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Tesis Doctoral

Letargo invernal en albaricoquero (*Prunus armeniaca* L). Análisis de diversos factores que afectan su evolución.

Dormancy in apricot (*Prunus armeniaca* L.). Factors affecting its evolution.

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I. SUMMARIES

I.I. SUMMARY (TESEO-SPANISH)

El letargo invernal es un mecanismo desarrollado por los árboles de clima templado, que crecen en climas con estaciones bien diferenciadas, para evitar el impacto de las bajas temperaturas invernales. La satisfacción de unas determinadas necesidades de frío invernal específicas para cada variedad es esencial para un adecuado desarrollo de las yemas florales y vegetativas, condicionando el momento y calidad de la floración, así como la posterior fructificación. Si bien se ha conseguido un notable progreso en las últimas décadas, todavía existen importantes incertidumbres en el conocimiento de su inducción, mantenimiento y salida. Así pues, el objetivo de este trabajo de investigación es intentar aportar nuevas evidencias que ayuden a esclarecer aquellos aspectos aún sin resolver asociados al complejo proceso del letargo invernal en albaricoquero, tales como: i) las similitudes y diferencias entre especies o variedades, así como entre localizaciones con diferentes condiciones climáticas; ii) la idoneidad de diversos métodos de estimación de las necesidades de frío para la salida del letargo en zonas de inviernos cálidos; iii) el efecto, a veces contradictorio en función de la bibliografía, de distintas temperaturas o combinaciones de éstas sobre la evolución y salida del letargo; iv) la eficiencia del sombreado así como la utilización de tratamientos químicos para romper el letargo, en condiciones de insuficiente acumulación de frío; v) la identificación de regiones del genoma (QTLs) asociadas a las necesidades de frío invernal o a la fecha de floración, etapa previa al desarrollo de marcadores moleculares específicos para su utlización en mejora genética asistida. La elección de la especie albaricoquero como modelo de estudio la justifica por un lado el hecho de que han sido escasas las investigaciones sobre letargo invernal realizadas en esta especie vegetal y, por otro, la sustancial importancia económica de este cultivo en el sureste de España. Además, el desarrollo de variedades de albaricoquero con bajas necesidades de frío resulta esencial para la introducción y cultivo de esta especie en áreas con una escasa acumulación de frío. Con el objetivo de obtener una visión global del proceso del letargo invernal en la especie albaricoquero, se evaluaron variedades que cubrían el rango completo de necesidades de frío de la especie, y parte de los ensayos realizados se llevaron a cabo en distintas localizaciones caracterizadas por clima mediterráneo, tales como Murcia (España), Toscana (Italia) y la Región de Western Cape (Sudáfrica).

I.II. SUMMARY (TESEO)

Dormancy is a mechanism developed by temperate fruit trees grown in climates with well-differentiated seasons against the impact of low winter temperatures. The chill requirement fulfilment for each cultivar is essential for achieving an optimum development of vegetative and reproductive buds, which will affect timing and level of flowering and fructification. Although considerable progress has been achieved in the last few decades, large functional gaps of knowledge still exist regarding bud-dormancy induction, maintenance and release.Thus, the objective of this research work is to supply new evidence that may contribute to the general understanding of those aspects that remain unclear in the complex trait of dormancy, such as: i) the similarities and differences among different species or cultivars, as well as among locations with different climatic conditions; ii) the suitability of different methods for assessing chilling requirements for breaking of dormancy in mild winter areas; iii) the effect, sometimes uneven according to the literature, of different temperatures or combinations of temperatures over dormancy progression and dormancy release; iv) the efficiency of shading during endodormancy and chemical rest breaking agents for breaking dormancy in conditions of insufficient chill accumulation; and v) the identification of regions of genome (QTLs) controlling chilling requirements or flowering time, which is the first step to develop specific molecular markers for using molecular assisted selection in breeding programmes. The apricot species has been used as plant material not only due to the fact that few published studies have addressed apricot dormancy, but also because of the economical importance of this crop in the southeast of Spain. The development of low chill apricot cultivars is a neccesary step towards the introduction and growing of this species in marginal chill areas. With the purpose of obtaining representative results related to dormancy in the apricot species, a group of cultivars ranging the chilling requirement of apricot was studied. In addition, the studies were approached through experimentation in different climatic conditions that characterize the Mediterranean climate, in regions such as Murcia (Spain), Tuscany (Italy) and the Western Cape (South Africa).

I.III. SUMMARY (SPANISH)

El letargo invernal es un mecanismo desarrollado por los árboles de clima templado, que crecen en climas con estaciones bien diferenciadas, para evitar el impacto de las bajas temperaturas invernales. Si bien se ha conseguido un notable progreso en las últimas décadas, todavía existen importantes incertidumbres en el conocimiento de la inducción, mantenimiento y salida del letargo (Saure, 1985; Faust *et al*., 1997; Arora *et al*., 2003). Por otra parte, el conocimiento de las necesidades de frío de una variedad tiene una notable importancia, tanto práctica como económica, en el control, mantenimiento y producción de plantas leñosas (Fennell, 1999), y resulta necesario para poder plantar las variedades de albaricoquero en las zonas más adecuadas para su cultivo. Una salida incompleta del letargo afecta el comportamiento del árbol en tres aspectos principales: retraso de la brotación, una escasa floración y una falta de uniformidad en la brotación y floración (Tabuenca, 1965; Legave *et al*., 1982; Gil-Albert, 1989; Viti and Monteleone, 1991; Viti and Monteleone, 1995; Erez, 2000). Por otro lado, cuando se cultivan variedades con bajas necesidades de frío, es decir variedades de floración precoz, en zonas con inviernos fríos, la floración puede tener lugar demasiado pronto puesto que las necesidades de frío son satisfechas rápidamente. En este caso, las bajas temperaturas podrían dañar las yemas en su estado fenológico más susceptible (Bartolini *et al*., 2006b), conllevando la posible aparición de anomalías florales (Clanet and Salles, 1972), y la aparición de heladas podría producir importantes pérdidas en los cultivos (Scorza and Okie, 1990).

Entre los aspectos aún sin resolver respecto al proceso de letargo invernal podemos citar las siguientes: similitudes y diferencias entre especies o variedades (Crabbé and Barnola, 1996; Paiva and Robitaille, 1978; Gilreath and Buchanan, 1981b), así como entre altitudes y localizaciones (Balandier, 1993a; 1993b), el efecto, a veces contradictorio, de acuerdo a la bibliografía, de distintas temperaturas o combinaciones de estas sobre la evolución y salida del letargo (Jacobs *et al*., 2002; Erez *et al*., 1979b; Couvillon and Erez, 1985b; Rageau *et al*., 1998; Naor *et al*., 2003); la relación de estos resultados diversos con la fase del letargo de la planta y el efecto sobre su estado fisiológico (Weinberger, 1950; Thompson *et al*., 1975; Couvillon and Erez, 1985a; Young, 1992; Tehranifar *et al*., 1998); o la eficiencia del sombreado durante diferentes etapas del letargo y de diferentes tratamientos químicos para romper el letargo compensando una insuficiente acumulación de frío (Gilreath and Buchanan, 1981a; Buchanan *et al*., 1977; Costa *et al*., 2004; Erez, 1987a). Por

otro lado, el desarrollo de variedades con bajas necesidades de frío resulta una etapa necesaria para la introducción de árboles de clima templado en áreas con una insuficiente acumulación de frío. Por otra parte, los recientes avances en marcadores moleculares ofrecen a la mejora genética un acercamiento alternativo, rápido y preciso, a la selección convencional para la mejora de caracteres cuantitativos (Tanksley and Hewitt, 1988). Mediante el uso de mapas moleculares de ligamiento se puede proceder al mapeo, evaluación genética y selección de caracteres cuantitativos (QTL). Sin embargo, y en el caso concreto del albaricoquero, todavía no se han presentado trabajos sobre la identificación de QTL asociados a las necesidades de frío o la fecha de floración, con la excepción de un trabajo preeliminar presentado por Olukolu *et al*. (2008).

Considerando el hecho de que por un lado, han sido escasas las investigaciones sobre letargo invernal llevadas a cabo en albaricoquero y, por otro lado es conocida la notable importancia económica de este cultivo en el sureste de España, el objetivo de este trabajo de investigación es intentar aportar nuevas evidencias que ayuden a esclarecer el complejo proceso del letargo invernal, utilizando como material vegetal el albaricoquero. Por otro lado, y siempre que los ensayos lo requerían y se dispuso de material vegetal suficiente, se evaluaron variedades que cubrían el rango completo de necesidades de frío de esta especie. Además, y con el objetivo de dar una visión global del proceso del letargo invernal, los ensayos realizados se llevaron a cabo en distintas localizaciones caracterizadas por un clima mediterráneo, a saber, España, Italia y Sudáfrica.

La presente tesis doctoral se ha estructurado en diferentes capítulos en función de los objetivos específicos planteados. En el primer capítulo se muestra un estudio de la progresión estacional del letargo invernal, mediante dos metodologías diferenciadas. En la primera parte, se realizó con la metodología de segmentos uninodales con el fin de que los datos obtenidos reflejasen el estado de la propia yema (endoletargo). Este estudio se llevó a cabo en tres variedades distintas localizadas en un área moderadamente fría, Cieza-Murcia (España). Los resultados mostraron que el letargo invernal, tanto en yemas reproductivas como vegetativas, alcanza un nivel poco profundo en nuestra latitud. A su vez, este estado fluctúa enormemente con el tiempo, probablemente asociado a cambios fisiológicos a su vez asociados a otras variables como por ejemplo, la temperatura. Tras la diferenciación de los órganos florales se produjo un aumento generalizado de la intensidad del letargo, si bien este fue más pronunciado en las yemas vegetativas que en las reproductivas. Por otro lado, se encontró una mayor profundidad de letargo en yemas terminales, si bien fue seguida por

una más rápida salida del letargo, en comparación con yemas laterales. Esto pone de manifiesto el importante efecto del estado fisiológico de la estructura estudiada sobre su letargo.

En cuanto a la segunda parte de este capítulo, el material empleado para la evaluación del letargo, fueron brotes del año. Con estos la información obtenida es más representativa, si bien tampoco exacta, del comportamiento del árbol. En este trabajo se cuantificó el nivel de letargo en diversas variedades cultivadas en localizaciones con condiciones climáticas diversas en el sureste de España, así como en la región de Western Cape en Sudáfrica, con el objetivo de caracterizar la evolucíon del letargo bajo condiciones de clima mediterráneo. En España se estudiaron tres zonas de la Región de Murcia, Campotéjar, Cieza y Barranda; mientras que en Sudáfrica se eligieron Ladismith, Villiersdorp y Ceres. Los genotipos estudiados en España fueron: 'Currot', 'Rojo Pasión', 'Dorada', 'Murciana', 'Búlida' y 'Orange Red'; mientras que en Sudáfrica se eligieron 'Supergold', 'Suapriseven', 'Palsteyn', 'Charisma', 'Canino' y 'Orange Red'. Para calcular el frío acumulado mediante diferentes modelos climáticos (Chill Units (CU) –Modelo Utah-, Porciones –Modelo Dinámico- y Horas bajo 7 ºC) se registraron temperaturas medias horarias durante el periodo de estudio. Se cortaron periódicamente, desde finales de verano hasta finales de invierno, grupos de 15 tallos del año para cada variedad y localización. Seguidamente los tallos se forzaron a 25 ºC e iluminación continua, hasta que al menos 5 de los 15 tallos habían brotado. El conteo de brotación se realizó tanto para yemas reproductivas como vegetativas. Las yemas entraron en letargo al final del verano, antes del comienzo de la acumulación de frío en áreas cálidas y moderadamente frías. Sin embargo, en el área más fría de las estudiadas en España, la máxima profundidad del letargo se alcanzó tras una importante acumulación de frío (400 CU), si bien la inducción y el comienzo de la acumulación también ocurrieron al final del verano. El descenso de temperaturas al final del verano, coincidiendo con la reducción del fotoperiodo, pudo causar la activación de la entrada en letargo. La hipótesis de que la inducción del letargo está relacionada con la acumulación de frío parece errónea de acuerdo con los resultados obtenidos en las áreas cálidas y moderadamente frías de ambos países. Asimismo, se observó una generalizada e importante disminución de la intensidad del letargo en Diciembre (Junio en el hemisferio sur), seguida de una pausa en el descenso y una subsiguiente salida total del letargo. Esta pausa podría estar asociada a una 'espera' para la llegada de temperaturas favorables para el crecimiento. Por otro lado, las yemas reproductivas mostraron una profundidad del letargo similar, si bien la salida del letargo fue ligeramente anterior. El frío acumulado hasta la fecha de salida del letargo, cuantificado mediante los diferentes modelos, en la fecha de salida del letargo, varió notablemente para una misma

variedad en localizaciones distintas. Los resultados obtenidos general serias dudas sobre la robustez de los modelos utilizados para determinar la salida del letargo en situaciones diversas. Las considerablemente diferentes condiciones climáticas mediterráneas estudiadas, lo cual ha sido evidenciado por las diferentes temperaturas registradas y, a su vez, la variable acumulación de frío, podría explicar, en parte, las diferencias en la progresión del letargo desde finales de verano hasta su salida.

En el segundo capítulo, se evalúa el efecto de factores como el genotipo y la localización sobre la salida del letargo. Para ello, en la primera parte del capítulo se estudiaron los genotipos 'Currot', 'San Castrese', 'Goldrich', 'Stark Early Orange' ('SEO') y 'Orange Red' en Venturina-Toscana (Italia) y en Cieza-Murcia (España). Este grupo de variedades cubre el rango completo de necesidades de frío de la especie albaricoquero en el clima mediterráneo. Para obtener una información más cercana a la productividad de estas variedades se estudiaron, no sólo las necesidades de frío, sino también los requerimientos de calor, fecha de floración, así como los porcentajes de floración y fructificación durante dos años consecutivos. Se recogieron temperaturas medias horarias y con estos datos se calcularon las CU mediante el Modelo Utah. Las variables condiciones climáticas, debido a las distintas localizaciones y años, determinaron una acumulación de frío considerablemente variable. Asimismo, se encontraron diferencias considerables en necesidades de frío entre las distintas variedades y años. Esta variabilidad sugiere la inexactitud del Modelo Utah para la determinación de las necesidades de frío en las condiciones climáticas estudiadas. Además, parece que la temperatura debería ser analizada junto con otros factores climáticos, como el momento de aplicación y la combinación de bajas y altas temperaturas, para así poder mejorar el cálculo de las necesidades frío.

Para complementar esta primera parte, se realizó un estudio similar dividido a su vez en dos partes. En la primera se calcularon las necesidades de frío y calor para florecer de diez variedades, las cuales cubrían también el rango completo de necesidades de frío invernal y de época de floración de la especie albaricoquero, durante cuatro años consecutivos y en una misma localización (Cieza-España). Por otra parte se calcularon las necesidades de frío de cinco variedades distribuidas en tres localizaciones distintas en la región de Western Cape en Sudáfrica. En ambos casos, se obtuvieron temperaturas medias horarias de ambas localizaciones y se calcularon las necesidades de frío en CU (Modelo Utah), Porciones (Modelo Dinámico) y Horas bajo 7 ºC. Los resultados obtenidos en las diferentes localizaciones y años fueron comparados y se realizaron correlaciones

entre las necesidades de frío y calor, los días entre la salida del letargo y la floración, así como con la fecha de floración. Las necesidades de frío para la salida del letargo de una determinada variedad fueron muy diferentes en función de su cultivo en un área muy cálida o en un área moderadamente fría. Un ejemplo es 'Canino', el cual cultivado en Ladismith (Sudáfrica) mostró unas necesidades de frío de 304 CU, mientras que en Cieza (España) muestra en torno a 780 CU. En este sentido, el rango de requerimientos de frío invernal para variedades comerciales difiere en función de las localizaciones. El Modelo Dinámico minimizó las diferencias entre años y localizaciones. Sin embargo, en 2006-2007, un año con insuficiente acumulación de frío, se observaron en campo síntomas de esa insuficiente acumulación, mientras que las necesidades de frío calculadas mediante el Modelo Dinámico fueron suficientes para satisfacer los requerimientos de casi todas las variedades. Esto puede explicar también la baja variabilidad encontrada entre años con este modelo, en comparación con los otros dos modelos estudiados. Por otro lado, no se encontraron diferencias significativas entre variedades respecto a las necesidades de calor para florecer, por lo que nuestros resultados indican que las necesidades de calor no son una característica intrínseca de la variedad en albaricoquero. Así pues, la fecha de floración está principalmente determinada por las necesidades de frío de la variedad, siendo mayor el periodo que media entre salida del letargo y floración para las variedades con bajas necesidades de frío debido a que las temperaturas cuando éstas salen del letargo son inferiores que cuando lo hacen las variedades con altas necesidades.

