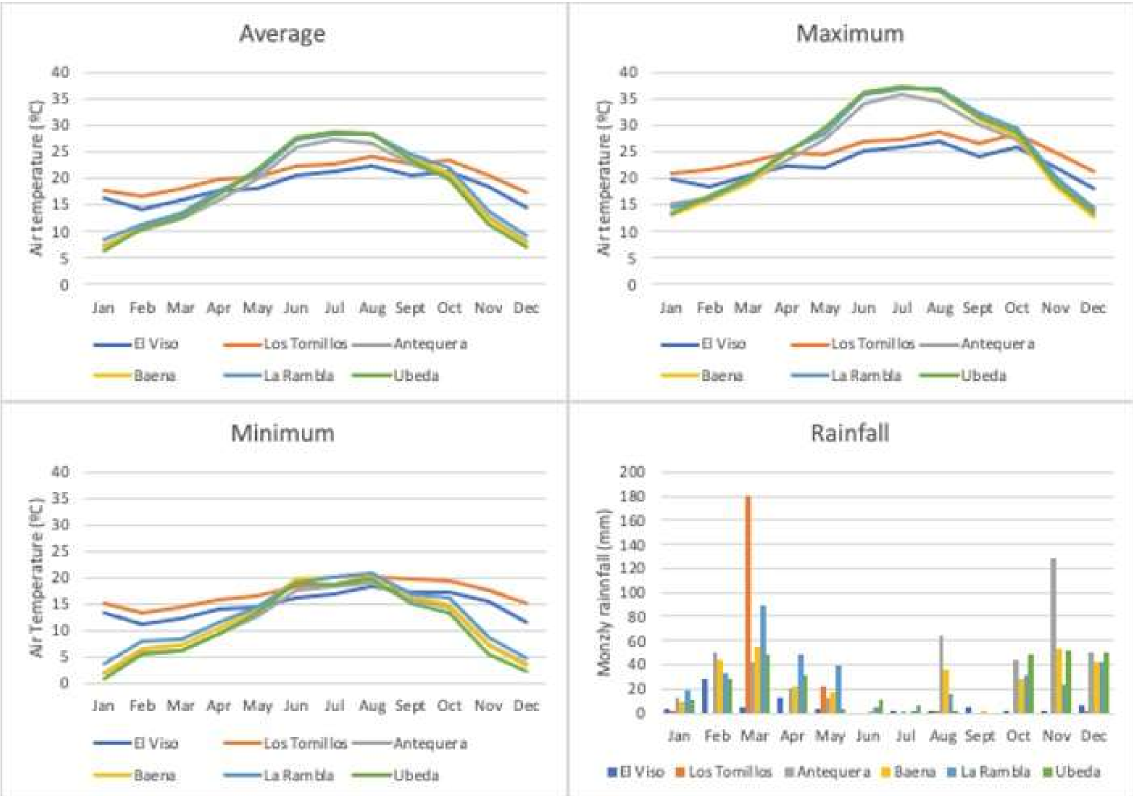

Fig. IV.2.- *Average, maximum and minimum daily temperature (monthly average in ºC) and monthly rainfall (mm/m^2) of the six locations considered. Temperature data were recorded hourly to make daily statistics.*

3. Fruit traits analysis

Ripening index was evaluated according to the procedure described by (Frias *et al.,* 1991). Afterwards, oil content was measured in three random sub-samples of 25 g of each sample. Each sub-sample was oven dried at 105 ºC for 42 h (Río and Romero, 1999) to ensure dehydration, and weighed to measure the percentage of oil content in a NMR fat analyzer.

4. Statistics

Analysis of variance was performed to evaluate the relative influence of location, harvest date and their interaction on the variability of phenolic content and composition. Comparison of means (Tukey) was used to test differences among locations, harvest dates and harvest dates-locations, when significant. Previous works have shown that the water availability is the main factor influencing phenol content (Gucci *et al.*, 2019). Therefore, in order to test the differences between the Mediterranean and Subtropical conditions of Andalusia and Tenerife regions, a separate analysis was performed with only the four irrigated locations (two in Andalusia and two in Tenerife). Finally, a person correlation was performed to test the correlations among phenol constituents and with fruit traits.

IV.IV. Results

The six locations involved in this work showed different climatic conditions during the experimental period, mainly associated with the region level (Figure 4.2). The two locations in Tenerife, Canary Islands, showed milder temperatures both in summer and winter, with around 7 ºC range of average maximum-minimum temperatures. Temperatures in Los Tomillos (located 200 m.a.s.l.) were 2-3 ºC higher than in El Viso (400 m.a.s.l.). Among the locations in Andalusia, Baena, La Rambla and Úbeda showed quite similar temperature patterns; while only slightly lower temperatures were observed in Antequera. Some differences in rainfall distribution among the Andalusian locations were also observed, particularly due to high rainfall in Antequera in the first week of November. Compared to the Peninsula, locations in Tenerife were characterized by limited rainfall throughout the year, with only exceptional rainfall observed in Los Tomillos in March.

The oil accumulation of 'Arbequina' in the six locations sampled showed a similar pattern considering that harvest 1 was done in August for El Viso and Los To- millos (in Tenerife) and in September for the Andalusian locations (Figure 4.3).

Among all the identified phenols in 'Arbequina' fruits, oleuropein represented around half of the total phenolic content (Table 4.1), and together with demethyloleuropein, comseogoside, ligstroside and verbascoside constituted 95% of the total phenolic content. All the phenols showed high variability (high coefficient of variance), being especially high for verbascoside and oleuropein.

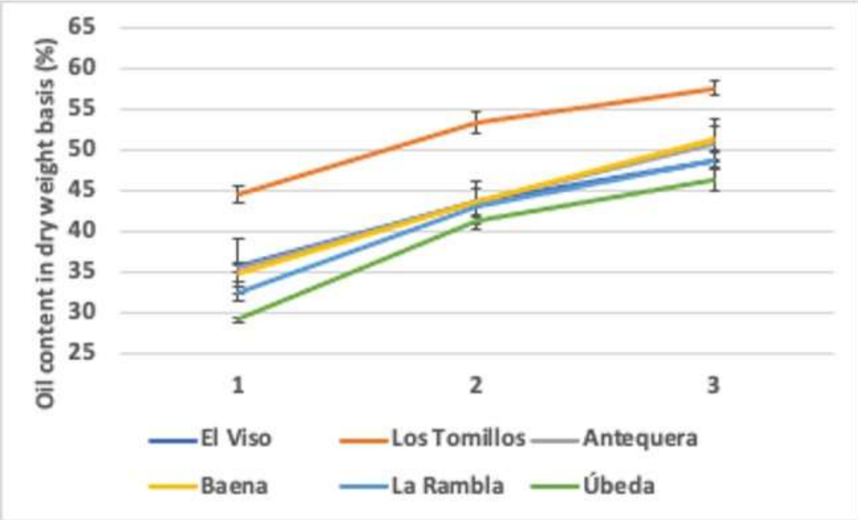

Fig. IV.3.- *Oil content on dry weight basis of 'Arbequina' fruits on three harvest dates in the four locations of Andalusia, Iberian Peninsula (Antequera, Baena, La Rambla and Úbeda) and in two locations of Tenerife, Canary Islands (El Viso and Los Tomillos). In Andalusia, harvests 1, 2 and 3 represent mid- September,mid-October and mid-November at the Iberian Peninsula locations. In Tenerife, oil accumulation occurs one month in advance. For that reason, harvests 1, 2 and 3 represent mid-August, mid- Sep- tember and mid-October. Three replicates per location and harvest date were averaged.*

This high variability in phenolic content and composition was mainly due to location effect for phenols and most of the components (Table 4.2) except for oleuropein and ligstroside, for which location and harvest date showed comparable variance and for demethylo- leuropein, which showed a double variance for the harvest date effect (41.9) compared to location (21.2) and their interaction (18.0). The interaction between location and harvest date represented the main contributor to total variance only for hydroxytyrosol-4-glucoside. Error variance represented more than half of the total variance for luteolin-7-O-glucoside. In any case, location, harvest date and their interaction showed a significant effect on phenolic content and all its components except for rutin and luteolin-7-O-glucoside, for which location was the only significant factor.

The phenolic components described were found in all locations and harvest dates (Table 4.3). The high variability due to location for total phenols was mainly due to the high values observed for Antequera and Baena (Table 4.3, Figure 4.4). While the decrease in total phenols with harvest date was, on average, more similar between harvest 1 and 2 than between harvest 2 and 3. However, for Antequera and Baena, this decrease was more evident between harvest 2 and harvest 3 just the opposite for the rest of the locations. Oleouropein, the major phenol identified, showed a similar pattern of variation to that of total phenols. The same could be said for ligstroside.

Different patterns of variation were observed for the rest of phenolic components (Table 4.3, Figure 4.4). De-methyloleuropein showed an increase between harvest 1 and harvest 2 in all locations except for Los Tomillos. In Antequera, this increase was also maintained between harvest 2 and harvest 3, maybe associated to a heavy rainfall at that time. Few variations among harvest dates were observed for comselogoside and verbascoside. Only in Antequera and Baena, the two locations with the highest values for both components, a significant decrease was observed in harvest 3.

Tabla IV.1.- *Descriptive statistics (from a total of 54 samples) of the phenols found in ‘Arbequina’ olive pulp. Data are presented in µg/g of fresh olive pulp.*

Compound	Average	SDb	CVb	Min	Max
Total phenols	23028	11647	51	8180	51767
Oleuropein	11176	10740	96	585	39985
Demethyloleuropein	7103	3722	52	555	14639
Comselogoside	1470	681	46	462	3279
Ligstroside	1103	972	88	171	4251
Verbascoside	916	927	101	30	4009
Hydroxytyrosol-4-glucoside	431	226	52	75	1061
Rutin	408	252	62	49	1046
Luteolin-7-O-glucoside	367	232	63	15	1335
Tyrosol-1-glucoside	53	18	33	35	97

[a]SD=Standard desviation, [b]CV= coefficient of variance

Tabla IV.2.- *Percentage of variance for location, harvest date and their interaction for the phenols found in ‘Arbequina’ olive pulp by the ANOVA analysis. Values in bold indicate significant differences for this source of variation at p<0.01. The analysis included 6 locations, 3 harvest dates and 3 replicates per location and harvest date.*

	Totalphenols	Oleuropein	Demethyloleuropein	Comselogoside	Liustroside	Verbascoside	Hydroxytyro- sol-4-glucoside	Rutin	Luteolin-7-O-glu-coside	Tyrosol-1-glucoside
Location	66.1	43.2	21.2	87.6	38.7	64.1	40.4	64.0	41.5	65.9
Harvest date	25.0	45.6	41.9	1.0	43.4	4.6	0.0	0.0	0.2	1.2
Location * Harvest date	5.0	5.2	18.0	4.1	8.2	10.7	47.4	4.9	0.0	7.1
Error	3.9	6.1	18.9	7.3	9.7	20.6	12.2	31.0	58.3	25.8

For the four components with lower contents in ‘Arbequina’ fruits (hydroxytyrosol-4-glucoside, rutin, luteolin-7-O-glucoside and tyrosol-1-glucoside), the highest values were observed in Antequera and Baena samples, probably due to the water stress. The unique exception was hydroxytyrosol-4-glucoside with very high levels in the fruits from La Rambla, and a significantly different accumulation pattern observed in Los Tomillos. This component is probably less influenced by water stress. Few variations with harvest date were observed for rutin, except for the very high values in Baena in harvest 2.

Fruit traits were also evaluated in the six locations and on three harvest dates. Fruit size, moisture and maturity index showed most of the variance due to the location effect. The oil content was mainly influenced by harvest date, as expected (data not shown). Antequera was the location with the smallest fruit size; while Los Tomillos showed higher oil content than the rest of the locations (Table 4.4). Fruit moisture was much lower in the two dry farming locations (Antequera and Baena), as expected. Maturity index was very delayed in Antequera, and very advanced in Úbeda, although intermediate in the rest of the locations.