La evaluación del efecto tanto de temperaturas frías, como de combinaciones de temperaturas frías y elevadas en ciclos diarios, aplicadas durante distintas fases del letargo constituye el tercer capítulo de la Tesis. En una primera parte, se evaluó la eficiencia de ciclos de temperatura respecto a la salida del letargo utilizando brotes del año de la variedad 'Palsteyn'. Se realizaron tres réplicas de los ensayos distribuidas en dos años, considerando una diferente acumulación de frío, y por tanto distinto nivel de requerimientos de frío satisfechos. Para cada réplica, se aplicaron ciclos de temperaturas durante un total de 60 días (temperatura continua a 5 ºC y ciclos de temperatura de 19h/5h con temperaturas de 5/15 ºC, 5/20 ºC, y 5/25 ºC, así como los mismos ciclos tras pre-tratamientos de 5 ºC durante 30 o 45 días). Después de cada tratamiento, se procedío al forzado de los brotes a 25 ºC en cámaras de cultivo, y se calculó el tiempo medio de brotación (TMB) para yemas laterales vegetativas, terminales vegetativas, vegetativas en general (englobando tanto laterales como terminales) y reproductivas. El estado del letargo, que tenían las yemas en el momento del corte, influenció considerablemente la eficiencia para la ruptura del letargo de los distintos tratamientos. Así, cuando todavía no se había acumulado frío antes de la

fecha de corte, el tratamiento de 5 ºC fue el más efectivo, seguido de el de 5/15 ºC. Sin embargo, cuando los tallos ya habían recibido una cierta acumulación de frío en el campo, el tratamiento de 5/25 ºC siempre fue el más eficiente, mientras que 5/15 ºC y 5/20 ºC resultaron igualmente eficientes que el tratamiento continuo a 5 ºC. Por otro lado, cuando se realizó el pretratamiento de 5 ºC cuando aún no se había acumulado frío en campo, la eficiencia de los tratamientos tendió a igualarse, especialmente cuando se aplicó un pretratamiento de 45 días. En todos los tipos de yema se observó una tendencia similar, si bien las yemas reproductivas mostraron unos requerimientos de frío inferiores. Asímismo, se observaron escasas diferencias de requerimientos de frío entre yemas terminales y laterales vegetativas. Por otro lado, la notable diferencia encontrada entre 5/25 ºC en comparación con 5/20 ºC y 5/15 ºC, tras una acumulación parcial de frío, podría indicar, más que un cambio cuantitavo, un cambio cualitativo en el efecto de temperaturas para la salida del letargo. Los resultados obtenidos mostraron que temperaturas como 25 ºC, pueden ser muy eficientes para la salida del letargo cuando se aplican tras una acumulación parcial de frío y en un ciclo diario junto con bajas temperaturas. Finalmente, merece la pena destacar que el frío aplicado fue considerablemente más eficiente cuando se aplicó en campo que en cámaras de cultivo.

En cuanto a la segunda parte de este capítulo, se realizó un estudio del efecto de temperaturas bajas (1, 4, 7, 10 ºC) sobre la progresión del letargo de brotes recogidos en campo con distintas cantidades de frío acumulado. Se realizó el corte de material vegetal cada 15 días desde mediados de Octubre hasta mediados de Enero, y se sometió a tratamiento de las diferentes temperaturas durante 60 días. Posteriormente se procedió al forzado y la determinación del TMB para establecer la intensidad del letargo tanto de las yemas reproductivas como de las terminales y laterales vegetativas. Para estas fechas el frío acumulado en campo osciló desde 0 hasta 731 CU, lo que correspondería al 70-90% de las necesidades de frío de las variedades estudiadas. En cuanto a los resultados, se obtuvieron valores similares entre yemas laterales y terminales. La máxima intensidad del letargo fue registrada a mediados de Noviembre (con 100 CU acumuladas en condiciones de campo), excepto en el caso del tratamiento de 10 ºC. Este tratamiento fue el que indujo una mayor profundidad del letargo en yemas laterales vegetativas, y además, fue el más efectivo para provocar la salida del letargo tanto en yemas terminales como laterales. El resto de temperaturas tuvieron un efecto similar entre ellas. Por otro lado, las yemas reproductivas mostraron un letargo más superficial, y una salida del letargo más precoz en comparación con las vegetativas. En yemas reproductivas el máximo letargo fue alcanzado a mediados de Octubre (con 0 CU acumuladas en condiciones de campo), el cual fue seguido de un descenso progresivo del MTB

hasta la salida completa del letargo. Los resultados mostraron que el estado del letargo tiene una importante influencia sobre el efecto de las temperaturas sobre el letargo invernal. Así, se observó un efecto no lineal a lo largo de la evolución del letargo, especialmente en el rango superior de las temperaturas que han sido consideradas adecuadas para la salida del letargo. La introducción de este efecto diferencial podría ayudar a la mejora de los modelos orientados a la estimación de la salida del letargo.

El cuarto capítulo aborda la evaluación del efecto del sombreado durante diferentes etapas del letargo invernal, en un área con inviernos relativamente cálidos, y también el estudio del efecto del tratamiento con una citoquinina, el tidiazuron ([TDZ] N-fenil-N-1,2,3-tiodiazol-5-il-urea), y aceite de invierno, sobre la salida del letargo. Para ello se estudiaron la fechas de floración y maduración, así como los porcentajes de floración, fructificación y aborto de pistilos. El estudio se llevó a cabo durante tres años consecutivos caracterizados por importantes diferencias en la acumulación total de frío. El sombreado durante el endo-letargo adelantó la salida del letargo, la fecha de floración y también la fecha de maduración. Además, en condiciones de insuficiente acumulación de frío, los tratamientos con TDZ+aceite y los de sombreado durante el endo-letargo y final del endo-letargo, incrementaron significativamente la fructificación e indujeron un incremento de la precocidad. Por otro lado, se observó una interesante interacción entre el aborto de pistilo, y la baja acumulación de frío, el año de baja acumulación de este. En este sentido, el TDZ+aceite por una parte incrementa la floración de forma significativa, si bien cuando no se acumuló frío suficiente se observó un mayor número de flores con aborto de pistilo que cuando hubo una acumulación suficiente. En nuestra opinión, el tratamiento de TDZ+aceite provoca una floración generalizada. Bajo este tratamiento, la aparición de flores defectuosas es escasa en condiciones de adecuada satisfacción de frío invernal, mientras que el porcentaje de aborto de pistilos se incrementa muy significativamente en el caso de que exista una escasa acumulación de frío. De esta forma, se podría deducir que los bajos porcentajes de floración normalmente obtenidos bajo condiciones de insatisfacción de las necesidades de frío, podrían estar provocados por anomalías en el desarrollo del pistilo, que pasarían desapercibidas sin la aplicación del tratamiento con TDZ+aceite. Por otro lado, y en condiciones de insuficiente acumulación de frío, la distribución de frío acumulado durante el letargo, parece ejercer un importante papel en la posterior salida del letargo, floración y fructificación. Finalmente, y considerando las posibilidades de cultivar el albaricoquero en latitudes tan bajas como las estudiadas en Sudáfrica, junto al sustancial incremento de la relación frutos/yemas de flor obtenidos en un año con escasa acumulación de frío, el sombreo

de árboles durante el invierno, así como el tratamiento con TDZ+aceite podrían producir interesantes resultados en cuanto al incremento de la precocidad y mejora del rendimiento de variedades de bajas necesidades de frío en zonas considerablemente cálidas. Además, la aplicación del sombreo en zonas con fotoperiodos más largos y escasa nubosidad durante el invierno, junto con la utilización de variedades con necesidades de frío más bajas que la utilizada en este ensayo, podrían optimizar el efecto del tratamiento.

Finalmente, en el quinto capítulo se realizó un estudio preeliminar para la identificación de QTLs asociados a la fecha de floración. Además, se describe la metodología de la 'megaplex PCR', la cual puede abrir nuevos horizontes en el uso multifuncional de los microsatélites en el campo de la mejora genética, multiplicando la eficiencia y reduciendo significativamente los costes de análisis. Con esta técnica se han desarrollado dos mapas de ligamiento genético preliminares en albaricoquero con 37 y 29 microsatélites SSR, respectivamente. Por otro lado, fue posible la identificación de un QTL asociado a la fecha de floración en el grupo de ligamiento 5, cerca del cuál encontramos asociado el marcador AMPA-105. No obstante, será necesario ampliar el trabajo realizado y completar la saturación del mapa para confirmar los resultados preeliminares obtenidos.

I.IV. SUMMARY

Dormancy is a mechanism developed by temperate fruit trees grown in climates with well-differentiated seasons against the impact of low winter temperatures. Although considerable progress has been achieved in the last few decades, large functional gaps of knowledge still exist regarding bud-dormancy induction, maintenance and release (Saure, 1985; Faust *et al*., 1997; Arora *et al*., 2003). Knowing the chilling requirement (CR) of a cultivar has a significant practical and economic impact on the control, maintenance and production of woody plants (Fennell, 1999), and is necessary for crop management of apricot cultivars in their most suitable areas. In this manner, incomplete dormancy release affects tree behaviour in three main ways: late bud break, a low level of flower bud break and a lack of uniformity of leafing and bloom, resulting in a higher flower bud drop (Tabuenca, 1965; Legave *et al*., 1982; Gil-Albert, 1989; Viti and Monteleone, 1991; Viti and Monteleone, 1995; Erez, 2000). On the other hand, in the case of cultivars with low chilling requirements (i.e. early-flowering cultivars) growing in cold winter areas, blooming happens too early because chilling requirements are quickly satisfied. In this case, the low temperatures can damage the swelling buds in their more susceptible phenological stages (Bartolini *et al*., 2006b), resulting in the appearance of floral anomalies (Clanet and Salles, 1972), and frost can induce an important loss of yield (Scorza and Okie, 1990).

Among the points that remain unclear with regard to dormancy are: the similarities or differences between different species or cultivars (Crabbé and Barnola, 1996; Paiva and Robitaille, 1978; Gilreath and Buchanan, 1981b), altitudes and locations (Balandier, 1993a, 1993b); the effect, sometimes uneven according to the literature, of different temperatures or combinations of temperatures (Jacobs *et al*., 2002; Erez *et al*., 1979b; Couvillon and Erez, 1985b; Rageau *et al*., 1998; Naor *et al*., 2003); the relationship of these diverse results with the dormancy status of the plant and the effect of these factors depending on the physiological state of the plant (Weinberger, 1950; Thompson *et al*., 1975; Couvillon and Erez, 1985a; Young, 1992; Tehranifar *et al*., 1998); and the efficiency of shading during different stages of endodormancy and chemical rest breaking agents to compensate insuficcient chill accumulation (Gilreath and Buchanan, 1981a; Buchanan *et al*., 1977; Costa *et al*., 2004; Erez, 1987a). Besides, the recent advances in DNA markers offer plant breeders a rapid and precise alternative approach to conventional selection schemes to improve quantitative traits (Tanksley and Hewitt, 1988). Using detailed molecular linkage maps, quantitative

trait loci (QTLs) affecting important traits can be mapped, genetically evaluated and selected through linked markers. However, no works regarding the identification of QTLs controlling chilling requirements have been reported on apricot species, excepting preliminary results that have been presented recently (Olukolu *et al*., 2008).

Given that few articles have addressed apricot dormancy and the economical importance of this crop in southeast Spain, the objective of this research on apricot was to contribute new evidence that would enhance the general understanding of the complex trait of dormancy. A group of cultivars ranging the chilling requirement of apricot was studied, with the goal of obtaining representative data for the apricot species. In addition, the trials were approached through experimentation in different Mediterranean climatic conditions: Italy, Spain and South Africa.

The Thesis has been divided into different chapters depending on specific objectives addressed. The first chapter contains an approach to the progression of dormancy. This chapter is subdivided into two sections. In the first section, an approach using the single node cuttings method was carried out in three apricot cultivars in a moderately cold area in Spain, aimed at determining the behaviour of the bud itself minimizing external influences. In conclusion, a shallow endodormancy state was observed in both vegetative and reproductive buds in apricot in these climatic conditions. An oscillating pattern in dormancy progression was observed. This pattern was markedly influenced by autumn and early winter temperatures, whereas the onset of dormancy occurred prior to the advent of chilling accumulation. After the differentiation of floral whorls, a higher increase in depth of dormancy was found in vegetative buds than in reproductive buds. A deeper dormancy, but earlier release of dormancy, was found in terminal vegetative buds compared to that in lateral vegetative buds. An effect of suboptimal chilling over acrotony was found.

Regarding the second section of the first chapter, detached shoots were chosen to obtain information closer to the behaviour of complete trees. In this section, bud dormancy progression was quantified in apricot under different winter conditions of the southeast of Spain and the Western Cape in South Africa in order to characterize the bud dormancy behavior of apricot species under a Mediterranean climate. Three areas with different chilling accumulation were chosen in Murcia (Spain) (Campotéjar, Cieza and Barranda) and in South Africa (Ladismith, Villiersdorp and Ceres). Two groups of six apricot cultivars representing the range of flowering time and chilling requirements of the apricot species grown in each country were selected. In Spain, ‘Currot’, ‘Rojo Pasión’, ‘Dorada’, ‘Murciana’, ‘Búlida’ and ‘Orange Red’ were studied, whereas in South Africa,

'Supergold', 'Suapriseven', 'Palsteyn', 'Charisma', 'Canino' and 'Orange Red' were chosen. Mean hourly temperatures were registered in each area, and chill accumulation expressed in Chill Units (CU) (Utah Model), Portions (Dynamic Model) and Hours-below-7 ºC (Chill Hours) was calculated. Bunches of fifteen one-year-old shoots of each cultivar and location were periodically cut from late summer to late winter. Budburst under forcing conditions (25 ºC and continuous illumination) was recorded until five out of fifteen shoots per bundle had sprouted. Scoring was done for both vegetative and reproductive buds. Buds entered into dormancy prior to the advent of chilling accumulation in warm and moderately cold areas. However, in the coldest area in Spain maximum depth of dormancy was achieved after a considerable accumulation (400 CU), even though dormancy induction also occurred in late summer. The decrease in temperatures in late summer, coinciding with the decreasing photoperiod, could trigger the onset of dormancy. The assumption that dormancy induction is related to chilling accumulation seems erroneous according to the results obtained in most of the warm areas and cultivars studied in both countries. An important decrease in the depth of dormancy was achieved by December (June in the southern hemisphere), followed by a pause in the releasing of dormancy, a period of waiting for favorable temperatures to initiate budburst. Reproductive buds showed a similar maximum depth of dormancy, but dormancy release was slightly earlier. The chill accumulated at dormancy release calculated by the three models varied considerably for the same cultivars in different locations and did not reflect the variation of bud dormancy progression, which raises doubts about the utility of the models to determine endodormancy induction and breaking of dormancy. The considerable differences among the different Mediterranean climatic conditions, evidenced by the different temperatures and chill accumulation using three estimation models, could partly explain the differences concerning endodormancy progression from late summer to its release.

The aim of the second chapter was to study the effect of different climatic conditions on the overcoming of dormancy in different apricot cultivars growing in different areas. In the first part of the second chapter, Tuscany, Italy and Murcia, Spain were chosen as locations representative of the Mediterranean climate. Trials were conducted for two consecutive years on the same genotypes: 'Currot', 'San Castrese', 'Goldrich', 'Stark Early Orange' and 'Orange Red'. These genotypes cover the range of chilling requirements in the apricot species in these Mediterranean areas. The chilling requirements (chill units, CU) for breaking of dormancy, heat requirements, flowering date and flowering and fruit set percentages were estimated. Temperatures were recorded and transformed into the corresponding CU by the Utah Model. The winter climatic conditions

determined a dissimilar chill unit accumulation in Tuscany and Murcia as well as an important effect of the year in both areas. While all cultivars with the exception of SEO overcame dormancy, significant differences regarding the chilling requirements of cultivars growing in the different environmental conditions were observed. The variability of results shows that the Utah Model was not completely accurate with regard to establishing the chilling requirements for dormancy release under a Mediterranean climate. Temperature should be analysed together with other climatic factors, such as time of temperature application and combination of cold and heat, in order to improve the chilling requirements assessment.

With regard to part two of the second chapter, CR for breaking of dormancy and heat requirements (HR) for flowering were studied for four successive years in the same location in ten apricot cultivars that spanned the range of flowering times in this species in the southeast of Spain. Additionally, CR were studied for two successive years in five apricot cultivars situated in three different climatic conditions in the Western Cape in South Africa. Hours-below 7 ºC, Dynamic and Utah Models for estimating CR were evaluated and compared, and correlations between CR, HR, days from endodormancy release to flowering and flowering date were established. Marked differences of CR for the same cultivar were observed when a moderately cold area and a very warm area were compared. This is the case of 'Canino' which cultivated in Ladismith (South Africa) registered only 304 CU compared to the 780 CU obtained in Cieza (Spain). The range of CR of the commercial apricot cultivars was significantly different among locations. The variability of CR due to year-by-year differences and location was minimized by the Dynamic Model. However, the Dynamic Model also minimized the year-by-year effect. In 2006-2007, a year when symptoms of insufficient chilling were observed in field conditions, the chill calculated by the Dynamic Model was sufficient to meet the CR of all cultivars. This can partly explain the lower variability found among years in the CR of the different genotypes calculated with Dynamic Model compared to those calculated with the Utah and Hours-below 7 ºC Models. No significant differences in HR were found among cultivars. Our results indicate that HR for flowering are not an intrinsic characteristic of the cultivar in the apricot species. Thus, flowering date was mainly determined by the chilling requirement of the cultivars, being the period when HR accumulated (ΔJD) higher for the low chill cultivars due to the lower temperatures registered earlier in the season.

As detailed in the third chapter, an evaluation of the effect of chilling temperatures and combinations of chill and high temperatures in a daily cycle was conducted. In the first part of this chapter, the efficiency of different temperature cycles with regard to dormancy release in one-year-old shoots of the apricot cultivar 'Palsteyn'was evaluated. Three replications of shoots were collected throughout two consecutive years from adult trees when different amounts of chill were accumulated in field conditions. Different temperature cycles were applied during 60 days in growth chambers (continuous temperature of 5 ºC and temperature cycles of 19h/5h at 5/15 ºC, 5/20 ºC, and 5/25 ºC, as well as the same temperature cycles after pre-treatments of 5 ºC for 30 or 45 days). After the temperature treatments, all shoots were forced at 25 ºC until budburst. The mean time to budburst (in days) of lateral vegetative, terminal vegetative, vegetative, and reproductive buds was evaluated. The efficiency of the different treatments was highly influenced by the state of bud dormancy when shoots were cut. When no chill had been accumulated prior to the cutting date, continuous 5 ºC was the most efficient treatment, followed by 5/15 ºC. However, when shoots had already received a certain chill accumulation in field conditions, 5/25 ºC was always the most efficient treatment, whereas 5/15 ºC and 5/20 ºC became as efficient as 5 ºC. After a pre-treatment of 5 ºC, and when no chill had been accumulated in field conditions, the efficiency of the treatments tended to equalize, especially after 45 days of pre-treatment. A similar trend in all treatments was observed within the different types of bud, even tough reproductive buds had lower CR. Almost no differences in CR were observed between terminal and lateral vegetative buds. The notable efficiency shown by the combination 5/25 ºC compared with that of 5/20 ºC and 5/15 ºC after partial chilling in field conditions could indicate a qualitative more than a quantitative change. Besides, the results showed that high temperatures, such as 25 ºC, can be very efficient for dormancy release when applied in a daily cycle with low temperatures after partial chilling has been accumulated. Chilling was substantially more efficient when applied in field conditions than in the growth chamber.