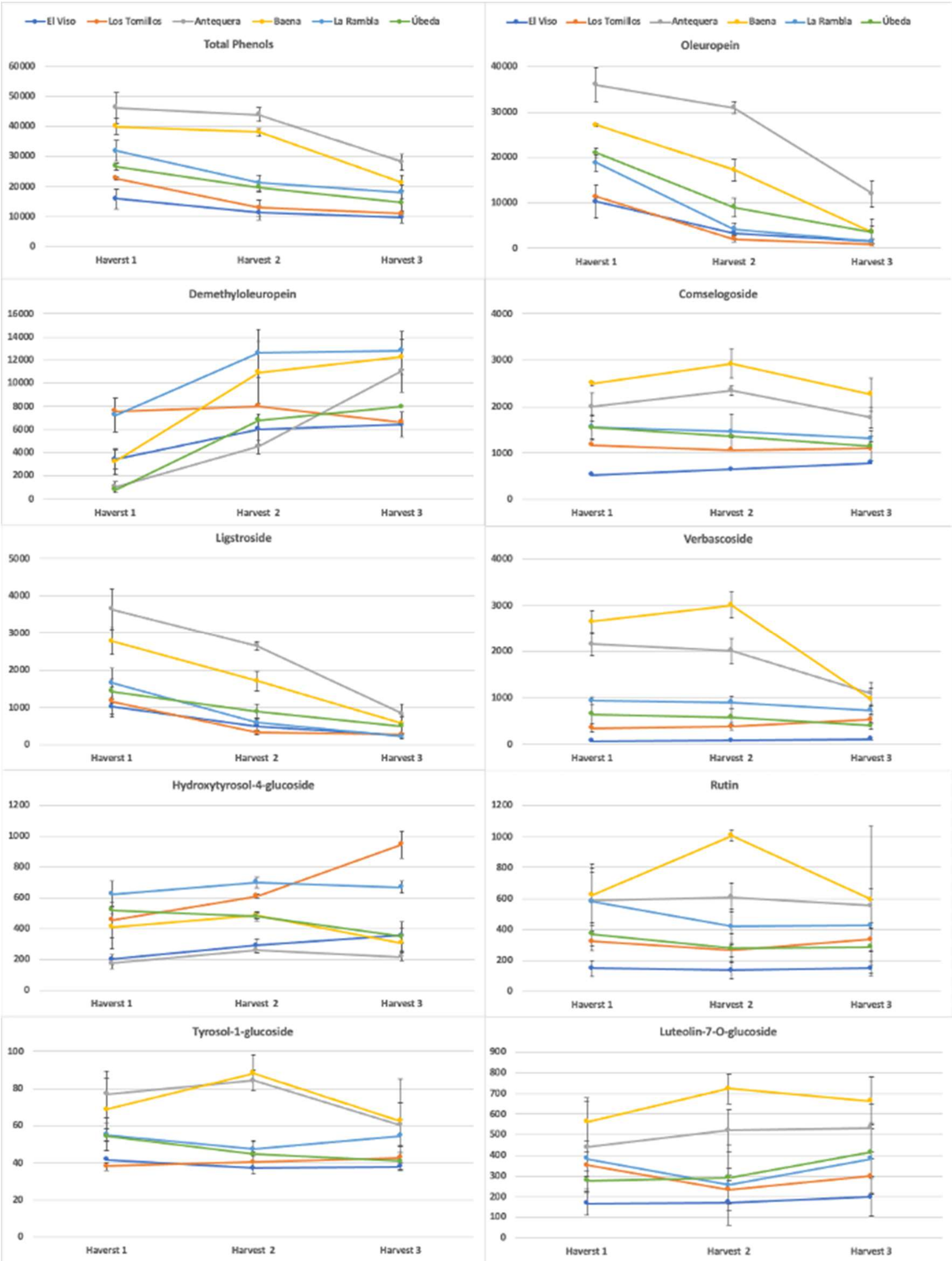

***Fig. IV.4.-** Variation in the main phenols found in ‘Arbequina’ fruits on the three harvest dates of the six locations considered. Data are presented in µg/g of fresh olive pulp. Three replicates per location and harvest date were averaged.*

Tabla IV.3.- *Total and main phenol means by harvest date, location and harvest* location in 'Arbequina'. Different letters indicate significant differences (p< 0.005) among means within each of the three groups of data (Tukey test). Data are presented in µg/g of fresh olive pulp.Three replicates per location and harvest were averaged.*

	El Viso		Los Tomillos		Antequera		Baena		La Rambla		Úbeda		Average	
Total Phenols														
Harvest 1	15834.4	ijk	22650.8	fg	46127.2	a	39922.6	bc	31775.7	d	26668.1	ef	30496.5	a
Harvest 2	11258.0	lm	12827.2	klm	43915.2	ab	38081.9	c	21231.0	gh	19747.0	ghi	24510.1	b
Harverst 3	9783.4	m	10911.0	lm	28063.9	de	21206.2	gh	18091.6	hij	14593.6	jkl	17108.3	c
Average	12292.0	d	15463.0	d	39368.8	a	33070.2	b	23699.4	c	20336.2	c		
Oleuropein														
Harvest 1	10242.6	f	11328.1	f	36031.0	a	27113.0	c	18728.1	de	21014.0	d	20742.8	a
Harvest 2	3360.8	gh	2045.8	gh	30914.5	b	17190.7	e	4235.9	g	9018.4	f	11127.7	b
Harvest 3	1454.5	gh	775.7	h	11946.2	f	3501.4	gh	1453.7	gh	3483.5	gh	3769.1	c
Average	5019.3	e	4716.6	e	26297.2	a	15935.0	b	8139.2	d	11172.0	c		
Demetiloleuropein														
Harvest 1	3429.5	d	7593.4	b	1030.6	ef	3226.2	de	7253.3	b	798.0	f	3888.5	c
Harvest 2	6054.5	bc	7995.4	b	4516.2	cd	10959.2	a	12628.2	a	6808.6	b	8160.3	b
Harvest 3	6439.9	bc	6629.7	bc	11057.3	a	12280.1	a	12852.0	a	7963.1	b	9537.0	a
Average	5308.0	d	7406.2	bc	5534.7	cd	8821.9	b	10911.2	a	5189.9	d		
Cosmelogoside														
Harvest 1	531.3	j	1176.1	gh	2002.0	cd	2501.5	b	1562.6	ef	1550.5	ef	1554.0	ab
Harvest 2	653.5	j	1065.3	hi	2357.2	bc	2922.8	a	1460.0	efg	1362.1	fgh	1636.8	a
Harvest 3	790.3	ij	1096.7	hi	1764.8	de	2269.7	bc	1311.2	fgh	1156.3	gh	1398.2	b
Average	658.4	e	1112.7	d	2041.3	b	2564.7	a	1444.6	c	1356.3	cd		
Ligustroside														
Harvest 1	1013.6	e	1155.5	de	3634.6	a	2774.4	b	1653.1	c	1433.6	cd	1944.1	a
Harvest 2	497.2	fg	322.3	g	2647.3	b	1704.9	c	583.5	fg	882.4	ef	1106.3	b
Harvest 3	245.9	g	262.9	g	833.4	ef	561.9	fg	215.6	g	495.0	fg	435.8	c
Average	585.6	d	580.2	d	2371.8	a	1680.4	b	817.4	cd	937.0	c		
Verbascoside														
Harvest 1	71.9	ef	352.2	def	2154.9	b	2646.2	ab	937.4	d	650.6	cde	1135.5	a
Harvest 2	83.4	f	383.5	def	2011.9	b	3005.0	a	901.4	cd	583.5	cdef	1161.5	a
Harvest 3	114.4	f	525.3	cdef	1099.9	c	969.5	cd	731.1	cd	405.2	def	640.9	b
Average	89.9	d	420.3	cd	1755.6	b	2206.9	a	856.6	c	546.4	cd		
Hydroxytirosol-1-Glucoside-1-														
Harvest 1	187.8	h	331.8	fghi	173.7	hi	407.7	efg	622.3	bcd	519.0	cde	373.7	c
Harvest 2	264.9	ghi	473.7	def	256.4	ghi	481.7	def	700.4	b	476.8	def	442.3	ab
Harvest 3	354.5	efgh	942.6	a	215.8	hi	306.6	fghi	665.5	bc	347.4	efghi	472.1	a
Average	269.1	cd	582.7	a	215.3	d	398.7	bc	662.7	a	447.7	b		
Rutin														
Harvest 1	148.5	fg	323.2	defg	585.0	bc	622.3	b	581.1	bc	370.8	cdef	438.5	ns
Harvest 2	135.5	g	268.0	efg	607.9	bc	1007.0	a	419.3	bcde	277.8	efg	452.6	ns
Harvest 3	148.1	fg	335.0	defg	552.9	bcd	591.0	bc	424.8	bcde	287.7	efg	389.9	ns
Average	144.0	d	308.8	cd	581.9	ab	740.1	a	475.1	bc	312.1	cd		
Luteolin-7-O-glucoside														
Haverst 1	167.6	e	352.4	bcde	438.5	abcde	562.7	abc	383.0	bcde	277.3	cde	363.6	ns
Harvest 2	170.9	e	232.8	cde	519.6	abcd	722.2	a	255.0	cde	292.7	cde	365.5	ns
Harverst 3	197.7	e	300.2	cde	533.1	abcd	663.4	ab	383.2	bcde	414.4	abcde	415.3	ns
Average	178.7	c	295.1	bc	497.0	ab	649.4	a	340.4	bc	328.1	bc		
Glucoside-1-tyrosol														
Harvest 1	41.7	ef	38.2	f	76.9	ab	68.7	bc	55.0	cde	54.2	cde	55.8	ns
Harvest 2	37.1	f	40.3	f	84.3	a	88.3	a	47.4	efg	44.7	ef	57.0	ns
Harvest 3	38.0	f	42.8	ef	60.5	cd	62.7	bc	54.5	cde	40.7	ef	49.9	ns
Average	39.0	c	40.4	bc	73.9	a	73.2	a	52.3	b	46.5	bc		

Tabla IV.4.- *Fruit trait means by harvest date, location and harvest* location in 'Arbequina'. Different letters indicate significant differences (p< 0.005) among means within each of the three groups of data (Tukey test). Three replicates per location and harvest were averaged.*

	El Viso		Los Tomillos		Antequera		Baena		La Rambla		Úbeda		Average	
Fruit fresh weight (g)														
Haverst 1	0,86	g	1,29	ef	0,93	g	1,44	cde	1,40	cde	1,08	fg	1,17	c
Harvest 2	1,24	ef	1,66	bc	0,86	g	1,37	def	1,75	ab	1,37	def	1,37	b
Harvest 3	1,32	ef	1,85	ab	1,30	ef	1,76	ab	2,02	a	1,60	bcd	1,64	a
Average	1,14	cd	1,60	a	1,03	d	1,52	ab	1,72	a	1,35	bc		
Oil content in dry basis (%)														
Harvest 1	35,6	g	44,6	e	35,5	gh	34,9	gh	32,4	hi	29,1	i	35,4	c
Harvest 2	43,5	ef	53,4	b	43,6	ef	43,6	ef	43,2	ef	41,2	f	44,8	b
Harvest 3	48,8	cd	57,6	a	50,6	bc	51,4	bc	48,7	cd	46,4	de	50,6	a
Average	42,6	b	51,9	a	43,2	b	43,3	b	41,4	bc	38,9	c		
Fruit Moisture (%)														
Harvest 1	59,7	ab	50,9	fg	45,5	ij	50,0	fgh	59,1	abc	56,7	cde	53,7	a
Harvest 2	57,8	abcd	52,1	f	35,6	k	43,9	j	58,1	abcd	55,2	e	50,4	c
Harvest 3	56,3	de	48,7	gh	44,6	j	47,8	hi	60,5	a	56,2	de	52,4	b
Average	57,9	ab	50,6	c	41,9	e	47,2	d	59,2	a	56,1	b		
Maturity index														
Harvest 1	0,8	gh	1,4	ef	m.d.		1,0	fgh	m.d.		1,5	ef	1,5	c
Harvest 2	1,7	de	2,4	c	0,1	i	1,2	fg	m.d.		3,1	b	1,7	b
Harvest 3	2,0	cd	3,2	b	0,8	h	1,2	fg	m.d.		3,7	a	2,0	a
Average	1,5	b	2,3	a	0,4	c	1,2	b	m.d.		2,7	a		

Tabla IV.5.- *Percentage of variance of region, harvest date and their interaction for the phenols found in 'Arbequina' olive pulp by the ANOVA analysis. Only data for the four irrigated locations in Andalusia (La Rambla and Úbeda) and Tenerife (Los Tomillos and El Viso) were included. For each location, data of the 3 harvest dates and 3 replicates per location and harvest date were considered. Values in bold indicate significant differences for this source of variation at p<0.01.*

	Totalphenols	Oleuropein	Demethyloleuropein	Comselogoside	Liustroside	Verbascoside	Hydroxytyro- sol-4-glucoside	Rutin	Luteolin-7-O-glu-coside	Tyrosol-1-glucoside
Region	47,2	19,1	5,9	58,7	13,8	63,1	7,2	45,7	22,3	52,7
Date	38,3	71,0	22,5	3,8	68,1	4,2	8,9	1,8	6,5	4,2
Region * Date	2,4	0,6	11,0	4,1	3,3	4,8	27,3	1,9	7,0	0,3
Error	12,1	9,2	60,5	33,4	14,8	27,9	56,6	50,6	64,2	42,7

Tabla IV.6.- *Pearson correlation coefficients among phenols and fruit characteristics for the data of elementary plots by location and harvest date (18 data points) in 'Arbequina'. Values > 0.7 are highlighted.*

	Total phenols	Oleuropein	Demethyloleuropein	Comselogoside	Liustroside	Verbascoside	Hydroxytyro- sol-4-glucoside	Rutin	Luteolin-7-O-glu-coside	Tyrosol-1-glucoside	Fruit fresh weight	Fruit dry weight	Oil content in dry weight	Fruit moisture
Oleuropein	0.93													
Demethyloleuropein	-0.20	-0.55												
Comseogoside	0.82	0.61	0.16											
Ligstroside	0.91	0.96	-0.51	0.61										
Verbascoside	0.81	0.65	0.00	0.83	0.69									
Hydroxytyrosol-4-glucoside	-0.28	-0.38	0.31	-0.07	-0.36	-0.10								
Rutin	0.70	0.48	0.20	0.79	0.50	0.74	0.00							
Luteolin-7-O-glucoside	0.49	0.31	0.18	0.62	0.34	0.51	-0.11	0.87						
Tyrosol-1-glucoside	0.83	0.70	-0.02	0.84	0.70	0.80	-0.17	0.63	0.43					
Fruit fresh weight	-0.39	-0.59	0.61	0.00	-0.54	-0.13	0.63	0.09	0.15	-0.27				
Fruit dry weight	-0.19	-0.42	0.56	0.25	-0.36	0.10	0.54	0.31	0.37	-0.02	0.86			
Oil content in dry weight	-0.50	-0.65	0.53	-0.15	-0.61	-0.25	0.33	-0.10	0.05	-0.26	0.63	0.75		
Fruit moisture	-0.59	-0.50	0.04	-0.65	-0.49	-0.59	0.18	-0.53	-0.47	-0.65	0.22	-0.23	-0.23	
Maturity index	-0.62	-0.63	0.07	-0.41	-0.57	-0.39	0.56	-0.36	-0.25	-0.54	0.59	0.35	0.38	0.39

A separate analysis of variance was performed for the four irrigated locations to better test differences among the Mediterranean and Subtropical conditions of Andalusia and Tenerife regions (Table 5). Region was the main contributor to the total variance for total phenols, comselogoside, verbascoside, rutin and tyrosol-1-glucoside. This effect was mainly due to the higher values for those components in the two Andalusian locations (La Rambla and Úbeda), especially for the first two harvest dates (Figure 4.4). While oleuropein and lisgtroside showed higher variance for harvest date mainly due to the sharp decrease in their contents, especially between harvests 2 and 3.