The second part of Chapter 3 covers the studies conducted to determine the effect of chilling temperatures (1, 4, 7, 10 ºC) on vegetative and reproductive bud dormancy progression in excised shoots of apricot. Temperatures were applied to shoots collected every 15 days from mid-October to mid-January. On these dates, the chill accumulated in the field ranged from 0 to 731 CU (CU), corresponding to 70-90% of the chilling requirement (CR) of the cultivars. Forcing conditions were applied after a 60 day chill treatment on each sampling date, and mean time to bud break (MTB) was established in vegetative (terminal and lateral) and reproductive buds to determine the

dormancy intensity. Similar results were obtained in both lateral and terminal vegetative buds. Maximum depth of dormancy in vegetative buds was achieved by mid-November – when 100 CU had accumulated in field conditions - in all treatments except for 10 ºC in lateral buds. Treatment at 10 ºC seemed to induce maximum dormancy in lateral vegetative buds but also to release bud dormancy earlier thereafter in both terminal and lateral vegetative buds. The other temperature treatments resulted in similar behaviour. Reproductive buds showed a shallower endodormancy and an earlier dormancy release than vegetative buds. Maximum depth of reproductive bud dormancy was achieved by mid-October, when no chill had accumulated in field conditions. A gradual decrease of MTB was observed thereafter. Results show the stage of dormancy has a strong influence on the effect of the different temperatures. A non-linear effect of different temperatures along the dormancy cycle was obtained, especially in the superior range of temperatures traditionally considered to release dormancy. The introduction of this differential effect could help to improve the models to estimate dormancy release.

The fourth chapter aims to evaluate the effects of shading during different periods of endodormancy in an area with relatively warm winters, as well as the effects of a treatment of thidiazuron ([TDZ] N-phenyl-N-1,2,3-thiodiazol-5-il-urea) and winter oil on apricot flowering, fruit set and ripening. The study was carried out during three years that showed marked differences in chilling accumulation. Autumn shading did not affect flowering or harvesting. Regarding harvest date, 2-3 days of precocity relative to the control were achieved by shading during endodormancy. The TDZ+oil treatment increased flowering percentage, made flowering more uniform and brought forward the flowering (by 7 to 14 days) and ripening (3-8 days) dates. Pistil abortion percentage was strongly increased by using TDZ and winter oil when there was low chilling accumulation, which led to a reduced fruit set percentage. However this was the result of the high number of new flowers that blossomed after the TDZ+oil treatment. Nonetheless, an increase in productivity greater than 250% was obtained compared to the control when insufficient chill was accumulated. It can be suggested that TDZ and winter oil trigger a general blossoming of flowers and that fewer defective flowers appear when the winter fully satisfies the chilling requirement, whereas the rate of deceptive flowers increases when not enough chill is accumulated. Shading during late endodormancy induced a 5-day precocity in harvest date in the year with lower chill accumulation. Shading of trees during endodormancy and the TDZ+oil treatment could be suitable for increasing precocity in warm winter climates. Significant year-to-year variation is shown in flowering, pistil abortion, fruit set and fruit/bud percentages. Correlations among these variables are also discussed.

Under conditions of marginal chill accumulation, the distribution of chilling during autumn and winter had an important role in the overcoming of dormancy, flowering and fruit set. Considering the possibilities of growing apricot cultivars in warm conditions such as those studied in South Africa, together with the substantial increase of fruit/buds percentages obtained in a year with insufficient chilling accumulation, shading of trees during endodormancy and the TDZ+oil treatment could be suitable to both increase precocity and improve the productivity of low chill cultivars in warm winter climates. What is more, the application of shading in areas with a long winter photoperiod and a low level of clouds during winter, together with the use of cultivars with low chill requirements, could result in higher treatment efficiency.

Finally, in the fifth chapter, an approach to the identification of QTL associated to flowering time was conducted. In addition, the utilization of megaplex PCR is described. This technique can open new dimensions in the multifunctional use of microsatellites for breeders and genetics, multiplying the efficiency and significantly reducing the cost of the analysis. Using this technique, two linkage maps in apricot have been developed with 37 and 29 SSR markers, respectively. In addition, it was possible to identify in this map one QTL linked to flowering time in the linkage group 5. One SSR loci (AMPA-105) was linked to this trait in apricot. However, a saturation of the genetic map will be necessary to confirm these preliminary results. Besides, further studies with appropriate crosses between parents, which segregate for this trait, will be necessary to apply efficient MAS strategies in the breeding programmes.

II. GENERAL INTRODUCTION

II.I. APRICOT CULTURE

II.I.I. Taxonomic and botanical descriptions, and origin of the apricot species

Apricot (*Prunus armeniaca* Linnaeus syn. *Armeniaca vulgaris* Lammarck) is a species of genus *Prunus* (*Rosaceae* family, subfamily *Prunoideae*) subgenus *Prunus* and section *Armeniaca* that is commercially grown world-wide (Mehlenbacher *et al*., 1991; Faust *et al*., 1998). This section Armeniaca includes eight different species including *P. ansu* and *P. holosterica* (from North areas of China and resistance to frost), *P. mume* (from humid areas of China and resistance to fungus disease) and *P. sibirica* and *P. manshurica* (from North of China and characterized by its resistance to low temperatures) as the most related species to apricot (Bailey and Hough, 1975; Mehlenbacher *et al*., 1991; Lichou and Audubert, 1992).

All these apricot related species are diploids with eight pairs of small, but distinguishable, chromosomes (2n = 16) although some tetraploid mutants have been found (Bailey and Hough 1975; Layne *et al*., 1996). The apricot is a deciduous fruit tree to bloom in spring, with intermediate CR within the *Prunus* species. Apricot fruit, as well as *Prunus* species, is a drupe where the mature, stony endocarp together with the seed forms a propagation unit comparable to a botanical seed with a seed coat (testa) (Figure 1). Apricot trees present a globose bearing from 3 to 7 meters with a pivoting root. This species has hermaphrodite white and pink flowers. The fruit which weights range from 20 to 90 grams is characterized by its sweetness, which is around 13-15 ºBrix, combined with a good level of acidity around 2 grams of malic acid per 100 mL (Mehlenbacher *et al*., 1991; Lichou and Audubert 1992). Apricot is a predominantly self-compatible species although some cultivars present a self-incompatibility of the gametophytic type controlled by a single *locus* with multiple codominant alleles.

Figure 1. Apricot trees, flowers, and fruits.

Apricot is a species originated in China and Central Asia, from Tien Shan to Kashmir (Vavilov, 1951). These regions are also two primary centers of domestication of apricot cultivars together with the Near Eastern (from Iran to Turkey) also described as the secondary centers of origin and diversification (Bailey and Hough, 1975; Faust *et al.*, 1998).

The apricot crop was already known in China in the year 2000 BC. This crop came from Central Asia to Iran, as part of military economic and cultural handle of Alexander the Great in its insights into Turkistan during the fourth century BC. The expansion of the species into Europe (a third center of diversification) seems that occurred at two different times. The Romans knew him through his wars with the Persians in the first century AC. From Rome, it spreads throughout the rest of the continent, reaching Spain between the second and the fourth century AC. Apricot was also introduced into Spain by the Arabs in the seventh century from North Africa, from where it spread to America, South Africa and Australia in the seventeenth century (Figure 2) (Bailey and Hough, 1975; Mehlenbacher *et al*., 1991; Lichou and Audubert, 1992). It has been suggested that some present day cultivars originated directly from the primary centers, while others may have arisen from the hybridization of genotypes from the secondary centers (Bailey and Hough, 1975; Mehlenbacher *et al*., 1991; Lichou and Audubert, 1992; Layne *et al*., 1996; Faust *et al*., 1998).

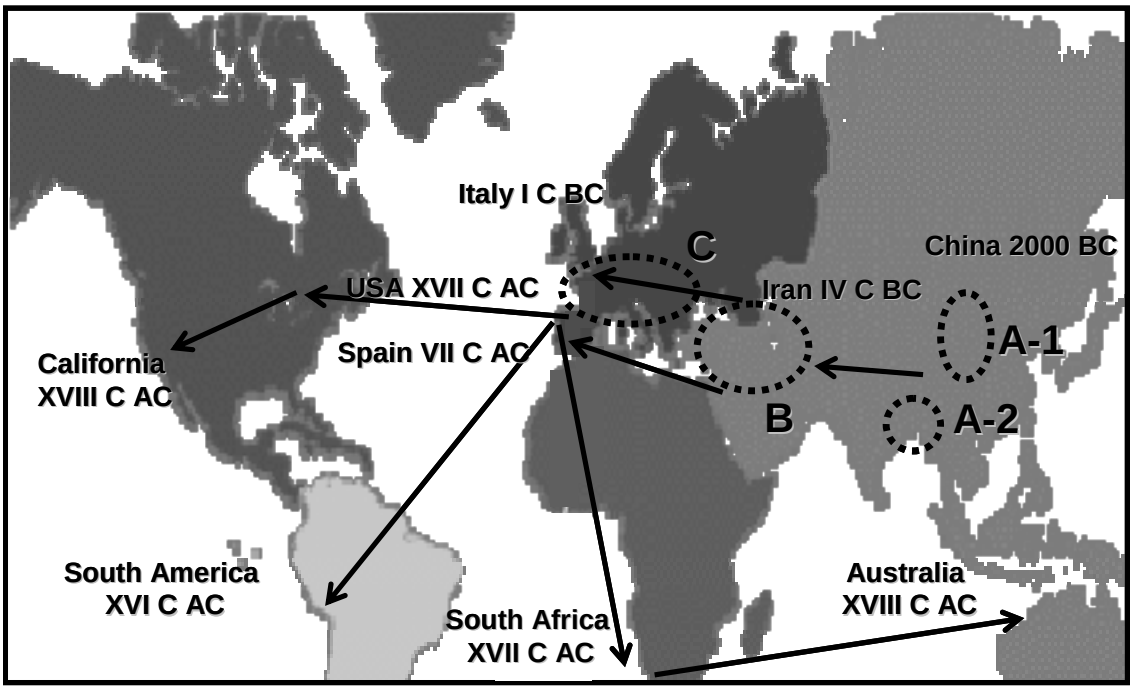


Figure 2. World map showing the origin for apricot species. Primary center of domestication in China (A-1) and Kashmir (A-2), the secondary center for diversification in Iran, the Caucasus and Turkey (B) and the third center of diversification in Europe (C). A schematic representation of the dispersion of the species around the world from China to Turkey and to Europe, and from Spain and Portugal to North and South America, South Africa and Australia is also indicated by arrows.

II.I.II. Genetic resources and economical importance of the crop

Apricot cultivars in the world are classified into four major eco-geographical groups (Kostina, 1964): Central Asian (the most ancient and with higher diversity), Iran-Caucasian (less vigorous trees than the other group), European (the most recent group with less diversity and including cultivars from North and South America, South Africa, Australia or New Zealand), and Dzhungar-Trasylian (from the border of Kazakhstan and China and characterized by its high CR). In general, the Central Asian, the Iran-Caucasian and the and Dzhungar-Trasylian groups show the richest variability, while the youngest European (including cultivars from North and South America, Australia, New Zealand and South Africa) is the group with the least diversity (Bailey and Hough, 1975; Mehlenbacher *et al*., 1991; Badenes *et al*., 1998).

More recently, Layne *et al*. (1996) suggested six main ecogeographical groups (Central Asian, East Chinese, North Chinese, Dzhungar-Zailij, Irano-Caucasian and European), whereas Hagen *et al*. (2002) proposed four cultivar groups (Diversification, Geographically Adaptable,

Continental Europe and Mediterranean Basin) based on the genetic diversity, geographical origins and agronomic characteristics.

Table 1. Main apricot germplasm banks in the World according to FAO 2009.

Country	Research Center	City	Nº accesions
	EUROPE		
Cezch Republic	Mendel University	Lednice	320
France	INRA-Avignon	Avignon	450
Hungary	Enterprise Extensión & Research	Budapest	442
Italy	ISF-Roma	Roma	350
Italy	Instituto de Coltivazione Arborea	Florencia	258
Italy	University of Bologna	Bologna	650
Romania	Fruit Research Institut	Pitesti	685
Slovak Republic	Research Breeding Station	Pietsany	145
Spain	CEBAS-CSIC	Murcia	60
Spain	IMIDA	Murcia	111
Spain	IRTA-Mas Bove	Reus	96
Ukraine	Institut for Fruticulture	Kiev	300
	ASIA		
China	Jilin Academy of Agricultural Sci.	Jilin	140
Japan	National Institut of Agrobiol. Sci.	Tokio	91
Iran	National Plant Gene Bank	Karaj	173
Turkey	Plant genetic resources Institut	Izmir	327
	AMERICA		
Argentina	INTA-Mendoza	Mendoza	52
Canada	Summerland Research Station	Summerland	150
Mexico	INIFAP	Mexico	165
USA	National Germplasm Repository	Fresno	165
	AFRICA		
Morocco	Station de Recherche sur Arbres	Rabat	68
South Africa	Fruit Technology Research Inst.	Stellenbosch	44
	OCEANIA		
Australia	Loxton Research Center	Loxton	615

Cultivated apricot is among the most polymorphic of all cultivated fruit and nut species (Martínez-Gómez *et al*., 2003a). Despite the plasticity expressed by the apricot species (grown in places as diverse as South Africa or Canada) and its great diversity, more than 1,300 different cultivars grow in Europe and around 10,000 accessions are described in the 225 Germplasm Banks of the world according to the Plant Genetic Resources for Food and Agriculture Commission of FAO (Table 1) (FAO 2009), there is a great specificity in the adaptation of the cultivars to each area. In addition around 80% of global production is based on fewer than 30 cultivars (Bailey and Hough, 1975; Mehlenbacher *et al*., 1991; Lichou and Audubert, 1992).

Apricot species is grown worldwide, but most production is concentrated in the Mediterranean area. Table 2 shows the main producing countries in the world during the period 2002-2006. Worldwide annual apricot production exceeds 3 million metric tons, being Turkey the main producing country (with around 500 thousand metric tons) followed by Iran (around 258 thousand metric tons). The second major apricot-producing area includes the European countries bordering the Mediterranean Sea, mainly Italy (the third leading country with 195 thousand metric tons on average), France (163 thousand metric tons), Spain (134 thousand metric tons) Algeria (116 thousand metric tons), Morocco (100 thousand metric tons) and in less degree Greece (79 thousand metric tons). Important producing countries exist in central and southwestern Asia including Iran (the second leading country after Turkey with 258 thousand metric tons in 2007), Pakistan (188 thousand metric tons in 2007), Uzbequistan (149 thousand metric tons) and China (80 thousand metric tons). Finally, we can include some of the traditional countries producing apricots with a less production in comparison with the emergent countries such as USA, South Africa, Japan or Syria (Table 2).

Regarding Spanish apricot production, it deserves special attention that the production of 'Región de Murcia' represents more than 65% of the total Spanish production (Table 3). Other important producing regions are 'C. Valenciana' and 'Castilla la Mancha' (12% and 8% respectively of total production. In Spain, the cultivar structure is characterized by the existence of a reduced number of cultivars cultivated in considerable surfaces. In this sense, 10 cultivars are producing 80% of total production. Even though there are also many cultivars which are cultivated only locally. 'Búlida' is by far the most important Spanish cultivar producing around 65% of total production, followed in order of importance by 'Canino'. They represent ca. 60% of the total surface cultivated of apricot in Spain. Other cultivars that are worthwhile mentioning are 'Moniqui', 'Real Fino', 'Paviot' and 'Ginesta' (Egea *et al*., 1994). In 'Región de Murcia', the most important cultivars have traditionally been 'Búlida', 'Pepito del Rubio', 'Mauricio', 'Currot' 'Real Fino' and 'Moniquí'. However, the cultivar distribution varies geographically. Thus, in 'Comunidad Valenciana' the most important cultivars are 'Canino', 'Palau', 'Currot', 'Ginesta' and 'Galta Rotja', whereas in Aragon 'Paviot' and 'Moniquí' are the most extended cultivars.

Table 2. Apricot production in metric tons (t) in the world (FAO 2002-2007).