The correlation between phenolic content components and with fruit traits were also studied (Table 4.6). Total phenolic content showed a high positive correlation with most individual phenolic components except for demethyloleuropein, which increased throughout the harvest season, and hydroxytyrosol-4-glucoside and luteolin-7-O-glucoside which did not show a clear decrease during ripening. Among the phenolic components, the stronger correlation was found between oleuropein and ligstroside. Although comselogoside, verbascoside and rutin seemed to also be highly correlated. Besides, tyrosol-1-glucoside showed very high correlation coefficients with verbascoside and comselogoside. Both have a similar chemical structure in which the glucose is directly linked to the phenolic alcohol moiety, hydroxytyrosol and tyrosol, respectively. No high correlations were found between the total phenolic content or specific phenolic components and fruit traits, with most of them being negative, except for demethy- loleuropein and tyrosol-1-glucoside. Ripening index showed low correlations with phenol content and composition and with the fruit traits evaluated. In particular, fruits having a high ripening index (more than 2) showed low phenol contents (less than 20,000 µg/g of fresh olive pulp); while fruits having a ripening index lower than 2 showed a very large range in variation in phenol contents (from 8,180 to 51,767 µg/g of fresh olive pulp).

IV.V. Discussion

The nine major phenolic components identified, in all locations and harvest dates, showed high variabil- ity (coefficient of variance higher than 50%, except for comselogoside and tyrosol-1-glucoside). Oleuropein was the main phenol as previously reported for olive fruits of other cultivars (Ben Ghorbal *et al.*, 2018; Valente *et al.*, 2020).

The high variability found was mainly due to a location effect for total phenols and for most individual phenolic components. Significant the effect of location was previously reported for phenols in several cultivars. This is the case of ‘Gemlik’ in Turkey (Ben Ghorbal *et al.*, 2018), ‘Arbequina’, ‘Manzanilla’ and ‘Arauco’ in Argentina (Bodoira *et al.*, 2015) and Italian cultivars from a central region of Italy (Mousavi *et al.*, 2019).

Most variability associated with location was caused by the high values observed for Antequera and Baena compared to the other four locations. The most out- standing difference between Antequera and Baena and the rest of the locations is that olive trees are managed in dry farming, while, in Los Tomillos, El Viso, La Rambla and Úbeda, drip irrigation is used. The great influence of water availability on phenolic content and composition of olive fruits and virgin olive oils was previously reported when olive dry farming and irrigation were compared (Gómez-Rico *et al.*, 2006; Cirilli *et al.*, 2017). It seems that water stress periods caused a higher concentration of phenolic compounds in in the dry farming locations of Antequera and Baena, which remained during most of the maturation period. Water availability in the dry farming locations was especially low at the beginning of the oil accumulation period with respect to the four irrigated locations. Previous reports have suggested that water stress in this period has a strong influence on the phenol content (Gucci *et al.*, 2019). This is maybe the reason why rainfall at the beginning of November in Antequera had little influence on phenol content. However, in some other works, a decrease in phenol content with higher stress has been reported (Valente *et al.*, 2020). Probably, in the latter case, the combination of water and heat stress gave a different response of the olives.

When considering only the four irrigated locations, two in Andalusia and two in Tenerife, higher content for total phenols and some components was observed in Andalusia. These differences were especially important for the first harvest date. The higher summer temperatures in Andalusia with respect to Tenerife could produce higher stress which could be the cause of those differences.

Previously, the Sub-Tropical temperatures of Tenerife have shown a great influence on the flowering phenology of the olive tree (Medina-Alonso *et al.*, 2020). This is important, since the Tenerife climatic conditions could help to predict the influence of climate change in the Mediterranean climate. In our case, it seems that the main factor associated with climate change that would impact phenol content is water availability more than changes in air temperature. However, the higher heat stress in summer, predicted in a climate change scenario, could also increase phenol content although have negative influence on other parameters such as oil content (Navas *et al.*, 2019). More experimentation is needed to accurately determine the influence of climate change on phenol content and composition.

Harvest date was also showed to influence the variability in total phenols and phenolic components. As observed in our trials, most of the previous works reported a decrease in total phenolic content with maturation (Abaza *et al.,* 2017; Ferro *et al.*, 2020), including ‘Arbequina’ in North-East Spain (Benito *et al.,* 2013). However, different patterns of variation were observed for individual phenolic components. In fact, the decrease in oleuropein content throughout the ripening process was concomitant with a parallel increase in demethyloleuropein, as previously reported (Gómez-Rico *et al.,* 2008). This was the only component with a significant increase with harvest date. Previous reports have also shown different variation patterns for the different phenolic components in fruit (Ben Ghorbal *et al.,* 2018; Talhaoui *et al.,* 2015; Ferro *et al.*, 2020). ANOVA analysis revealed the comparison of the relative influence of location and harvest date. For total phenols and some components such as comselogoside, tyrosol-1-glucoside, verbascoside and rutin, location had a much greater influence than harvest date. On the contrary, in the case of demethyloleuropein, harvest date was more important. And for other components such as oleuropein and ligstroside, both factors were equally important. Therefore, the relative influence of harvest date and location very much depend on the phenolic component. No previous study on the comparative variance analysis has been performed for olive phenols, except for the case of ‘Baladi’ in Lebanon (El Riachy *et al.,* 2018), where a greater influence of location was observed with respect to harvest date for total phenol content determined spectrophotometrically using the Folin-Ciocalteu method. Different influences of location and genotype for the different phenolic components were previously reported in a set of breeding trials (Pérez *et al.,* 2018).

As expected, all phenolic components showing similar behavior across locations and harvest dates were highly correlated. The extremely high correlation found for oleuropein and ligstroside suggest a common biosynthetic pathway for both secoiridoid glucosides. While the negative correlation between oleuropein and demethyloleuropein support the hypothesis on the interconversion of oleuropein into demethyloleuropein (Obied *et al.,* 2008). On the contrary, demethyloleuropein, hydroxytyrosol-4-glucoside and luteolin-7-O-glucoside showed no correlation with the rest as having a different pattern of variation. Different correlations than the ones reported here were found in a previous set of breeding trials (Pérez *et al.,* 2018). This is probably due to the fact that the variability here is only due to environmental factors; while in the previous work, the phenol variability is attributable to different environments but also to different genotypes. The correlation of phenols with oil and moisture contents in the fruit was low and negative, which seems to indicate different metabolic pathways.