Country	2002	2003	2004	2005	2006	Average 2002-2006
Turkey	352,000	499,000	350,000	860,000	460,182	504,236
Iran	284,000	285,000	166,373	275,578	280,000	258,190
Italy	200,110	108,320	213,425	232,882	221,994	195,346
Pakistan	129,700	210,882	214,800	197,239	189,533	188,431
France	169,418	123,814	166,136	176,950	179,568	163,177
Uzbekistán	97,000	82,000	162,000	170,000	235,637	149,327
Spain	127,549	143,840	121,486	137,167	141,400	134,288
Algeria	73,733	106,469	87,991	145,097	167,017	116,061
Japan	112,600	88,300	113,600	123,000	119,800	111,460
Morocco	86,200	97,950	85,000	103,600	129,440	100,438
Syria	100,902	104,900	75,700	65,513	85,000	86,403
China	72,218	81,874	86,509	77,937	83,001	80,308
Greece	70,272	59,854	89,,538	84,135	93,709	79,502
Egypt	103,070	70,424	72,523	73,000	74,000	78,603
USA	81,647	88,541	91,716	74,070	40,530	75,301
Ukraine	68,500	110,500	99,300	94,200	28,000	80,100
South Africa	56,509	50069	97,774	43,741	83,639	66,346
World (Total)	2,670,234	2,887,956	2,861,184	3,498,820	3,221,416	3,027,922

Apart from the traditional cultivars, imported cultivars had been introduced in the last years. Even though they do not represent a vast extension nowadays, it could be cited ‘Goldrich’, ‘Orange Red’, ‘Goldbar’ and ‘Aurora’. In some areas the unsuitability of the climate for the cultivar entails an inadequate chill satisfaction, which is associated to erratic production. More recently, since 2004, a reconversion of traditional cultivars, especially ‘Búlida’, is taking place in Spain, and especially in Murcia. The new releases of the breeding-program of CEBAS-CSIC ‘Rojo Pasión’ (Egea *et al*., 2004b), ‘Murciana’ (Egea *et al*., 2005a) and ‘Dorada’ (Egea *et al*., 2005b), among others, represent a good alternative considering their productivity, fruit quality and appropriate CR.

Table 3. Mean area in hectares (ha) and production in metric tons (t) of apricot in Spain from 2003 to 2006 (MAPA).

Region	Surface		Production	
	ha	%	t	%
R. Murcia	10536	54.07	91865	65.69
C. Valenciana	4359	22.37	16579	11.86
Castilla la Mancha	2125	10.91	11893	8.50
Aragón	856	4.39	9308	6.66
Cataluña	308	1.58	2867	2.05
Baleares	771	3.96	2197	1.57
Others	510	2.62	5008	3.58
Spain	19485	100	139841	100

II.I.III. Crop difficulties

Apricot is one of the most important and desirable temperate tree fruits, with total world production reaching more than 3 million metric tons (Table 2). Although apricots are grown principally in temperate and subtropical areas worldwide, mainly in Mediterranean climates, apricot culture is also developed in places as diverse as Saharan oasis or Central Asian deserts as well as in very cold places as Siberia or Himalaya. However, despite the plasticity expressed by the apricot species, the main apricot cultivars show a low adaptability to different climate conditions that those where they are established.

In apricot species, erratic yields are frequent (Mechlenbacher *et al.*, 1991; Egea and Burgos, 1998). There are many genotype-dependent factors related with the floral biology, that influenced fruit set and, consequently, productivity such as flower bud production, flower bud drop, flowering time, ovule development stage at anthesis, pollen germination, height difference between the stigma and the superior plane of the anthers, aborted pistils and the autogamy level. Many of these factors e.g. flower bud production, flower bud drop or aborted pistils are closely related to the above cited lack of adaptability of apricot cultivars. However, the main factor associated to the cultivar adaptation to certain climate conditions are the CR for breaking of dormancy. Moreover, CR satisfaction influence greatly the expression of others floral biology factors.

The knowledge of the chilling requirement of a cultivar is necessary for cultivating apricot cultivars in the most suitable areas. In this way, if a cultivar is established in an area where its CR are not satisfied adequately, the vegetative and productive behaviour of the cultivar will be affected negatively (Coville, 1920; Weldon, 1934; Black, 1952; Samish, 1954; Samish and Lavee, 1982). On the contrary, in the case of cultivars with low CR (i.e. early-flowering cultivars) growing in cold-

winter areas, blooming happens too early because CR are quickly satisfied, and LT can induce an important loss of yield by frost (Scorza and Okie, 1990). Nowadays, a dynamic varietal renewal process is taken place on apricot species, and many new cultivars and foreign cultivars are being grown in new areas different from their origin. In addition, many of these new cropping areas are characterized by very mild climates. For this reason, the lack of adaptability due to inadequate satisfaction of CR is a very important problem which must be taken into consideration to a large extent.

The scarce data available for the apricot species, which is the least-studied temperate fruit with regard to CR, as well as the lack of knowledge regarding the mechanisms and factors controlling the dormancy process, do necessary the development of studies aimed at these subjects.

II.II. DORMANCY IN TEMPERATE FRUITS

II.II.I. Background

Dormancy in temperate-zone deciduous fruit trees is a phase of development that allows the trees to survive unfavourable conditions during the winter (Faust *et al*., 1997). Diverse factors can lead to meristem inactivity. Unfavorable environmental conditions, for example, generally determine this inactivity. That is the case of winter temperatures, which under a certain threshold impede the processes that lead to growth, preventing any indication of activity at an external level. Nonetheless, it can not be said, as it will be discussed later on, that no internal physiological activity is developing. This activity affects the future potential development and growth. Likewise, processes that inhibit the development of other vegetative meristems are well known. These processes are catalogued as apical dominance or inhibition such as that imposed by the leaves over the axillary buds. Globally, the processes are known as correlative inhibition. This factor, which determines the inhibition in the two cases previously mentioned, is external and usually distant to the inhibited meristem. These inhibitions generally operate in all woody plants, independent of the origin or adaptation areas, and frequently have substantial implications in the morphogenetic factors that determine the tree structure (Champagnat, 1983). At the beginning of the 20th century, Coville (1920) pointed out an unusual phenomenon that affected equally native shrubs and trees from cold northern areas, which consisted in, contrarily to the accepted idea that the trees entered into dormancy through the progressive influence of low temperatures, trees kept in greenhouse conditions for breeding purposes during winter stopped their growth in autumn, shed their leaves and entered into dormancy. Equally unusual, was the fact that the plants kept in greenhouse conditions were unable to flower in spring. This highlighted a type of growth inhibition characteristic of the woody species specifically grown in temperate zones. This type of inhibition is a development phase of plants, whose habitat is characterized by well defined seasons with cold winters, bestows endurance to unfavorable winter conditions and, particularly, a delay in the reproductive processes, i.e. flowering and fruit set, allowing the survival of the species through the preservation of its descendants.

Contrary to the characteristics of the apical dominance and the dormancy associated with the unfavorable environmental conditions, this inhibition seemed to arise from the inhibited structure

itself. That is to say, it was an endogenous factor of the meristem, installed in both vegetative and reproductive buds. Chouard (1956) attempted to classify the different causes of the growth inhibition observed in woody temperate species. This classification created the following groups: quiescence, the inhibition determined by the environmental conditions; correlative inhibition, the inhibition mediated through dominance between different parts of the plant and dormancy, as the inhibition whose control resides in the inhibited structure itself. Subsequently, Saure (1985) defined these inhibitions as imposed-, pre- and true-dormancy. Finally, and this is the most used nowadays, Lang *et al*., (1987), classified the inhibitions as ecodormancy, which is found in late winter and spring and is imposed by temperatures unfavorable to growth; paradormancy, equivalent to the correlative inhibition or apical dominance; and endodormancy, which is the deep dormancy or winter dormancy. The latter is, as we have previously mentioned, the genuine dormancy that characterizes the woody plants in temperate zones, and has been the objective of many studies that have shown the enormous complexity of this phenomenon.

After an initial stage when the adaptation problems associated with partial endodormancy release, which generate problems similar to those described by (Coville, 1920), were attributed to diverse causes (Weldon, 1934), they were associated progressively with the climatic conditions. As Coville (1920) indicated, a relationship was established between dormancy release and the action of low temperatures. Despite the initial disagreement regarding the establishment of the temperature threshold for the dormancy breaking, 45 ºF (7.2 ºC) was finally adopted (Weldon 1934; Samish, 1954; Vegis, 1964). By approximation, 7 ºC was adopted as the threshold for the useful temperatures, with respect to overcoming of endodormancy. Concomitantly, the concept of chilling requirement (CR) needed to overcome dormancy or to flower was established. This parameter was considered to be cultivar-specific and was interesting because it allowed knowing beforehand the possibility of the successful adaptation of a cultivar in a pre-determined environment.

However, it was soon perceived that this parameter, expressed as Hours below 7 ºC, tended to be very variable and dependant on the year and location. This questioned its consistency and suitability for measuring the quantity of cold required to overcome dormancy over a particular period. This ascertainment triggered the development of new approaches to solve this problem. Numerous have been the proposals in this sense, which indicates the notable difficulties associated with delimiting the phenomenon (Richardson *et al*., 1974; Gilreath and Buchanan, 1981b; Shaltout and Unrath, 1983; Erez and Couvillon, 1987; Fishman *et al*., 1987a, 1987b; Cesaraccio, 2004).

Common elements among these proposals are the consideration of temperatures noticeably higher than 7 ºC as useful for overcoming dormancy and the negative effect on the chill accumulated due to temperatures above a certain threshold, especially when combined with low temperatures in a daily cycle (Erez and Lavee, 1971; Overcash and Campbell, 1955; Erez *et al*., 1979a, 1979b; Couvillon and Erez, 1985b). A limited period between the application of low and high temperatures is required for the negative effect of high temperatures because, when this period increases, the negative effect disappear as the action of the low temperature has actuated in a permanent way (Erez *et al*., 1979b). Another important contribution in relation to the effect of high temperatures on dormancy progression was the establishment of the synergic effect of moderate temperatures (13-15 ºC), when combined with low temperatures in daily cycles. Moderate temperatures do not have a positive effect on dormancy release by themselves, but when they occur after cold treatment, augment substantially its positive effect (Guerriero *et al*., 1985b; Erez and Couvillon, 1987). As can be seen, several corrections to the Utah Model (Richardson *et al*., 1974), one of the oldest and most used, have been presented, either because of the discovery of the effect of temperatures originally not considered or because their application in species different to peach or in areas with climatic conditions different to those of Utah led to dormancy progressions divergent to those initially postulated by the model (Shaltout and Unrath 1983; Linsley-Noakes and Allan, 1994). Recent studies in an already-cited species, apple, have shown fairly-disparate results compared to the previous reports. Thus, temperatures close to 0 ºC were considered very efficient with regard to overcoming of dormancy, whereas in previous models, a very limited efficiency had been assigned (Naor *et al*., 2003).

In contrast to what could be denominated ‘Classic school’, in terms of research into dormancy in fruit trees, and to which belong previously mentioned researchers who have developed the different models described and who consider that the induction, control and dormancy exit are apparently regulated by a balance of inhibitors and promoters of growth (Amen, 1968); we can find the ‘French school’, which considers dormancy as a morphogenetic factor under the control of several correlative influences. According to this, a gradual transition is observed from the correlative inhibition of the bud growth to the deep dormancy localized in the bud itself (Champagnat, 1983). Crabbé (1994) concluded that the classic theory of the hormonal control of dormancy had failed, which does not exclude that hormones have a key role under certain circumstances. Thus, certain biochemical markers could indicate the relative level of dormancy in organs, tissues or even cells. The metabolism of nucleic acids and the permeability of the cell

membrane could be two examples. The 'nucleotide test' determines the capacity of the tissues to transform adenilic nucleotides (ATPs) into non-adenilic nucleotides (NTPs). The dormant tissues have a diminished ability to carry out this conversion and the energetic compounds needed for the normal development of metabolic processes are blocked (Lavarenne *et al*., 1982). The second biochemical test measures the permeability of the cell membrane to a weak acid 5,5 dimethyl-oxazolidine-2,4-dione (DMO). The non-dissociated form of the acid passes through the cell membrane and is dissociated, being the degree of dissociation variable with the intracellular pH. Gendraud and Laflenuriel (1983) showed that the values of DMO concentrations inside and outside the cell were dependent of the state of dormancy in the different tissues and the pH. Tamura *et al*. (1998) suggested that the 19-kDa protein may be a suitable marker for measuring the degree of bud dormancy in Japanese pear (*Pyrus pyrifolia* Nakai).

In general, the studies about dormancy in apricot (*Prunus armeniaca* L.) are scarce, and the results have partially questioned the utility of the methods frequently used in other species. These studies suggest the use of biological indicators and the consideration of the specific climatic characteristics of each area (Hatch and Walker, 1969; García *et al*., 1999). Apricot is a species widely distributed in Mediterranean areas. In Europe, almost the totality of the apricot production is located under Mediterranean climate. However, the Utah Model, which is the most used method, was developed in Utah, where long and cold winters are frequent. The Dynamic Model (Fishman *et al*., 1987a, 1987b) was developed in Israel and its aims were to include new advances in the knowledge of dormancy (the effect of temperature cycles, fixed accumulation, etc), and to solve the inaccuracy presented by the Utah Model in warm-winter areas. Nonetheless, there was still an open question regarding which of these models would be more adequate for intermediate areas, basically represented by climates generally denominated as Mediterranean. The variability in the dormancy requirements, associated to the year-by-year variation and the diverse cultivar response conditioned by the contrasting dormancy intensity of different cultivars (Saure, 1985), demand studies which include a high number of cultivars and annual repetitions to obtain solid conclusions about the suitability of the model and, in a posterior phase and with the inclusion of the latest advances, render possible the formulation of new, more complex and accurate models.

II.II.II. Dormancy induction

Understanding the evolution along the annual cycle of the general process of dormancy, and the plant responses to environmental conditions in each stage, is of interest from both scientific and

practical viewpoints. Induction of dormancy takes place at an early phase of the cycle. Thus, when young, non-lignified shoots are extending in spring, the suppression of the terminal buds triggers, in a short period of time, the development of apparently-dormant, distal axillary buds (Hillman, 1984). Without this action, the axillary buds would NOT remain dormant until the end of the vegetative cycle. The dormancy of the axillary buds was imposed remotely by an active terminal bud. This correlative inhibition is denominated 'apical dominance' and it seems to be imposed through the preferential consumption by the main apex of water, nutrients (such as mineral salts and glucides), and growth regulators such as cytokinins, which mainly have a root origin. In this inhibitory process, the auxins, which are abundantly produced in young leaves of the terminal bud, are responsible of this preferred acropetal circulation and thus of the relative impermeability of the tissues that join the base of the axilary buds to the vascular tissues of the shoot (Champagnat, 1992, Crabbé and Barnola, 1996). As the season progresses and the terminal bud reduces or even ceases its growth, neither decapitation nor defoliation are effective at resuming growth in the axillary buds. The bearing axis itself now plays the inhibitory role in bud development (Arias and Crabbé, 1975). In fact, when limbs of a shoot are removed and each axillary bud is cut with several centimetres of the adjacent internode and is situated in a growth chamber with a high relative humidity and temperature; growth will be visible within a few days. This procedure has been called 'single node cutting' and has been widely used to assess endodormancy (Guerriero *et al.*, 1985a, Balandier et al, 1993 a; Falusi and Calamasi, 1997; Bonhomme et al, 1999). On the other hand, if potted trees are defoliated and forced under the same forcing conditions, no growth will appear. These results can be obtained in numerous species during July and part of August. From July to October, the behaviour of 'single node cuttings' in the same conditions shows an acute increase in the time until the resumption of growth and a higher proportion of them shows a complete dormancy. It could be though that either the bud itself is dormant or the short shoot segment impedes the growth. The answer to this question is not easy as an isolated bud hardly burst. The 'in vitro' culture of isolated buds implies important lesions that modify the meristem physiology and also interfere with the growth regulators, which should be added to the culture medium (Champagnat, 1992). In general, the terminal meristems, which exert but do not suffer the apical dominance, behave in a different way from laterals. Terminal meristems are the latest to stop growth and form buds. This involves a different dormancy progression: terminal buds achieve a deeper dormancy but are released from dormancy more easily than lateral buds (Williams *et al*., 1978; Mauget and Rageau, 1988; Champagnat, 1992).

It is also worth mentioning that the response will be strongly influenced by the species or cultivar studied. Thus, apical dominance, and thence the inhibition of lateral buds, is greater in pome fruits and in cherry than in peach, plum or apricot. In apple, Lespinasse and Delort (1986) distinguished the spur types, with strong dominance for cultivars such as 'Granny Smith'. These characteristics acquire special relevance in warm climates. It is worth noting on a tree, or even on the same 1-year-old shoot, buds can have a different reaction amidst this chain of influences. Each bud has its own network of correlative inhibitions and reacts accordingly to them. Thus, the progression and depth of dormancy have morphogenetic consequences. In general, the depth of dormancy in a current shoot in autumn decreases from the apex to the basal part, showing a basitonic gradient in the growth ability of the buds (Champagnat *et al*., 1975). This heterogeneity of circumstances originates differences in the expression of dormancy when complete trees, shoots or single node cuttings are used to determine the dormancy progression.

II.II.III. Influence of diverse factors on the onset and depth of dormancy

If dormancy is a plant mechanism to survive the adverse conditions of winter in temperate and cold climates, the onset of dormancy before the arrival of extreme temperatures is essential. This survival depends on both the injuries in the tree itself and the interference in the reproductive processes that preserve the future of the species studied. To make this possible, it is necessary to activate the physiological process that leads to dormancy, through environmental signals with a seasonal origin. The trendsetting discovery of Garner and Allard (1923), who observed the key role of the short photoperiod in the induction of dormancy, has been documented thoroughly in a wide variety of woody plants in temperate climates (Kramer, 1936; Wareing, 1956; Nitsch, 1957; Heide, 1974; Heide and Prestud, 2005; Heide, 2008). In species such as *Acer pseudoplantanus* L., and *Betula pudescens* J.F. Ehrh. (Kawase, 1961), the exposure of plants to short photoperiods halted growth whereas the plants exposed to long photoperiods showed a continuous growth. An important exception to the control of growth through short photoperiods in woody plants was observed by Garner and Allard (1923) in apple (*Malus pumila* Mill.). This exception was confirmed and extended to other genera of the *Rosaceae* family (Nitsch, 1957). Considering that no other signal was identified as responsible factor to induce dormancy in these plants, it was considered that the ceasing of growth was regulated totally by internal factors (Wareing, 1956; Battey, 2000). This had also been the conclusion extracted from the experiments carried out by Coville (1920) and Samish (1954), where dormancy was achieved in plants kept during autumn at warm temperatures and with

artificial light. Nevertheless, it has been demonstrated that, under determined temperature conditions, short days can trigger the dormancy induction (Nitsch, 1957; Heide, 2008) - with a link between short days and dormancy induction. Besides, recent data indicate that low temperatures control growth cessation and that dormancy induction in pear and apple is independent of the photoperiod (Heide, 2008).

There was a time when growth inhibitors where seen as causal agents of dormancy. Abcisic acid (ABA), which was denominated 'abscisin II', 'dormin' or 'dormancy inductor' (Addicot, 1983), was considered the most important growth inhibitor. ABA is effective in delaying budburst in apple buds (Dutcher and Powell, 1972); if inyected, prevent budbreak in sour cherry (*Prunus cerasus*, L.) (Mielke and Dennis, 1978) and in peach (*Prunus persica* L. Batsch). Other authors have questioned the action of ABA as a dormancy inductor. Mielke and Dennis, (1978) indicated that though the defoliation of sour cherry in autumn prevented the increment of ABA, the dormancy intensity did not change. Saure (1985), in his extensive review, declared that ABA *per se* does not regulate budburst. Trewavas and Jones (1991) stated that several environmental signals act through a change in the biosynthesis of ABA. In the case of *Acer pseudoplatanus* L., it was established that both short days and ABA induce dormancy in this species. Moreover, there are conditions, such as drought, that causes an increment of ABA and induces dormancy. ABA, through its action on dehydrins or membrane permeability, could have an effect on dormancy progression (Jacobsen and Shaw, 1989; McAnish *et al*., 1991). In trees, levels of dehydrins have been associated with cold hardiness, winter dormancy and content/state of water in the tissue (Arora *et al*., 1997; Erez *et al*., 1998; Karlson *et al*., 2003; Kalberer *et al*., 2006). Production of dehydrins (the Group D-11 LEA (late embryogenesis-abundant) proteins) have been found in many plants species (Close, 1996). Production of dehydrins is induced by environmental stresses, such as low temperature or dehydration, or by treatment with ABA (Close, 1996). Dehydrins alter the thermodynamic interactions between macromolecules and water via solute exclusion or direct binding (Close *et al*., 1993a, 1993b). Thus, they may provide stability to macromolecules, such as nucleic acids and proteins, during desiccation by preventing denaturation or ice crystal formation. However, there is no direct evidence to support this hypothesis (Close 1996). Dehydrin proteins are induced in response to chilling accumulation and cold acclimation in flower buds of blueberry (Muthalif and Rowland, 1994). Transcript accumulation of a peach bark dehydrin is also induced by cold acclimation (Arora *et al*., 1992; Artlip *et al*., 1997). Yamane *et al*. (2006), working in *Prunus mume* (Siebold & Zucc.) supported the findings of earlier work comparing dehydrin expression in the bark

tissue of the evergreen and deciduous peach genotypes (Arora *et al*., 1992; Artlip *et al*., 1997), and suggested that the role of dehydrin during the dormant season is common to all *Prunus* species. Interestingly, the accumulation pattern of dehydrins in mid-season 'Nanko' and early-flowering 'Ellching' cultivars was different. The maximum accumulation of dehydrin was achieved on December 17 in 'Nanko' and November 12 in 'Ellching' (Yamane *et al*., 2006).

However, Yakovlev *et al*. (2008) found in Norway spruce (*Betula pubescens* Ehrh.) that the expression of some of the dehydrin genes (including PaDhn1, PaDhn4.6, PaDhn5, PaDhn6, PaDhn2, and PaDhn3) decreased gradually when approaching flushing. These changes interrelate both with decreasing hardiness and with ontogenetic development leading to flushing. Thus, the observed changes can hardly be related to winter dormancy as the chilling requirement for bud burst in Norway spruce is already fulfilled in winter (Yakovlev *et al*., 2008).

The role of temperature in the dormancy induction has been introduced progressively. To begin with, it is convenient to highlight that dormancy depth is especially influenced by the genotype, and the characteristics of the dormancy for each genotype are influenced strongly by the conditions that precede its establishment (Champagnat, 1983). For instance, flower buds of cultivars with low CR do not enter into dormancy or enter very progressively when the autumn temperatures are elevated. This could lead to autumn flowering, sometimes very teeming, when budbreak is stimulated by a precocious loss of leaves, by irrigation after a dry period, or by the application of chemical agents that break the shallow dormancy. The response described is very unfavourable as the flowers, as the flowers can complete fruit set but the fruits cannot reach commercial quality in those dates (Erez, 2000). A considerable number of studies indicate that low temperatures can intensify dormancy in autumn (Hatch and Walker, 1969; Walser *et al*., 1981; Kobayashi *et al*., 1983; Ben Ismail, 1989). Hatch and Walker (1969) observed an increment in dormancy, measured through the gibberellic acid (GA_3) concentration needed to stimulate the expansion of vegetative buds in autumn, although an accumulation of CU took place. Cook *et al*. (2005), showed that a pre-treatment with temperatures slightly lower than 0 ºC clearly deepened dormancy in one-year-shoots of 'Granny Smith' apple cultivar; whereas warm temperatures delayed but did not impede the onset of dormancy and even caused an increment in the depth of dormancy of trees subsequently exposed to low temperatures. Ben Ismail (1989), working with the 'single node cuttings' technique, showed that the growth of vegetative buds in apple was inhibited when they were exposed to low temperatures at the beginning of October. The response was dependant on the time of sampling and

the period of exposure to low temperatures. If chilling temperatures intensify and reduce dormancy in different stages of the annual cycle, the moment when chilling temperatures are applied results critical (Dennis, 1994).

As the manifestation of dormancy begins at the end of summer, temperatures of this stage could be implicated in the intensity of dormancy and, consequently, in the time of budburst.

Jonkers (1979) demonstrated that there was a tendency for dormancy to deepen when the temperature at which the buds had been formed was higher, and suggested that the delay of foliation was not only a result of the marginal accumulation of cold in winter, but also was influenced by the high temperatures of the preceding summer. Heide (2003) showed that high temperatures applied during dormancy induction through short days significantly increased the CR for dormancy breaking in *Alnus glutinosa* (L.) Moench. Moreover, September temperatures explained 20% of the year-by-year variation of budburst times.

Notwithstanding, Chuine and Cour (1999) documented that even though summer temperatures could be related to the dormancy intensity, they do not significantly affect the date of budburst. Thus, budburst date is determined mainly by the thermal conditions from quiescence (ecodormancy) to budburst and secondarily by the temperatures during endodormancy. These results coincide with those found by Mauget (1977, 1980). Westergaard and Eriksen (1997), found a close relationship between autumn temperatures and dormancy intensity: high temperatures combined with short days induced the deepest dormancy. Junttila *et al*. (2003) showed that northern ecotypes of birches (*Betula pubescens* and *Betula pendula*) exhibited the fastest development of dormancy at intermediate temperatures of 15-18ºC, whereas dormancy development was delayed at lower and higher temperatures. Heide (2003) demonstrated that a high temperature during dormancy induction by short days significantly delayed bud burst in *Alnus glutinosa* and *Betula* species growing under controlled conditions as well as under field conditions.

Altitude has been shown to influence the depth of dormancy that a particular genotype can reach. Thus, in the same latitude, dormancy was deeper in walnut trees grown at 400 m than in those at 800 (Mauget, 1977). The effect of latitude has also been shown. Champagnat (1983), working on 'Golden Delicious' apple, showed that the depth of dormancy, from October to January, was higher in Clermont-Ferrand than in Tunisia. Balandier *et al*. (1993a, 1993b), working on peach, showed extraordinarily-different CR and time to budbreak under forcing conditions for the same cultivars situated in different locations and altitudes in Clermont-Ferrand and in Reunion island.

Conifer populations localized in coastal areas generally have lower CR than populations located in inland areas, suggesting a lower depth of dormancy (Campbell, 1974; Sorensen, 1983).

Moreover, latitude has been related to photoperiod, temperature and light quality requirements. Li *et al*. (2003a) showed that the response to photoperiod in birch (*Betula pendula* Roth) varied with latitudinal origin of the ecotype: northern ecotypes (67º N) had a greater sensitivity to photoperiods and a longer critical photoperiod, i.e. they showed earlier growth cessation and cold acclimation than the southern ones (58ºN) when exposed to shortening photoperiods. Li *et al. (*2005), showed that the response to low temperatures in birch varied with latitudinal origin of the ecotype, and the northern ecotype (67º N) having a more rapid cold acclimation and development of freezing tolerance than the southern ones (58ºN), resulting in more rapid cold acclimation and development of freezing tolerance in actively-growing plants, and an earlier enhancement of freezing tolerance and bud dormancy release in the dormant plants. Finally, Olsen (2006) stated in his review that in most temperate- and boreal-zone woody plants the critical night length (photoperiod) increases clinally with increasing latitude or altitude of origin of the population, and it is considered to be an adaption to the length of the growing season (Olsen, 2003; Olsen et al., 2004). Besides, a clinal variation in light quality requirements has been demonstrated also in woody species, at least for northern populations. Earlier studies of *Picea*, *Pinus*, *Betula* and *Salix*, using light sources enriched in far-red (FR) or red (R) light, have indicated a clinal variation the FR light required to maintain growth; the demand increased with increasing northern latitude of origin (Håbjørg, 1972; Junttila and Kaurin, 1985; Clapham *et al*., 1998, 2002).

Apart from the factors mentioned, the depth of dormancy is also determined by diverse factors inside the genotype itself. Thus, the vigour of the tree can have a noticeable influence. The more vigorous the tree, or even the shoot within the tree, the greater its depth of dormancy (Saure, 1985). Thereby, culture methods that tend to debilitate the vigour are frequent in those growing areas where CR is not properly satisfied. Otherwise, young trees show, in general, higher CR than adult trees of the same cultivar. Culture methods such as the application of a growth retardant restrain growth, enabling dormancy breaking (reviewed in Erez, 2000). Further, dwarfing rootstocks or with a low affinity with the cultivar can favour an earlier flowering and budburst. Besides, it has been suggested that high vegetative vigour, induced by high temperatures during the growing season, can reduce the intensity of floral initiation (Tromp, 1976, 1980), which can exert an important influence over productivity.

Bud break is achieved earlier in horizontally oriented branches than in upright ones. This effect definitely arises from the change in the balance of hormones in the buds and from the reduction of vigour produced if bending is performed during the growing period (Erez, 2000). Likewise, branch bending seems to decrease the auxins transport altering the intensity of dormancy (Crabbé, 1984). The time and type of pruning, which have and important effect over the vigour of the tree and the reinforcement of the apical dominance, influence the intensity of dormancy: the latter the pruning is done, the lower the effect over the dormancy breaking of lateral vegetative buds would be.

Some results in the literature indicate an effect of the time of defoliation on the depth of dormancy. Spiers and Draper (1974) found that defoliation notably reduced the dormancy of vegetative buds in the blueberry (*Vaccinium virgatum*) cultivar 'Tifblue de Rabbiteye'. Moreover, Walser *et al*. (1981) found that the time of leaf fall in autumn was related with the duration and intensity of the dormancy of terminal buds of the peach cultivar 'Gleason Elberta'. A possible cause of this could be the movement, from the leaves to the buds, of compounds that control the depth of dormancy. Chemical defoliation at the beginning of autumn can be beneficial for dormancy breaking if it is not performed so early as to cause a suppression of dormancy (Erez, 1982).

As has been shown, numerous are the factors that can affect the depth of dormancy of a genotype. So, this parameter, which has frequently been considered as a constant characteristic of the genotype, is dependant on the variations introduced by different variables. This, together with the higher or lower efficiency of the models used to determine the CR, would explain the high inter-annual and inter-locality variability usually observed.

II.II.IV. Endodormancy breaking

From the end of summer on, and throughout autumn, endodormancy is being installed and, in most parts with temperate climate, the maximum intensity of this parameter seems to be reached at the end of October or during November, depending on the cultivars, species and climatic conditions of the autumn (Brown, 1957; Guerriero, 1976; Fuchigami *et al*., 1977; Hauagge and Cumming, 1991b; Naor, 2003). These same authors frequently have associated an external signal, the defoliation, with the critical point when the effect of temperatures over dormancy shows a crucial variation. Before the onset of maximum depth of dormancy, the first cold days of autumn produce an intensification effect on the rest, whereas, from this point on, chilling temperatures seem

to be the critical factor for overcoming dormancy (Chandler and Tufts, 1934, Samish, 1954; Erez and Lavee, 1971; Walser *et al*., 1981). Thus, it is interesting to determine the point of inflexion in the curve of dormancy progression - to be able to establish precisely the CR of the different fruit cultivars. In this regard, some proposals should be highlighted in terms of the point from when chilling temperatures should be considered. Among others, we can make reference to the Utah Model (Richardson, 1974) - which indicates that the accumulation begins in the autumn, the day after the maximum negative accumulation. Other proposals point out that the onset of chill accumulation should begin when 50% of defoliation has taken place (Guerriero, 2002), or when the chill accumulation is produced in a persistent way (Richardson *et al*., 1974; Erez *et al*., 1979b; Guerriero *et al*., 2002; Ruiz *et al*., 2007). Knowledge of the CR of a cultivar has a practical and economic significance for the control, maintenance and production of woody plants (Fennell, 1999), as the value of this parameter partly decides the adequate areas in which a cultivar can be grown. To determine the CR, it is necessary to know not only the exact time of the beginning of chilling accumulation, but also the moment when it has been satisfied. Thereafter, and applying the most-adequate model, CR could be calculated if the adequate climatic data are available. Notwithstanding, dormancy release is not achieved abruptly; thence, it is a requisite to establish a common judgement for the end of dormancy - for example, two weeks to reach a determined level of flowering. These results are very interesting with regard to the measurement and comparison of experimental results, even though it can not be said that they correspond exactly to the moment when the growth inertia has disappeared (Seeley, 1996). However, the lack of unification of criteria is evident in the literature. Along these lines, Felker and Robitaille (1985) enumerated several criteria established by many authors: response of apricot and peach buds to GA_3; 50% of green tip in reproductive buds after 3 weeks of forcing conditions; 50% of vegetative buds of sour cherry after 14 days of forcing conditions; an increment of 30% in the weight of flower buds combined with, at least, 30% of buds at Baggionlini´s stage B-C (1952) in seven days; beginnig of pollen meiosis; etc. Couvillon and Erez, (1985b), working on apple, peach and cherry, considered that dormancy was overcome when 50% of the buds had sprouted in two weeks at 24 ºC. Tamura *et al*. (1998) suggested that the 19-kDa protein may be a useful marker for measuring the degree of bud dormancy in Japanese pear. Considering all this heterogeneity, an effort to unify the different protocols for determination of endodormancy release is advisable. The recent discovery of a potential gene marker for CR fulfillment in grape (Mathiason *et al*., 2009), could provide new tools for the determination of dormancy overcoming in fruits.

Nowadays, the notable rise of plant material interchange and the noteworthy production of new released cultivars, the breeding of which has been carried out in unequal climatic conditions, have accentuated the necessity of knowing their CR. Even though certain variability is associated to the CR values due to the previously-mentioned interactions, it must be highlighted that this parameter is an indicator that can avoid the problems associated with the growth of a cultivar in an area where their CR are not satisfied. As a matter of fact, the information regarding the CR of a cultivar has at least the same importance as characters such as productivity, flavour, size, aspect, organoleptic qualities, etc. However, at present and with excessive frequency, this information is not available and new cultivars are introduced in new areas with the resulting failure in the correct adaptation of the cultivar to the new environmental conditions. When CR data are lacking, it is convenient to use another parameter intimately associated to it, the flowering time (Ruiz *et al*., 2007). Low-chill cultivars have precocious flowering time and vice versa. According to the idea that maximum endodormancy is produced at the end of October to early December, Weinberger (1950) established that chilling temperatures are especially effective at overcoming dormancy in the period between the beginning of November and mid-February, corresponding to beginning of May and mid-August in the southern hemisphere. Although the stages where the accumulation occur within this period can affect the effectiveness of the total amount of chill received (Weinberger, 1950; Thompson *et al*., 1975; Young, 1992). It is evident that the chill accumulation in this period is a key variable characterizing an area in regard to its potential for the successful adaptation of different cultivars. Nonetheless, it is necessary to continue investigating the consequences of the distribution of the chilling temperatures along that period in order to ascertain why similar total accumulations entail different responses for the same cultivars.

In the view of the fact that a scarce difference in the temperature value can result in an accumulative effect or a negation effect over the chill accumulated (Richardson *et al*., 1974; Erez and Couvillon, 1987), different aptitudes for adaptation of determined cultivars are frequently seen between nearby growing areas. In the same growing area, a cultivar grown in the foothill or the valley can show unequal behaviours in terms of adaptability or precocity of the ripening time. Following on from this, it would be interesting to accomplish studies, starting from data of the stations network of the meteorological services, aimed at determining the real potential of certain areas with respect to growing stone-fruits cultivars depending on their CR. This would serve growers' aim of establishing new plantations.

Special emphasis should also be put on the fact that the CR are usually calculated with temperatures obtained by meteorological services. These data are registered in meteorological stations, not influenced by the direct solar radiation. Hence, in areas with a noteworthy solar radiation in winter, the bud temperature is usually fairly higher than the official temperatures registered by the meteorological stations, which results in an overestimation of the real CR of the cultivars. Another point that should be taken into account when establishing the CR is the different requirements of reproductive and vegetative buds (Weinberger, 1950; Guerriero *et al*., 1987; Erez, 2000, Naor *et al*., 2003). Thence, if the information available only corresponds to the reproductive CR and there are important differences between reproductive and vegetative CR, which are genotype-dependant, there can be problems associated to a lack of adaptation in mild winter areas, even though the CR for flowering are fulfilled.