Ripening index seems to be also negatively correlated with phenolic content and most of the phenolic components. In fact, ripening index has been proposed as an indicator of fruit composition (Sánchez de Medina *et al.,* 2014). However, the correlation coefficients obtained in this work were not very high and varied greatly across locations. Overall, low phenolic content was found in fruits with high ripening index (2.0 to 4.0). However, at lower ripening indexes (0.8 to 2.0), both fruits with high and low phenolic contents were found. These results could explain some contradictory results reported in previous studies. Indeed, Gomez-Rico *et al.* (2008) and Morelló *et al.* (2004) showed both high correlation and lack of correlation of ripening index with phenolic content and composition in the ‘Arbequina’ cultivar. Therefore, a general relationship between fruit color and phenolic content should be taken cautiously. A strong association of phenol content with harvest time but not with ripening index could be explained by the fact that there is not a consistent pattern of variation in the ripening index with harvest time in the different locations considered.

IV.VI. Conclusions

In summary, water availability and harvest date seem to play a more important role than air temperature on the phenolic content and composition of ‘Arbequina’ fruits, even when very different climatic conditions such as Sub-Tropical and Mediterranean are compared. Therefore, in order to obtain olive fruits with high phenolic content, low water availability together with an early harvest should be considered. Taking into account that those two factors would also reduce the oil content in fruit. Among the phenol components studied, comsegoloside seems to be the one with the greatest variance among locations. Therefore, future studies might consider this phenol as a good marker for plant stresses, especially water availability. Further research is needed to determine the relative influence of water availability and harvest date on other cultivars with different phenolic profiles than the ‘Arbequina’ one here considered.

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CAPÍTULO V. Discusión general

V.I. Floración del olivo

Numerosas investigaciones han demostrado el relevante papel que juega la temperatura del aire en la fenología de floración de los frutales templados (Rohde y Bhalerao, 2007). Por ello, numerosos modelos han sido desarrollados para conocer los requerimientos de frío de las especies y variedades arbóreas de interés para la agricultura. En relación con el olivo, estos modelos e investigaciones han sido llevados a cabo, tradicionalmente, en zonas de clima Mediterráneo donde los requerimientos de frío durante el invierno anterior a la floración son ampliamente cubiertos (Alcalá y Barranco, 1992; De Melo-Abreu, 2004). Sin embargo, la reciente expansión del cultivo del olivo a otras áreas fuera de la cuenca del Mediterráneo, así como el evidente aumento de la temperatura media del aire, originado por el calentamiento global debido al cambio climático (AEMET y OECC, 2021) han llevado a realizar diversos estudios encaminados a predecir la fecha y la calidad de floración en esas condiciones (Aybar *et al.*, 2015; Brugnara y Sabião, 2022). Otras investigaciones se han centrado en aumentar artificialmente la temperatura del aire, empleando estructuras de tipo invernadero que simulan estas condiciones futuras (Benlloch-González *et al.*, 2018). Pero no existen apenas estudios en condiciones naturales de ausencia de frío invernal.

En los estudios aquí presentados se ha comparado la fenología de floración del olivo en dos climas muy diferentes, como son el clima mediterráneo andaluz y el clima subtropical de Tenerife. Durante los años que han comprendido el presente estudio (2017 a 2021), se ha comprobado que el clima de Tenerife es bastante similar en cuanto a las temperaturas medias a un clima subtropical y se caracteriza por tener unas temperaturas invernales suaves, más cálidas que las registradas para los mismos períodos de tiempo en Andalucía. La amplitud térmica a lo largo del año y también entre el día y la noche ha sido mucho menor en Tenerife respecto a Andalucía. Además, en los ambientes estudiados en Tenerife, en muy pocos momentos se alcanzó la temperatura óptima para la acumulación de frío en olivo, que ha sido fijada en 7-12ºC (De Melo-Abreu *et al.*, 2004; Aybar *et al.*, 2015; Rubio-Valdés *et al.*, 2022).

Las diferencias en la floración entre Andalucía y Tenerife, ponen de manifiesto la alta influencia que tienen las condiciones ambientales en este proceso fenológico, tanto en la fecha como en la duración de la misma (García-Mozo et al, 2010). Además, existe una influencia significativa de las condiciones ambientales del año, como también han descrito previamente otros autores (Navas-Lopez *et al.*, 2019; Brugnara y Sabião ,2022). En el presente estudio, no se evaluó la calidad de la flor emitida en las condiciones ambientales del clima subtropical. Sería interesante incluir este parámetro en futuras investigaciones, puesto que, en estudios previos (Navas-Lopez *et al.*, 2019), se observó que este parámetro estaba condicionado tanto por el ambiente como por el genotipo.

La floración en los ambientes de Andalucía, durante los años estudiados, tuvo un comportamiento esperado en cuanto a fechas y duración, siendo similar a la descrita en estudios previos (Aguilera y Ruiz Valenzuela, 2009; Oteros *et al.*, 2013). Las mayores diferencias entre los ambientes de esta región se apreciaron en Gibraleón, localidad con inviernos más cálidos que el resto coincidiendo con lo descrito con anterioridad por Navas-Lopez *et al.* (2019). En cambio, existen grandes diferencias entre la fenología observada en Tenerife y la de Andalucía. La fecha de floración en Tenerife fue anterior en todos los casos y el período de floración fue mucho más largo. En primer lugar, la floración en condiciones de inviernos cálidos se inició antes y la plena floración se produjo antes. Numerosos estudios ya habían señalado este extremo, mediante modelos de predicción de condiciones climáticas futuras (Aguilera *et al.*, 2013) o sometiendo a los árboles a incrementos de temperatura (Malik y Bradford, 2009).

Estas características diferenciales de la floración en Tenerife se encontraron en todas las variedades estudiadas, tanto en las dos variedades principales en la Isla (‘Arbequina’ y ‘Picual’), como en otras cinco procedentes del Banco Mundial de Germoplasma de Olivo del IFAPA (Belaj et al, 2022) y originarias de diversos países, como son ‘Hojiblanca’ (España), ‘Picholine Marocaine’ (Marruecos), ‘Coratina’ (Italia), ‘Koroneiki’ (Grecia) y ‘Martina’ (procedente del programa de mejora de olivo de Córdoba). Por lo tanto, el factor ambiente fue el principal responsable de las variaciones en la longitud del período de floración y en la fecha de floración. Hasta el momento, la intensidad de floración había sido un parámetro de estudio para valorar la adaptación del olivo (Aybar *et al.*, 2015). Sin embargo, la longitud del período de floración parece un carácter interesante en la descripción de la fenología de la floración y que nunca antes había sido considerado como tal y que debería ser incluido en posteriores investigaciones. Sobre todo, debería de ser considerado en los modelos de predicción del comportamiento fenológico del olivo ante el cambio climático.

Consecuentemente, en los olivos de Tenerife, la diferencia entre el estadio más avanzado y el más retrasado para una misma fecha fue mucho más amplia que en Andalucía. Además, la floración en Tenerife fue independiente en cada una de las yemas del brote sin que existiera un orden en el inicio de la floración. Rubio-Valdés *et al.* (2022) expone que cada yema tiene diferentes requerimientos de frío, lo que podría explicar la floración independiente de las yemas. Esta asincronía en la floración fue una característica muy particular que presentaron los árboles en Tenerife y que sólo ha sido también registrada en olivos en Brasil (Brugnara y Sabião, 2022). Una consecuencia de esta asincronía, fue el hecho de que en Tenerife se observaron dos momentos de plena floración en un año para un mismo árbol. Esta circunstancia no ha sido descrita con anterioridad en olivo.