On the other hand, models currently used to calculate the chilling accumulation, and thence the CR, circumvent the possibility of a differential effect of the temperatures according to the moment of application. The incorporation of this reported evidence (Weinberger, 1950; Thompson *et al*., 1975; Couvillon and Erez, 1985a; Young, 1992) in the future models for the estimation of CR would be convenient; the Dynamic Model included the new evidences available at that time. In this sense, more exhaustive investigations should be performed regarding the suggestion that the application during brief daily periods of temperatures which theoretically negate the chill accumulated does not have this effect (Couvillon and Erez, 1985b). These brief periods of moderate or high temperatures can be correlated to some days from December to February in mild winter climates with a high radiation during winter. Due to the high radiation, real bud temperatures should be considered.

II.II.V. Dormancy and fruit production

The productivity problems derived from dormancy are not restricted to the fruit production, as other activities such as the silviculture industry can be affected. Nonetheless, the focus will be on the problems associated to fruit production. As it has been discussed, among the temperate fruit species, each cultivar requires an amount of cold (CR), which is predominantly determined by the genotype, although different factors can contribute to its modulation (Champagnat, 1983). Most of the species and cultivars of temperate areas were originated and cultivated originally in areas situated, approximately, between the parallels 34 and 48 of the northern hemisphere (Faust, 2000). Independently of the continentality, which plays a key role in the climate, and the latitude, the

cultivars of the aforementioned area have their climatic history tagged to them. In spite of the climatic diversity we can find between these parallels, cultivating these traditional cultivars in one or other further way could involve negative consequences to their adaptation. These latter effects could be related to, on the one hand, aspects such as photosynthesis, respiration, etc. and, on the other hand, to the capacity of new growing areas to fulfil adequately the CR of the different cultivars. Numerous are the areas on the planet, particularly in the tropics and subtropics, which lack the climatic conditions adequate for dormancy breaking. In these areas, only in prairies situated at high altitude could be possible the accumulation of enough chill to ensure the natural fulfilment of the CR for dormancy overcoming. Unfortunately, the problems are not restricted to these areas and many difficulties have been reported with regard to growing profitable plantations within the geographical limits mentioned. These difficulties are related to the unsuitability of the cultivar to the area, or to the noticeable year-by-year variation of chill accumulation registered, which lead to problems of productivity and longevity in the plantations.

At the end of the 19th century, plantations of temperate fruit crops were set up in areas warmer than those traditionally cultivated. As the cultivars grown where the traditionally-used ones, the CR were not adequately fulfilled (Chandler, 1925; Weinberger, 1950). These were the difficulties that led to the study and identification of the problem (Coville, 1920; Weldon, 1934), as it has been mentioned previously. The problem of colonizing warm areas to grow temperate fruit trees was approached in two different ways. On the one hand, a selection from among the traditional cultivars of low chill requirements was carried out, aimed at breeding new cultivars with commercial quality and low CR, which is a heritable character (Byrne *et al*., 2000; Hauagge and Cummins, 2000). On the other hand, new cultural practices were developed to avoid or reduce the negative consequences of an insufficient chill accumulation (Erez, 1987a; Erez, 1987b; Erez and Lerner, 1990). The most-interesting option for temperate crops in warm areas has frequently been the combination of low chill cultivars and the application of cultural practices to break or avoid dormancy. Although the breeding for low chill cultivars has been developed in different species of the *Rosaceae* family, it is in peach where the furthest advances have been made. In different breeding programmes, cultivars with CR lower than 100 CU have been obtained. For the others species, only cultivars with a range of CR between 300 and 500 CU have been obtained (Byrne, 2000). In Spain, especially in Murcia which is the main producing area and the most specialized in early market production of apricot, the most precocious cultivars have ca. 500-600 CU (Ruiz *et al*.,

2007) and an ameliorable quality. The main objectives for the improvement of these cultivars are: precocity and quality.

Although it is possible to transcend the superior border of the temperate fruit growth (parallel 48) as no dormancy problem would be found, the counterpart would be that the general conditions are not optimum for the growth of fruit trees since they are excessively cold, such that fruits do not even reach ripening. On the other hand, below the parallel 34, summer conditions are long and warm enough, sometimes in excess, for the development and fruit ripening. In this case, two different conditions can be found: a) tropical climates, characterized by the total absence of temperatures adequate for the breaking of dormancy (always above 20 ºC) (Faust, 2000) and b) sub-tropical climates, where there is a limited contribution of suitable temperatures for breaking endodormancy, causing an incomplete dormancy release. In the tropics, the only possible way to cultivate temperate fruits under traditional conditions -even the low-chill ones- is in mountainous areas, where there are adequate minimum temperatures for the overcoming of dormancy (Edwards, 1987). At low altitudes, where moderate temperatures prevail, a culture method has been developed in which the potential of continued growth is allowed by the temperatures: the rest avoidance. It seems clear that dormancy can be avoided if a strong stimulus is applied before the induction of endodormancy (Erez and Lavi, 1984; Edwards, 1987; Erez, 1987b). In practice, it has been found that defoliation can trigger budburst if it is carried out at a precise moment, before buds reach endodormancy (Saure, 1973; Janick, 1974; Edwards, 1987). Budburst can also be achieved through drought and a posterior irrigation (Bederski, 1987). Optimizing these treatments, a second cycle could begin six to eight months after the previous one; also, rest breaking agents can overcome dormancy. This culture practice involves more than one crop in one year. Logically, the best cultivars are those with low CR.

However, from our perspective, the analysis of the temperate fruit production in the warm-winter areas is of major interest. The mild winter areas contain some of the world´s most important areas of stone fruit production. This is especially true for ‘Mediterranean-type’ subtropical climates, typically situated along the western sides of continents in such locations as: the borderlands of the Mediteranean Sea, central and coastal southern California, central Chile, the southern tip of South Africa, and parts of southernmost Australia (Figure 3) (Byrne *et al*., 2000).

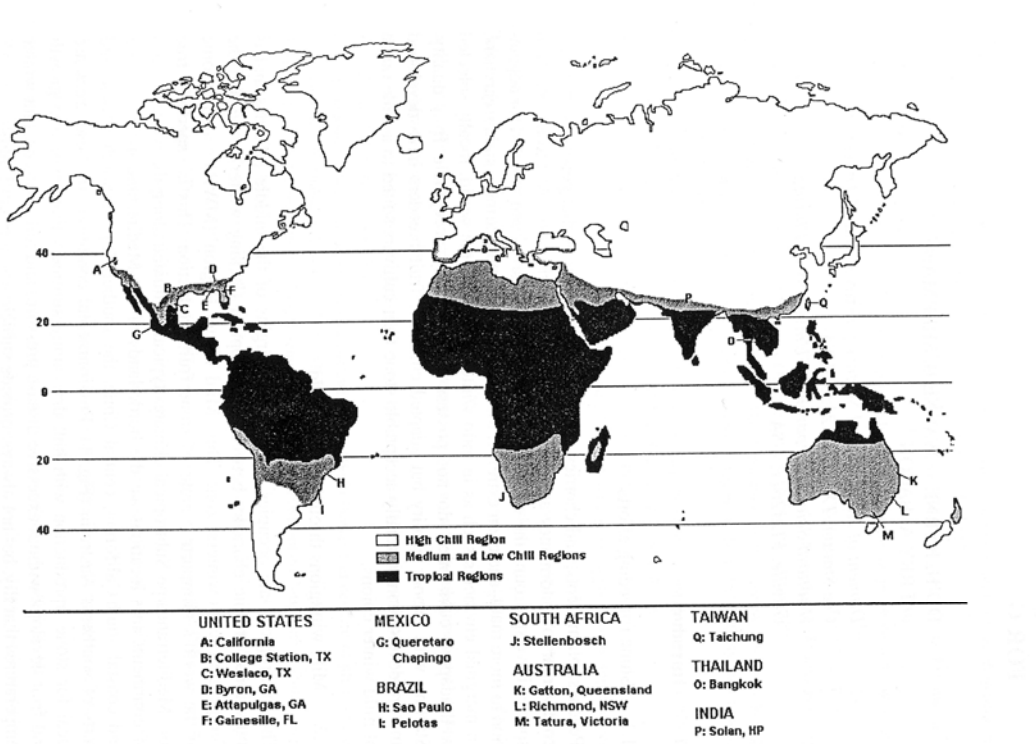


Figure 3. Medium and low chill regions of the world with locations of major stone fruit breeding programs -adapted from Byrne *et al*. (2000)-.

When the chill accumulated in a determined area during the autumn-winter period is not sufficient for a cultivar to break rest, there is a characteristic response which is proportional to the degree of insufficiency: budburst delay, low budburst rate and lack of uniformity. These dysfunctions have, at the same time, important economic consequences, due to their impact on the production and quality of the fruits, and by their effects over longevity of the trees (Erez, 2000). Knowing the potential of an area to supply cold as well as the CR of the cultivars, it will be possible to avoid the problems related to the establishment of cultivars in inadequate areas. However, the experience shows that, either for the lack of availability of information regarding CR of cultivars or the year-by-year climatic fluctuations (sometimes very important) or to the attractiveness of certain cultivars or the lack of others more adequate, this problem seems ubiquitous in warm-winter areas. In these areas the optimal solution is the utilization of low chill cultivars adapted to those areas (Melgarejo, 1996). If these cultivars are not available, the best option would be to choose cultivars that meet the quality standard and have the closest CR to the chill potential of the area. To palliate the difficulties that would appear in this case, a wide group of culture practices have been

developed to alleviate the negative consequences of unadequate CR fulfillment (Erez, 1995; Melgarejo, 1996).

Couvillon and Erez, (1985a) found that the main cause that limits the budburst in many areas is the high daily temperatures. According to Richardson (1974), temperatures above 16 ºC have a chill negation effect. At any rate, the application of treatments to reduce the temperatures by a few degrees during the insolation hours will have a favorable effect on the chill accumulation. Two main types of treatments have been developed to meet this objective. On the one hand, the sprinkling of water over the trees and its consequent evaporation produces a reduction of the bud temperature that can reach several centigrade degrees (Gilreath and Buchanan, 1981a; Erez and Couvillon, 1983; Erez, 1995; Nir *et al*., 1988). Also shading can produce the aforementioned effect of reducing temperatures in the insolation hours. These effects are proportional to the insolation hours during the period of chill accumulation and are especially important in some Mediterranean climates, where winters are dry. The hypothesis is that decreasing the temperature a few degrees could reduce the currently-assumed negative effect of high temperatures. Besides, moderate temperatures (13-15ºC), unlike high temperatures, have a synergic effect with low temperatures for dormancy overcoming. Other physical means to control or to reduce the difficulties associated to insufficient chilling are applied. These methods are related to the vigor of trees, the horizontal training and the late pruning.

Although the physiological processes related to the dormancy throughout time still remain unclear, a group of chemical products have been identified, either by chance or trial-and-error, to have a positive effect on dormancy overcoming when applied correctly. Dinitro-ortho-cresol (DNOC) has been one of the chemical compounds most used, in combination with mineral oils, to break endodormancy. However, the use of this product is currently forbidden because of its dangerous environmental effects. The mineral oils also have an important effect on the dormancy release (Erez *et al*., 1971; Erez, 1987a). Another example is potassium nitrate (KNO_3). It is effective, although its efficiency is lower than a combination of the previously-mentioned compounds. Additionally, there is nitrogen cyanamide, usually commercialized as Dormex®, the efficiency of which has been tested in many woody plants and particularly in apple, plum, apricot and peach trees with high CR (Erez, 1987a; De Benito, 1990). Among the growth regulators, gibberellins and cytokinins have also been used (Wang *et al*., 1986; Lloyd and Firth, 1993). The

cytokinin Thidiazuron ([TDZ] N-phenyl-N-1,2,3-thiodiazol-5-il-urea) deserves a special mention since, in combination with mineral oil, it also shows an important impact on dormancy release.

Nevertheless, there are still some gaps in our knowledge regarding not only the physiology of its action but also its application. The clarification of these gaps would help to reach the maximum efficiency in its application. It seems that the applications generally should be carried out when a considerable part of the CR has been satisfied (Erez, 1987a). However, it seems a more-complex matter, and further investigation should be performed for different crops and locations. This derives from the fact that, in general, plant follows a dynamic different to the usual when the treatment is not performed. Another point that should be clarified, for its economic and practical relevance, is when to apply these treatments, because their efficacy and phytotoxicity depend on the stage and depth of dormancy (Erez, 1987a; Fernández-Escobar and Martín, 1987). The knowledgement of the limit when these treatments should be applied to achieve a beneficial effect, in terms of date, CR of the cultivar and chilling accumulation of the area would improve the crop management. Besides, it has been shown that regular application of some treatments not only increases the percentage of budburst but also has a depressive effect over the vegetation (Costa *et al*., 2004).

On the other hand, it is worthwhile mentioning the consequences of the residual effect of dormancy, which sometimes seem imperceptible but have notable implications in the productivity (Spiegel-Roy and Alston, 1979; Couvillon and Erez, 1985b; Felker and Robitaille, 1985; Powell, 1986).

II.II.VI. Molecular control of dormancy

In last few years, with the development of modern techniques, new horizons in the study of the complex trait of dormancy have opened up. In this last part, an overview of recent advances in the control of the establishment, maintenance and release of dormancy is shown.

Daylenght measurement depends on the ability of plants to detect light and the existence of a timekeeping mechanism referred to as the circadian clock (Jarillo *et al*., 2008). The participation of the circadian clock in the control of biological activities allows plant species to anticipate and adapt to periodic environmental changes, maximizing their opportunities to survive successfully (Mas, 2005; McClung, 2006; Hotta *et al*., 2007). Photoreceptors perceive the light, representing the main input pathway to the clock; the pace of the clock is reset by light every day allowing the

progressive adjustment of the clock to the time of dawn, so that the mechanism of the oscillator remains synchronized with external cycles of light and dark (Jiao *et al*., 2007). Plants have evolved an array of photoreceptors to detect light over a large range of fluence rates and wavelengths, including the PHYTOCHROMES (PHY), which absorb in the red and far-red region of the spectrum, and the CRYPTOCHROMES (CRY), PHOTOTROPINS (PHOT), and the ZEITLUPE (ZTL)/ LOV KELCH PROTEIN 2 (LKP2)/FLAVIN BINDING KELCH REPEAT F-BOX 1 (FKF1) family, all of which absorb blue and UV-A light (Yanovsky and Kay, 2003; Jiao *et al*., 2007; reviewed in Jarillo *et al*., 2008). Despite the fact that the key role of phytochromes in perceiving the length of the photoperiod has long been established (Williams *et al*., 1972), the knowledge at the molecular level of the induction of winter dormancy by short days has been scarce until recently (Allona, 2008). The PHY system plays a key role in the detection of the daylength in woody species (Olsen, 2006). In higher plants, PHY is constituted by families with both distinct and overlapping functions in light perception (Quail, 2002). In black cottonwood (*Populus trichocarpa* Torr. & Gray), three phytochrome genes have been found, PHYA, PHYB1 and PHYB2 (Howe *et al*., 1998). With regard to PHYB2, an adaptive response in this gene to local photoperiodic conditions has been suggested (Ingvarsson *et al*., 2006). Besides, this gene has been mapped to a linkage group which contains a QTL for bud flush and bud set. This has been confirmed by several authors (Frewen *et al*., 2000; Chen *et al*., 2002). A role for phytochrome in daylength detection was found on transgenic hybrid aspen (*Populus tremula*× *tremuloides*) overexpressing oat PHYA gene (Olsen *et al*., 1997b). While wild-type (wt) plants respond to short days by growth cessation, bud set, cold acclimation and dormancy development, different lines of PHYA overexpression plants show insensitivity to daylength when grown at constant temperature (Olsen, 2006), since PHYA regulates FT transcription by modulating CO (Yanovsky and Kay, 2002). It should be mentioned that both transgenic (PHYA) and wt plants showed a similar cold acclimation after exposure to low temperatures (Welling *et al*., 2002), which can indicate the independent activation of cold acclimation by low temperatures and short days in hybrid aspen.

The phytochrome system interacts with biosynthesis of plant hormones like GAs and ABA, as well as responsiveness to hormones, apparently by the interaction of plant hormones with cell cycle regulation (Olsen, 2006). However, the role of plant hormones in the onset of dormancy remains partially unclear (Tanino, 2004; Olsen, 2006; Rohde and Bhalerao, 2007). In woody plants, the level of GAs decreases rapidly under short day conditions (Olsen *et al*., 1995b, 1997a). Further, GA content under short days is correlated with arrested cell divisions in the subapical meristematic

area of the stem (Hansen *et al.*, 1999). In *Populus*, transcriptional down-regulation of a gene encoding a key biosynthetic enzyme, GA 20-oxidase, has been observed and plants over-expressing this enzyme produce a delay in growth cessation (Eriksson and Moritz, 2002). This illustrates that a proper interaction between the phytochrome system and GA biosynthesis is an integrated part of the mechanism involved in growth cessation and winter bud formation in response to short days (Olsen, 2006), although microarray analyses suggest that it is a negative regulator of GA signaling who restricts growth in the autumn (Druart *et al*., 2007).

For dormancy establishment, growth cessation is necessary. Growth cessation is caused by environmental signals such as cold, photoperiod or drought. The role of photoperiod in the control of growth cessation is well known (Nitsch, 1957). Inactivity is reached in the apex through a signal emitted by leaves, which perceives photoperiod (Hemberg, 1949; Wareing, 1956). However, it was only recently, except for phytochrome, when the components of the signal transduction chain acting downstream of the critical daylength were determined (Howe, 1996). The identification of *FT* (FLOWERING LOCUS T) and CONSTANT (*CO*) as mediators of short-day signals for growth cessation represented a milestone in the elucidation of the control timing of flowering and seasonal growth cessation in trees (Böhlenius *et al*., 2006). The *CO*/*FT* module seems to have a mode of action similar to that described in *Arabidopsis* (revised in Kobayashi and Wigel, 2007). In the presence of light, a high level of *CO* gene expression, controlled by the circadian molecular clock, activates the *FT* transcription. In LD (long day) conditions, *CO* transcriptions peaks at dusk when there is still light, which induces expression of the *FT* gene and the consequent induction of flowering. Under short day conditions, *CO* mRNA levels remain low during daylight hours and do not rise until nighttime, so *FT* is not expressed (Suárez-López *et al*., 2001; Yanovsky and Kay, 2002; Valverde *et al*., 2004; Imaizumi *et al.,* 2005; Sawa *et al.,* 2007; in Allona *et al*., 2008). Similarly, Böhlenius *et al.* (2006) showed that in Poplar *CO* expressions levels remain low during the light period when the days get shorter as autumn sets in, thus *FT* is not expressed, which induces growth cessation and bud set. This control mechanism could be related to the clinal variation in critical photoperiod required to induce growth cessation with variations in the latitude of the place of origin of some woody species (reviewed in Olsen, 2006). Gyllenstrand *et al*. (2007) found a significant and tight correlation between growth rhythm (both bud set and bud burst), and expression pattern of a FT homologue gene, suggesting FT as a key integrator of photoperiodic and thermal signals in the control of growth rhythms in gymnosperms. Additionally, the substitution of a single amino acid can transform an FT protein from an activator to a suppressor in flowering

(Hanzawa *et al*., 2005). Thus, some gaps still exist in our knowledge of the role of FT genes in the endodormancy of angiosperms and gymnosperms.

A connection between the induction of poplar homologues of the FCA, a gene controlling flowering time in *Arabidopsis* (Macknight *et al*., 1997), and the fact that the binding protein FCA may be an ABA receptor (Razem *et al*., 2006) has been suggested (Allona, 2008). However, a recent publication revealed that FCA does not bind ABA (Risk *et al*., 2008), suggesting ABA-binding properties of these proteins should be carefully re-evaluated and that alternative ABA receptors are likely to be discovered. Nonetheless ABA has been suggested to play a key role in dormancy; its exact function in bud dormancy is still poorly understood. Studies correlating ABA levels, with bud dormancy have provided contradictory results. Under short day conditions, ABA has been shown to increase (Li *et al*., 2003b). Despite the fact that, short day conditions can induce dormancy in an ABA deficient birch (Rinne *et al*., 1998) the induction of dormancy through exogenous ABA application have been shown to be ineffective (Li *et al*., 2003a, 2003b). On the other hand, ABA has been suggested to play a role in the photoperiodic control of cold acclimation rather than in the induction of endodormancy (Arora *et al.*, 2003), as ABA-deficient mutants have shown reduced tolerance to low temperatures compared to wild types in birch (Rinne *et al.*, 1998). Regarding ethylene, it has been shown that transgenic ethylene insensitive birches under short day conditions, ceased growth, terminal bud was not formed and endodormancy induction was delayed, suggesting that development and dormancy onset could be independent (Ruonala *et al*., 2006). More recently, Ruttink *et al*. (2007) stated that light, ethylene, and abscisic acid signal transduction pathways consecutively control bud development by setting, modifying, or terminating these processes. Ethylene signal transduction was positioned temporally between light and abscisic acid signals and is putatively activated by transiently low hexose pools. Besides, the identification of a large set of genes commonly expressed during the growth-to-dormancy transitions in poplar apical buds, cambium, or *Arabidopsis thaliana* seeds suggests parallels in the underlying molecular mechanisms in different plant organs (Ruttink *et al*., 2007).

The role of the low temperatures in the establishment of winter dormancy is being elucidated, in spite of the fact that after two decades of molecular plant science temperature sensors have still not been identified. The analysis of quantitative trait loci (QTL) associated with dormancy in hybrid poplars has indicated that genetic differences in photoperiodic responses only partly explain genetic differences in bud set timing under natural field conditions (Allona, 2008). Thus, it

could be suggested that responses to other environmental factors, such as temperature, could help to complete the emerging picture (Howe *et al*., 1999, 2000; Chen *et al*., 2002). Benedict *et al*. (2006) showed, for transgenic *Populus,* that expression of C-repeat binding factor 1 (CBF1) from *Arabidopsis* (AtCBF1) increase cold acclimation of non-acclimated plants compared to wt plants. In chestnut, it has been shown that a low temperature treatment stalls the rhythmic expression of the circadian clock components LHY and TOC1 (Ramos *et al*., 2005). Warming the plants was sufficient to restart the clock, but this effect did not depend on the dormancy of the buds (Ramos *et al*., 2005). So it is possible that the circadian clock also has a role in the temperature-regulation of bud dormancy in trees (Penfield, 2008), although the results also indicate that the alteration of the circadian clock is not intrinsic to dormancy (Olsen, 2006). There is also evidence for a role for the plant circadian clock in mediating temperature signalling itself. For example, the circadian clock regulates CBF expression and the magnitude of its transcriptional activation by cold. CBF expression is increased when cold is experienced 4 h after dawn, compared with a similar experience 4-h after dusk (Fowler *et al*., 2005). It has recently been found that CBF gene expression can be promoted at nonchilling temperatures by manipulating light quality (Franklin & Whitelam, 2007). These authors found that under far-red rich light regimes the CBF regulon can be activated in *Arabidopsis* plants growing at 16 ºC in the absence of chilling (Franklin & Whitelam, 2007). This activation depends on the regulation of CBF transcription by the circadian clock, such that the role of far-red light greatly increases the amplitude of the normal circadian regulation of CBF transcription. Strikingly, a low red: far-red ratio or phytochrome deficiency can even confer freezing tolerance on plants that have not been cold-acclimated (Penfield, 2008). Interestingly, the ruin lizard (*Podarcis sicula*), a hibernating ectothermal vertebrate, shows a similar clock disruption in response to cold (Chiara Magnone *et al*., 2005; Vallone *et al*., 2007). The basic mechanisms of clock function in plants and animals are similar, although their oscillator genes are unrelated. This parallelism between two such evolutionarily-distinct organisms suggests that the stopping of the circadian clock in response to cold could be part of a general adaptive strategy that enables living organisms that undergo dormancy or hibernation to survive the winter (Allona *et al*., 2008).

Once dormancy is established, it is maintained by hitherto unknown mechanisms. From a purely mechanistic point of view, meristem cells could be insulated from growthpromoting signals, such as gibberellins. Indeed, meristematic cells become symplasmically isolated upon transition to endodormancy, by disconnection of the plasmodesmatal circuitry linking them to neighbouring cells (Rinne and van der Schoot, 1998; Rinne *et al*., 2001). Whereas plugging the plasmodesmatal

connections with callose will prevent gibberellin transport, auxin (and other regulatory molecules) might rely on different transport systems. Auxin is transported by specialized carriers, the mRNAs of which are detectable in poplar cambium after endodormancy is established (Schrader *et al*., 2004). Furthermore, auxin levels do not change in cambial cells throughout the entire activity–dormancy cycle, but the responsiveness to auxin does (Little and Bonga, 1974; Uggla *et al*., 1996). The role of gibberellins, and the putative significance of their exclusion from the meristem, is unclear in growth re-initiation. At least in poplar cambium, gibberellin application even after chilling exposure does not induce cell division, in contrast to auxin (Rohde and Bhalerao, 2007).

As has been stated, dormancy is overcome through long-term accumulation of chilling temperatures. Similarities between vernalization and dormancy release have been noted and assessed (Chouard, 1960). An important characteristic of vernalization in *Arabidopsis* is the mitotically stable repression of *FLOWERING LOCUS C (FLC)* after prolonged exposure to low temperatures (Sung and Amasino, 2005). FLC-like genes have been shown to be regulated differentially during the satisfaction of CR in vegetative buds poplar (Chen and Coleman, 2006), and more recently, in lateral vegetative buds of Japanese apricot (*Prunus mume*) (Yamane *et al*., 2008). Thus, *Pmdam6* gen might be involved in endodormancy function as an internal suppressor gene to delay or inhibit bud growth under conditions favourable for bud burst when none of the external inhibitory effects such as apical dominance or unfavourable environmental conditions exist (Yamane *et al*., 2008). Bielenberg *et al.* (2008) revealed a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation in evergrowing peach. Similar genes were differentially expressed following dormancy transitions in raspberry (*Rubus idaeus* L.) (Mazzitelli *et al*., 2007) and apricot (Yamane *et al*., 2008). These MADS-box genes (named DORMANCY ASSOCIATED MADSBOX or DAM genes) are related to SHORT VEGETATIVE PHASE (SVP) and AGAMOUS LIKE24 (AGL24) of *Arabidopsis* but form a separate clade within this group (Bledelenberg, 2008). In *Arabidopsis*, mutations in SVP promote early flowering (Hartmann *et al.*, 2000). Interestingly, experimental evidence indicates that SVP negatively regulates expression of FT in *Arabidopsis* by binding to its promoter (Lee *et al*., 2007). AGL24, a floral promoter, is up-regulated during vernalization (Michaels *et al.*, 2003). Analysis of publicly available microarray data indicate that both AGL24 and SVP are preferentially expressed during short day conditions relative to long day in micro-dissected apical tissue harvested 0, 3, 5, and 7 days after the shift to LD. Thus, SVP and AGL24 are regulated by environmental conditions known to impact bud dormancy in perennial species (Horvarth *et al*., 2008). Furthermore, several studies

have been done, in addition to DAM genes and FT/CENL1 with known or suspected involvement in bud dormancy/growth, on the impact of various dormancy transitions on the whole transcriptome of buds in kiwi fruit (Actinidia deliciosa) (Walton *et al*., 2007), grape (*Vitis riparia*) (Mathiason *et al*., 2009), raspberry (Mazzitel *et al*., 2007), potato (*Solanum tuberosum*) (Campbell *et al*., 2008), and in the terminal buds and cambial meristem of poplar (*Populus tremuloidies*) (Schrader *et al*., 2004; Druart *et al*., 2007; Ruttink *et al*., 2007). These studies have shown that dormancy signals impact numerous physiological processes including cell division, oxidative stress, and flavone biosynthesis. Additionally, these studies have demonstrated changes in expression patterns of numerous known regulatory genes that could play a vital role in dormancy transitions, or in regulating physiological processes impacted by dormancy transitions (Horvath *et al*., 2008). On the other hand, Halaly *et al*. (2008) suggested that similar mechanisms might be triggered by alternative external stimuli, such as heat shock and hydrogen cyanamide, that induce dormancy release in grape buds. This would support the previous hypothesis that temporary oxidative stress and respiratory stress may be parts of the mechanism leading to budburst and suggest that some molecular events that occur during dormancy release are mechanistically involved. Then again, in a recent transcriptome analysis in leafy spurge (*Euphorbia esula* L.), suggested that the dormancy transitions require specific alterations in transport functions (including induction of a series of mitochondrial substrate carriers, and sugar transporters), ethylene, jasmonic acid, auxin, gibberellic acid, and abscisic acid responses, and responses to stress (primarily oxidative and cold/drought). Besides the comparison to other dormancy microarray studies led to the identification of a particular MADS-box transcription factor related to the DORMANCY ASSOCIATED MADS-BOX genes from peach and the hypothesis that it may play a direct role in dormancy induction and maintenance through regulation of FLOWERING LOCUS T (Horvath *et al*., 2008). Mathiason *et al*. (2009) have recently done an interesting transcript profiling in *Vitis riparia* during chilling requirement fulfillment. Circa 1500 differentially expressed genes were identified, involved in metabolism, cell defense/stress response, and genetic information processing. An inhibition of genes involved in carbohydrate and energy metabolism and activation of genes involved in signaling and cell growth was observed along the satisfaction of CR. Moreover, several candidate genes were identified that may serve as indicators of bud chilling requirement fulfillment. Special attention deserves a rhodopsin receptor, which is a sensory signal mediator involved in the first events in the perception of light and may be involved in response to blue light (Paolicchi *et al*., 2005). The rhodopsin receptor, which exhibited a decrease in expression early in the chilling period but an increase

between 1,500 and 2,000 hours of chilling at 4 ºC, may be important as an early signal indicating fulfillment of the CR of the buds (Mathiason, 2009). These results suppose a milestone regarding the development of molecular markers for the determination of the CR of the seedlings of a breeding program. Up to now, scarce work has been carried out regarding identification of QTLs controlling CR in apricot, excepting preliminary results which have been presented recently (Olukolu *et al*., 2008). In this work, QTL analysis using two linkage maps with phenotypic trait data of dormancy budburst resulted in seven QTLs on linkage groups (LG) 1, 2, 3, 5, 6, 7 and 8 (Olukolu *et al*., 2008). A similar recent study conducted in peach for detection of vegetative bud dormancy QTLs also found three QTLs related to CR located in LGs 1, 4 and 7 of the *Prunus* reference map (Chaparro and Beckman, 2008). On the other hand, one QTL for the blooming date was also found in the linkage group 4 both in a genetic map of almond (Sánchez-Pérez *et al*., 2006) and a genetic map of rose (Hibrand-Saint Oyant *et al*., 2008).

Another interesting point regarding dormancy is the resetting. The resetting of dormancy allows a posterior dormancy induction and the subsequent need for chilling to reach dormancy overcoming. In the event that the induction and maintenance of dormancy entails epigenetic marks, it would be necessary its elimination or resetting. In the case of annual plants, the repressed state of FLC, which represses FT activity and consequently, flowering; is retained through successive mitotic divisions throughout the development of the plant after the period of low temperature ends, and the gene, is then rest to an active transcriptional state in the next sexual generation (Sheldon *et al.*, 2000). The repression of *FLC* by vernalization is controlled by changes in *FLC* chromatin, which is an example of epigenetic control of gene expression. Thus, the control of FLC activity ensures that FT is repressed before winter so that the long day photoperiod of spring is able to induce FT activity, with flowering occurring at an optimal time (Sheldon, 2000). There are still some uncertainties regarding the mechanism of resetting FLC gene activity. Choi *et al*. (2009) revealed some interesting cues regarding when it does take place and proposed that FLC reprogramming is composed of three phases: (i) repression in gametogenesis, (ii) reactivation in early embryogenesis and (iii) maintenance in late embryogenesis. However, crucial differences exist between vernalization and release from dormancy. Whereas, vernalization is thought to occur in actively dividing cells, endodormancy release occurs by exposure to low temperatures after termination of cell division (Wellensiek, 1964). Thus, the memorization of experienced cold through a mitotically stable repression is not required for dormancy: the cells that cease and resume growth are identical meristem cells. In annual plants, resetting of an epigenetically fixed, vernalized

state happens during meiosis and before seed dormancy (Rohde and Bhalerao, 2007). Nonetheless, it has been suggested that switching between perennial and annual habits occurred many times during evolution (Thomas *et al.*, 2000), which is in accordance to the half-way behaviour, between perennial and annual, of beet, foxglove and carrot. These species can be converted to a perennial habit if prevented from flowering (Harper, 1977). Moreover, an alternative devernalization mechanism triggered by accumulated heat in summer has been also suggested (Chouard, 1960; Prince and Marks, 1982; Battey, 2000).

II.III. OBJECTIVES

Is a well known fact that at the end of summer, like other woody species, temperate fruit trees progressively enter in a phase of reduction of growth - that promptly comes to a complete stop, even if there still are favorable conditions of light and temperature to maintain the vegetative activity. Nonetheless, if forcing conditions with temperatures relatively–high temperatures (25 ºC) are applied during the first phase of the process, growth can be resumed. The time of the application needed increases progressively until reaching a point where resumption of growth is not possible. This point basically coincides with the deepest phase of endodormancy (Lang *et al*., 1987).