A consecuencia de esta asincronía, las variedades en el clima subtropical de Tenerife tuvieron un comportamiento errático, sin ningún patrón que pueda definir variedades con floraciones más tempranas o períodos de floración más largos. Las diferencias en las fechas de plena floración entre variedades e incluso entre individuos de una misma variedad, dificultan la polinización cruzada, reduciendo con ello el cuajado de los frutos como se ha descrito para algunas variedades de olivo (Díaz *et al.*, 2006; Pinillos y Cuevas, 2009). Los largos períodos de floración registrados en Tenerife traen consecuencias negativas para el cultivo puesto que la flor está expuesta durante más tiempo a fenómenos meteorológicos adversos y/o a la incidencia de plagas, factores que pueden repercutir en un cuajado deficiente (Rapoport, 2014). Además, la asincronía de floración lleva a una madurez irregular en los frutos, lo que proporciona inestabilidad en el aceite obtenido (Beltrán *et al.*, 2005). Por el contrario, en el clima mediterráneo andaluz, en el que los requerimientos de frío son ampliamente cubiertos, no se observó esa asincronía y las diferencias de floración entre variedades son mucho menos evidentes.

A pesar de que, como se comentó anteriormente, las temperaturas invernales de Tenerife rara vez bajaron de los 10 – 12 ºC, no hubo ausencia de floración en ninguna de las variedades estudiadas. En cambio, en otros estudios similares donde se consiguió un aumento de la temperatura invernal mediante la introducción de las plantas en invernaderos, se registraron plantas en las que no hubo floración en esas circunstancias (Koubouris *et al.*, 2019). Esto hace pensar que la floración no está regida, exclusivamente, por la temperatura del aire si no que existe otra serie de factores ambientales implicados, como puede ser la amplia diferencia de radiación solar existente entre Tenerife y Córdoba, así como la diferencia en la temperatura del suelo entre ambas localizaciones.

Aunque el genotipo parece tener poca influencia en la floración, es conveniente avanzar en la búsqueda de variedades que se adapten mejor a bajos requerimientos de frío invernal. En este sentido, los programas de mejora juegan un papel importante en esta búsqueda, introduciendo este carácter en la selección de nuevas variedades. Para ello, el uso de acebuches autóctonos canarios como *Olea europea subsp. guanchica* (Belaj *et al.*, 2018) puede ser una estrategia adecuada para aportar genes de adaptación a inviernos cálidos a esas nuevas variedades potenciales. Hasta el momento no existen estudios que detallen la fenología de floración en individuos de esta subespecie. Habrá que comprobar si entre la variabilidad genética existente se pueden identificar genotipos con floración sincrónica, regular y abundante que puedan ser utilizados como parentales en futuros programas de mejora. Como se ha mencionado en trabajos previos con recursos silvestres, la utilización de estos materiales (Klepo *et al.*, 2013), dado su pobre comportamiento agronómico para muchos caracteres de interés en la producción de aceite de oliva (pequeño tamaño de fruto, bajo rendimiento graso, dificultad de recolección, etc.) supone una serie de desventajas muy importantes a la hora de abordar un programa de mejora que habría que valorar con cuidado. Por otro lado, para mejorar la producción olivarera en las zonas subtropicales como Tenerife, sería conveniente el desarrollo de ensayos relacionados con técnicas de manejo que favorezcan la floración (Castillo-Llanque,2014; Cabezas-Luque, 2022) y/o uso de productos químicos como se hace en otros cultivos (Erez, 1995; Almanza M. *et al.*, 2010)

En conclusión, el inminente aumento de la temperatura en la cuenca Mediterránea como consecuencia del calentamiento global (Giorgi, 2006) prevén que, en un futuro próximo, los inviernos andaluces sean tan cálidos que, la acumulación de horas de frío puede verse comprometida, especialmente en la costa Atlántica y en el suroeste. En consecuencia, los estudios en campo en condiciones climáticas tropicales y/o subtropicales pueden ayudar a disminuir la incertidumbre a la que hace frente el olivar, sector agrícola estratégico en el sur de España, en el contexto de cambio climático.

V.II. Contenido y composición de fenoles en olivo

La composición fenólica de las aceitunas es un factor importante y que va a condicionar la calidad del aceite producido. Es ampliamente conocida la influencia que tiene el contenido en polifenoles en las características organolépticas, como el amargor (Beltrán *et al.*, 2007) y con las propiedades saludables atribuidas al aceite de oliva virgen extra (Bendini *et al.*, 2007). En este caso, se ha estudiado la composición fenólica de las aceitunas de la variedad ‘Arbequina’ producidas en explotaciones situadas en cuatro ambientes de Andalucía (dos en riego y dos en secano) y dos en Tenerife (en riego).

Como ya se ha explicado anteriormente, estas dos regiones presentan climas muy diferentes, especialmente en cuanto a la temperatura media del aire, siendo más suaves (más cálidas en invierno y más frescas en verano) en Tenerife respecto a Andalucía. En relación con la pluviometría, ambas localizaciones presentan un régimen pluviométrico similar, con una estación seca marcada en verano.

En este estudio, se observó una alta variabilidad en el contenido en fenoles totales y para un gran número de compuestos fenólicos individuales, atribuible a las diferentes condiciones ambientales de las localidades estudiadas. Esta importante influencia que tiene el ambiente en la composición total de fenoles se había descrito con anterioridad para 'Arbequina' (Bodoira *et al.*, 2016) y para otras variedades (Gargouri *et al.*, 2013; Ben Ghorbal *et al.*, 2018). En algunos ensayos de mejora también se ha estudiado la influencia relativa de la ubicación y el genotipo para los diferentes compuestos fenólicos, como en el caso de algunas variedades italianas recolectadas en la región de Apulia (Baiano *et al.*, 2013). Incluso existen trabajos que relacionan el contenido de fenoles con la edad de los olivos (Bedbabis *et al.*, 2016).

Esta alta variabilidad asociada a la ubicación fue debida a las diferencias significativas registradas en dos de las localidades andaluzas (Antequera y Baena) con el resto. Sin embargo, las diferencias en cuanto a las condiciones climáticas entre estas dos localidades y el resto de Andalucía no son tan importantes como el hecho de que, en este caso, se trataba de explotaciones de secano. Así, se apreció la relevancia del régimen hídrico y de la disponibilidad de agua en el contenido fenólico. Previamente ya se había descrito el papel fundamental que jugaba este factor en la composición de los frutos y de los aceites de oliva vírgenes (Salas *et al.*, 1997; Gómez-Rico *et al.*, 2006) comparando olivares de secano y de regadío.

Analizando sólo aquellas localizaciones de regadío (dos en Andalucía y dos en Tenerife), se observó que los valores totales de fenoles fueron mayores en Andalucía. También ocurrió lo mismo para ciertos componentes fenólicos. Las altas temperaturas estivales que se registraron en Andalucía, principal diferencia con el clima de Tenerife durante el verano, y el estrés que éstas generan, podrían ser la causa de este diferente contenido. Asimismo, teniendo en cuenta que se prevé un aumento de las temperaturas debido al cambio climático, y, especialmente, una sequía cada vez más acusada (AEMET y OECC, 2021), esto se traducirá en un aumento del estrés de los árboles durante la síntesis del aceite. Este aspecto sería positivo para los aceites obtenidos en estas condiciones, aunque habría que tener también en cuenta que influyen negativamente en otros caracteres, como la cantidad de aceite sintetizado (Navas-López *et al.*, 2020). Para profundizar en las consecuencias que el cambio climático tendrá en la composición del aceite de oliva, Tenerife puede ser una localización ideal, ya que, en las condiciones de clima subtropical, el ciclo de cultivo del olivo se ve alterado y la síntesis de aceite y la recolección coinciden con el verano (Mérida-García, 2018).

Por otro lado, al analizar el contenido total de polifenoles en relación con la fecha de cosecha, se ha comprobado que su relación es inversamente proporcional; es decir, que el contenido total disminuye conforme la fecha avanza. No obstante, esta relación no se estableció igual para todos los compuestos individualmente. De este modo, algunos componentes como la dimetiloleuropeína aumentaban con la fecha. En el caso del comselogosido, tirosol-1-glucósido, verbascósido y rutina, la fecha de la cosecha no fue determinante en su contenido, siendo la ubicación el factor con mayor influencia sobre ellos. Esto implica que la influencia de la fecha de cosecha y la ubicación depende en gran medida del componente fenólico del que se trate. Esta influencia relativa de ambos factores sólo ha sido estudiada previamente para la variedad 'Baladi' en Líbano (El Riachy *et al.*, 2018). También se comparó la evolución del contenido fenólico con el índice de madurez del fruto y se demostró que no hay una relación consistente entre ellos, en contra de lo que se ha creído tradicionalmente. Esto concuerda con la falta de relación entre el índice de madurez y otros caracteres del fruto, tal como se ha observado por ejemplo respecto a la pauta general de acumulación de aceite (Navas-López *et al.*, 2019).

Las relaciones observadas entre los diferentes compuestos fenólicos individuales analizados están de acuerdo con el conocimiento disponible sobre las rutas biosintéticas de los mismos. Aquellos componentes fenólicos que mostraron un comportamiento similar en todos los ambientes y fechas de cosecha tenían una fuerte correlación, positiva en algunos casos donde se comparte la ruta de biosíntesis (oleuropeína y ligstroside) y negativa en casos de interconversión entre compuestos (oleuropeína y demetiloleuropeína) (Obied *et al.*, 2008). También, otros ensayos de mejora arrojaron resultados de correlación similares a los aquí obtenidos (Pérez *et al.*, 2018). En cualquier caso, las rutas de síntesis de los fenoles en los frutos del olivo son complejas y dependen de multitud de factores y, en función del lugar de estudio puede tener más influencia el ambiente, el genotipo, el estrés o la fecha de cosecha. Por lo tanto, es importante seguir profundizando en este tipo de ensayos.

V.III. Referencias

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CAPÍTULO VI.   Conclusiones generales

1. Las condiciones climáticas contrastantes del clima subtropical de Tenerife comparado con el clima mediterráneo de Andalucía tienen efectos muy importantes sobre la fenología de floración en olivar, pero no tanto en componentes de calidad de los aceites producidos como la composición fenólica de los frutos.
2. Las claras diferencias en fenología de floración observadas en las condiciones experimentales ensayadas confirman la utilidad de estos estudios como indicador fiable de los efectos del calentamiento climático.
3. La ausencia de frío invernal en olivo adelanta y alarga el período de floración. Como consecuencia, se produce una asincronía de la floración en todas las variedades estudiadas, a pesar de provenir de zonas de origen con condiciones climáticas muy dispares, desde Grecia a Marruecos. Por tanto, si las previsiones de cambio climático se cumplen, cabe esperar importantes efectos en la floración del olivo, con los consiguientes problemas de manejo que ello puede implicar.
4. La ausencia de frío invernal no implica la ausencia de floración para ninguna de las variedades de olivo aquí estudiadas (‘Arbequina’, ‘Picual’, ‘Ocal’, ‘Martina’, ‘Koroneiki’, ‘Hojiblanca’, ‘Coratina’ y ‘Picholine Marocaine’). Por tanto, se propone incluir la duración del período de floración como un parámetro más informativo en el desarrollo de modelos de simulación del cambio climático.
5. Se propone seguir explorando un rango más amplio de variedades para identificar materiales más adaptados a las condiciones previstas por el calentamiento global, incidiendo en aquellas variedades tradicionales de zonas con inviernos suaves y explorando recursos silvestres como *Olea europaea* subsp. *guanchica*, originaria de las Islas Canarias.
6. Asimismo, como medida de adaptación a los potenciales efectos del cambio climático en el olivar, se hace urgente que los programas de mejora en olivo introduzcan los bajos requerimientos de frío como un carácter de selección, como ya se hace en programas de mejora de muchos otros frutales.
7. La disponibilidad de agua de riego y la fecha de recolección desempeñan un papel determinante en el contenido y la composición fenólica de los frutos de ‘Arbequina’, aunque estos se desarrollen en condiciones climáticas muy diferentes. Así, dosis bajas de riego junto con una recolección temprana darán lugar a frutos con bajo contenido graso, pero con alto contenido en polifenoles en esta variedad.
8. El marcado efecto de la localidad en el contenido del compuesto fenólico comsegolosido hacen de este un buen marcador potencial de las condiciones de estrés de un árbol, especialmente en lo relacionado con el estrés hídrico de la planta.
9. Los resultados obtenidos en este trabajo para ‘Arbequina’ se deben contrastar con un mayor panel de variedades para comprobar su generalización o no a otros materiales vegetales de olivo.

CAPÍTULO VII. Transferencia de conocimiento

VII.I. Publicaciones en revistas ISI elaboradas durante el período de tesis doctoral y directamente relacionadas con el contenido de la tesis

Medina, G., Sanz, C., León, L., Pérez, A. G., & De La Rosa, R. (2021). Phenolic variability in fruit from the 'Arbequina' olive cultivar under Mediterranean and Subtropical climatic conditions. Grasas y Aceites, 72(4), e438. https://doi.org/10.3989/gya.1002202

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VII.II. Publicaciones en revistas ISI elaboradas durante el período de tesis doctoral relacionadas con el contenido de la tesis

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VII.III. Comunicaciones a congresos realizadas durante el período de tesis doctoral directamente relacionadas con el contenido de la tesis

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VII.V. Otras actividades de formación y divulgación realizadas durante el periodo de tesis doctoral relacionadas directamente con el contenido de la tesis

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