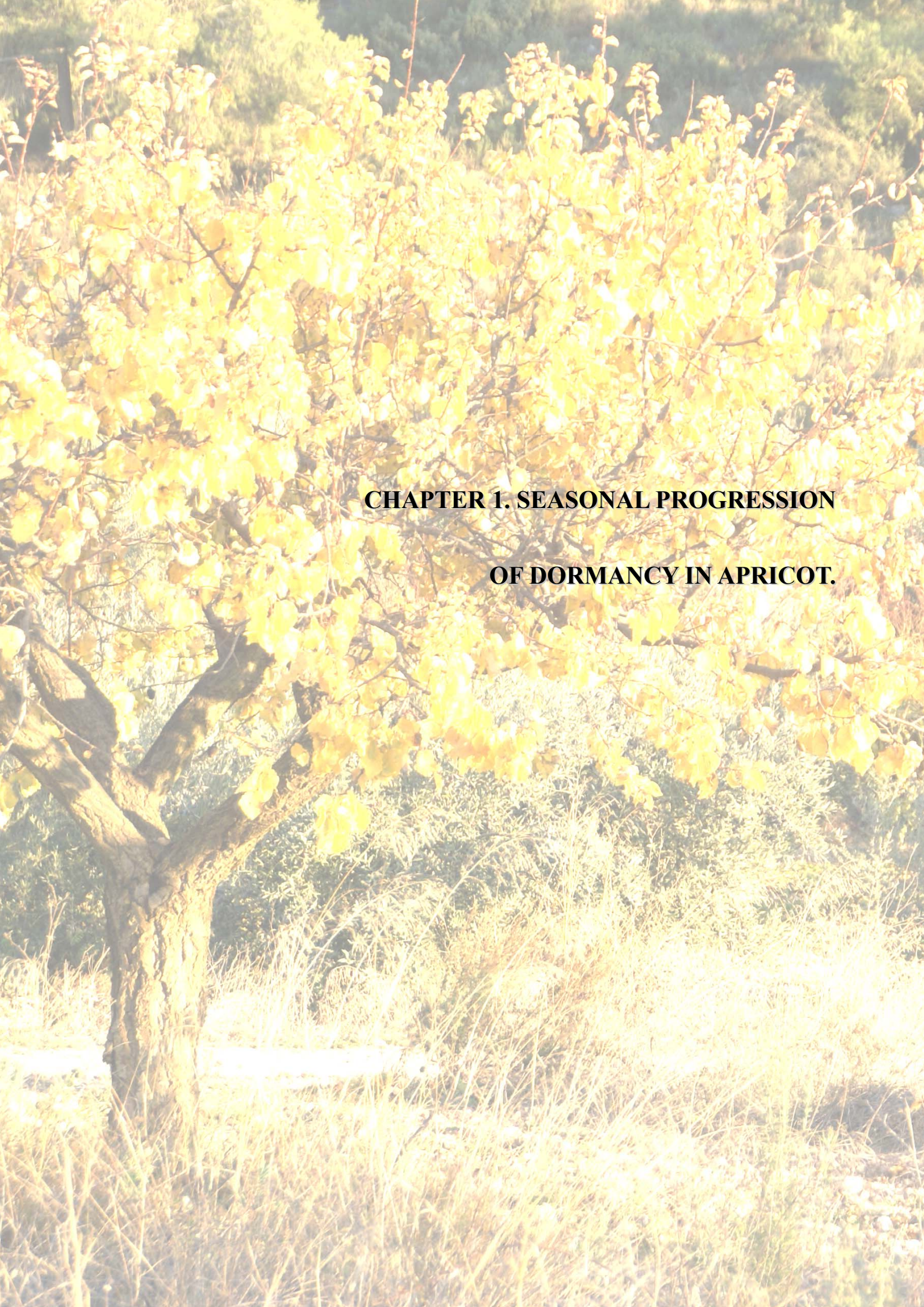
Although there are clear indications that factors such as light, fog, heat, etc., affect the evolution of dormancy, it is well documented that the most decisive factor is low temperature. From this ascertainment arose the need to determine which temperatures were really useful for endodormancy to be overcome as well as the effect of those temperatures ineffective in the release of endodormancy. Also was evaluated the effect of temperature combinations in daily cycles, emulating the temperature in field conditions. The result of this approach was the development of different models to quantify the chill registered in a specific location. At the same time, new evidence showed the differences in the amount of chill needed for different species and cultivar to overcome dormancy, which indicated that the chilling requirement could be also influenced by the climatic conditions where trees were cultivated.

Although the numerous uncertainties and gaps in our understanding related to this phenomenon demand an important scientific attention, it is also true that the recent advances have led to new approaches to solving problems associated with production. Growers are interested in planting fruit cultivars in areas where its chilling requirement will be adequately satisfied to avoid negative effects in the production and quality: for example, delaying flowering time to avoid frost

injuries or bringing forward to sell in the more-profitable early market; etc. Nowadays, all these interests can be adequately solved. The correct manipulation of dormancy can lead to interesting productive alternatives.

During this work, diverse methodologies will be employed to study aspects of the dormancy which are intimately related with the processes of dormancy onset and overcoming, the determination of the inflection point between both phases and its position in the annual cycle; the effect of low temperatures depending on the moment of application; the incidence in dormancy release of the application of daily cycles of temperature combinations with different relative durations to the previously used in this sort of experiments; the effect of the conditions during the onset of dormancy over its depth; the role of apical dominance in the behavior of shoots collected in different phases of endodormancy; the adequacy of different models of chill accumulation to Mediterranean climatic conditions; the variability of dormancy progression in different locations under Mediterranean climate, etc. Besides and closely related to production, the effect of the incidence of solar radiation in the process of chill accumulation and its consequences over dormancy overcoming will be studied. The aim of this study was to establish the possibilities of influencing the earliness of flowering and ripening of the fruits.

Finally, the recent advances in DNA markers offer plant breeders a rapid and precise alternative approach to conventional selection schemes, with regard to quantitative traits (Tanksley and Hewitt, 1988). Using detailed molecular linkage maps, quantitative trait loci (QTLs) affecting important traits could be mapped, genetically evaluated and selected through linked markers. Thus, a preliminary study of a QTL for flowering time developed with an innovative PCR technique will be shown in the last chapter.



CHAPTER 1. SEASONAL PROGRESSION OF DORMANCY IN APRICOT.

1. INTRODUCTION

Dormancy of buds of temperate-zone fruit trees is a complex trait developed as a mechanism against the impact of low winter temperatures. Other authors also envisage dormancy as a morphogenetic factor (Champagnat, 1983). Dormancy is not a mechanism achieved suddenly by plants, but a progressive process developed during autumn, increasing its depth until reaching the so-called deep rest (Fuchigami *et al*., 1977, Amling and Amling, 1980, Lang *et al*., 1987). The chilling period has been considered the main factor controlling growth cessation and dormancy progression in temperate woody species (Jacobs *et al*., 2002, Heide and Prestrud, 2005). A recent study has also reported the interaction of photoperiod and temperature in the control of dormancy process in *Prunus* species (Heide 2008).

Although considerable progress has been achieved, large functional gaps of knowledge still exist in bud-dormancy induction, maintenance and release (Arora *et al*., 2003). According to Lang *et al*. (1987), dormancy can be determined by different factors: paradormancy (regulated by physiological factors outside the affected structure, i.e. correlative inhibition); endodormancy (regulated by physiological factors inside the affected structure); and ecodormancy (regulated by environmental factors). Correlative inhibition begins to operate just when the bud is formed (Champagnat, 1983; Hauagge and Cummins, 1991b; Crabbé and Barnola, 1996); true dormancy or dormancy ascribed to the bud itself is installed progressively while the climate induces ecodormancy, mainly in the phase preceding bud burst. Transition from correlative inhibition to true dormancy begin at the end of August in red-osier dogwood (Cornus sericea L.) and reaches maximum intensity at the beginning of November, more or less at the onset of natural defoliation (Fuchigami *et al*., 1977). Hauagge and Cummins (1991b) found a similar situation in apple (*Malus domestica* L.). The moment of maximum intensity of dormancy also seems to be determined by the species or variety (Crabbé and Barnola, 1996), and its intensity modulated by the climatic conditions in which the onset of endodormancy occurs (Jonkers, 1979, Westergaard and Eriksen, 1997). Amling and Amling (1980), stated that in pecan (*Carya illinoinensis* (Wangenh.) K. Koch), the onset of dormancy occurs prior to the advent of chilling temperatures, but that further intensification occurred when chilling began. Cook and Jacobs (2000), observed that 'Granny Smith' and 'Golden Delicious' apple shoots from a cold area reached maximum dormancy before any considerable chilling had accumulated (<100 Chill Units (CU)), whereas those from a warmer area reached maximum dormancy after 600 CU.

However, there is limited information concerning seasonal progression of dormancy in apricot (*Prunus armeniaca* L.). Brown (1957), worked with regressions of temperatures prior to bloom in the 'Royal' apricot cultivar, and stated that the dormancy influence in the buds gradually deepened after late June, reached a maximum in late October and early November and declined rapidly thereafter. By mid-January buds were quite responsive to increasing temperatures. According to Hatch and Walker (1969), the rest intensity in 'Chinese apricot' formed a bell shaped curve, with a peak of maximum rest in November. The maximum depth of dormancy did not seem to be influenced by either the fluctuating temperatures or by the coldest part of the winter because the peak was reached before the cold weather occurred and dormancy was completed during the cold period of the winter.

So far, chill-unit accumulation has been typically used to estimate the depth and progress of bud dormancy largely due to the absence of visual bud changes during dormancy and/or to the lack of endogenous markers available for dormancy status (Arora *et al*., 2003). Several authors have worked on the assessment of dormancy release dates of different apricot cultivars. These studies have used both physical and physiological parameters to determine the date of breaking of dormancy, such as flower bud weight (Tabuenca, 1964); flower bud weight after forcing and phenological stage (Guerriero *et al*., 2002; Ruiz *et al*., 2007); histology of flower buds (Bailey *et al*., 1978, 1982); and histology and flower bud weight after forcing (Viti *et al*., 2003). Julian (2008), studied the flower bud development in apricot and its relationship with dormancy and stated that flower buds underwent dormancy with the floral whorls differentiated.

Dormancy progression in apricot has been studied evaluating periodically the responsiveness of buds to favorable temperatures to growth (Brown, 1957; Ruiz *et al*., 2007). Detached shoots have been widely used to determine the dormancy progression and CR of apricot cultivars (Arias and Crabbé, 1975; Cook and Jacobs 2000; Ruiz *et al*., 2007). When detached shoots or complete trees are used, results are determined by both the correlative inhibition at long and short distances and the endodormancy. Nevertheless, the single node cutting test has frequently been used to study dormancy (Guerriero *et al.*, 1985a; Balandier, 1993a; Balandier, 1993b; Falusi and Calamassi, 1997; Bonhomme *et al*., 1999), and results have been confirmed by biochemical tests (Crabbé and Barnola, 1996). Falusi and Calamasi (1997), reported that the use of one-node cuttings seemed to be the best method to define bud growth inertia as dormancy in its strict meaning since it can be ascribed to the bud itself (or to the adjacent portion of the branch). When the single node test

is used, it determines the intensity of inhibition developed in the same bud and the adjacent portion of the branch (Champagnat, 1992; Falusi and Calamasi, 1996). Dennis (2003), also recommended the use of a small experimental unit, such us single-node cutting, to study the physiological basis of rest. Thus, the use of short single node cuttings appears to cluster a group of advantages such as lower paradormancy effect and higher capacity of gas exchange, compared to the use of complete shoots, and an easy management and lower wound effect. The intensity of the inhibition is quantified by the period of time until budbreak occurs under forcing conditions. However, the use of the meristem has some drawbacks, such as the difficulty in its management and high stress due to the proximity of the cut to the meristem itself. On the other hand, single node cuttings are of little use for determining the response of whole trees (Dennis, 2003).

The position of the bud on the shoot also has an influence over budburst development. In this way, an acrotonic tendency in budburst development in shoots has been observed, e.g. in apple (Cook *et al*., 1998), and the terminal bud appears to exert a primigenic dominance (Bangerth, 1989). Regarding the position of shoot in the tree, Viti *et al*. (2003) stated that sampling from different canopy positions had an influence over the evaluation of flower bud dormancy in apricot.

On the other hand, the CR is not a constant factor (Tromp *et al.*, 2005). It is genetically determined, but other factors such as latitude, elevation or climatic conditions during endodormancy inception can affect its value (Lang, 1989). The different behavior of climatic models aimed at assesing the CR in different areas (Ruiz *et al*., 2007; Chapter 2), suggests that environmental conditions play a key role in relation to dormancy progression.

Detached shoots have been widely used to determine the dormancy progression and CR of cultivars (Arias and Crabbé, 1975; Cook and Jacobs, 2000; Ruiz *et al*., 2007). As for the criteria for determining depth of rest, time to budburst appears to be superior to recording the percentage of budbreak during a specific time interval (Dennis, 2003). Thus, it seems more appropriate to use the time to budbreak in detached shoots than to use single node cuttings to assess the dormancy progression in apricot trees.

The purpose of this chapter was to determine the seasonal progression of dormancy of vegetative and reproductive buds in apricot under Mediterranean climatic conditions. The chapter has been divided into two parts, since two different methodologies have been used with different aims: 2.1.) The single node cutting method has been used to understand dormancy progression at bud level, avoiding interactions such as the long-distance paradormant effect. In addition, influence

of genotype; type of bud (reproductive or vegetative, lateral or terminal); orientation and position of the lateral bud on the shoot; and year-by-year variation was evaluated. 2.2.) The aim of the second part of this chapter was to determine the seasonal progression of dormancy in apricot in different environments through the study of detached shoots in a group of apricot cultivars that covered the range of flowering time in each climatic condition. This experiment was carried out in two different countries under Mediterranean climate, Spain and South Africa. Within each country, several locations were chosen to assess the variability of the apricot species cultivated in different environments.

2. SEASONAL PROGRESSION OF BUD DORMANCY IN A MEDITERRANEAN CLIMATE. A SINGLE NODE CUTTING APPROACH.

2.1 Material and methods

2.1.1. Plant material

The study was carried out in two consecutive periods, from June 2005 to March 2006 -to simplify, it will be referred as 2005-06 in text- and from June 2006 to March 2007 –referred as 2006-07-. Plant material was obtained from an experimental orchard belonging to the CEBAS-CSIC, situated in the South East Spain (Cieza-Murcia, altitude 241 m, lat. 38º16'N, long. 1º16'O). Trees were grafted onto 'Manicot 1236' rootstock, trained as open center (vase) with a planting distance of 4 x 5 m and drip irrigated.

Two apricot cultivars, 'Rojo Pasión' (Egea *et al*., 2004a) and 'Murciana' (Egea *et al*., 2005b), and the advanced selection, 'S 405/17', were used. The CR for breaking of dormancy of plant material in the climatic conditions of the experimental orchard spanned from low to medium-high: 800 ('S 405/17'), 913 ('Rojo Pasión') and 1,009 ('Murciana') Chill Units (Ruiz *et al*., 2007). Material was sampled from ten trees per genotype.

2.1.2. Experimental design

Ten trees per cultivar or advanced selection were chosen from the experimental orchard. Nine one-year-old shoots with similar vigour were collected per genotype and sampling date. The shoots were randomly collected within three different orientations, south, north-west and north-east (3 repetitions each orientation). Shoots were ca. 40 cm long and had ca. 1 cm diameter. Collecting dates were ca. each 15 days in 2005-06 and ca. each month in 2006-07. Collecting data started in the 23rd of June in both 2005 and 2006.

2.1.2.1 Single node cutting test

The progression of flower and vegetative bud endodormancy was estimated for each cultivar following the single node cutting test used by Balandier (1993a, 1993b).

Five consecutive 5 cm long cuttings were dissected from each shoot. Each cutting had a single node 1 cm from its apex. Each node contained one vegetative and two flower buds. During 2005-06 the terminal buds were not used. During the 2006-07 season the terminal buds constituted the most distal segment, so that the effect of the terminal bud on the other four lateral cuttings could be observed. The basal part of the cutting was placed in tap water with 1 g/L of aluminium sulphite to avoid micro organism proliferation. The top was covered with Parafilm® to limit water loss. Water was replaced every 2-3 days. The cuttings were placed in growth chambers at 25 ºC (±1ºC) in 16h light/8h dark daily cycle under artificial fluorescent lighting (200 mol$\mu m^{-2}s^{-1}$). Cuttings were kept in forcing conditions for up to 50 days. The date of budbreak of each vegetative and reproductive bud was recorded by observations performed every weekday. Bud break was considered when a green tip was visible among bud scales, which corresponds to B-C Baggiolini´s stage (1952). The mean time to budbreak (MTB) in days, of each vegetative and reproductive bud population (arithmetic mean of the individual burst lapses) was calculated. The fluctuation in MTB values during the sampling period reflects similar changes in the bud intrinsic growth inability (Balandier *et al*., 1993a). Standard error for MTB was also calculated.

2.1.2.2. Floral whorls´differentiation

Observation of the differentiation of floral whorls was carried out during the two consecutive years, to determine the date of flower whorls formation as a reference prior to the establishment of dormancy. Flower buds which responded to forcing conditions were observed visually at C Baggiolini´s stage in order to verify if its floral whorls were completely differentiated.

2.1.2.3. Chilling accumulation

Hourly temperatures were collected with an automatic data-logger (Escort ® Datalogging Systems, Buchanan, Virginia, USA, 2002). The starting date for chilling accumulation was considered to be when a consistent chilling accumulation occurred and the temperatures producing a negative effect (chilling negation) were scarce (Richardson *et al*., 1974; Erez *et al*., 1979b; Guerriero *et al*., 2002). These dates corresponded to November 5 and 10 in 2005 and 2006, respectively. Chilling accumulation was assessed by CU of the Utah Model (Richardson *et al*., 1974). Previous studies (Ruiz *et al*., 2007) have reported a high correlation between the CR calculated by CU and Portions under our climatic conditions, that is to say, between the Utah and

Dynamic (Fishman *et al*., 1987a, 1987b) Models. Thus, to simplify the results, only chill accumulation by Utah Model will be shown.

2.1.2.4. Statistical analysis

Statistical analytical procedures were performed using SPSS ® 13.0 software for Windows (Lead Technologies Inc., Chicago, IL). Differences between genotypes, treatments and years were established by ANOVA.

2.2. Results

2.2.1. Chill Units accumulation and flowering time

Chill accumulation in the field conditions of this study usually begins during the first two weeks of November, although the pattern of accumulation is very variable from year to year (Ruiz *et al*., 2007). Chill accumulation varied greatly between the two seasons. By the first of December 2005, 270 CU had accumulated but by the same date in 2006, only 79 CU had accumulated (Figure 1). This led to a difference of more than 27% by the end of the stage of chilling accumulation for breaking endodormancy.

Flowering time (F_{50}-50% of flowers opened-) was determined for each variety in the two periods studied. In 2006, 'S 405/17' flowered on March 5, 'Murciana' on the 8 and 'Rojo Pasión' on the 9. In 2007, 'S 405/17' flowered on February 25 and both 'Murciana' and 'Rojo Pasión' flowered on March 2. In spite of the earlier and higher chilling accumulation registered in 2005-06 (Figure 1), flowering dates were around one week later than in 2006-07. These results can be related to the low temperatures registered in February 2006 (Figure 2), which led to a longer ecodormant period in 2005-06.

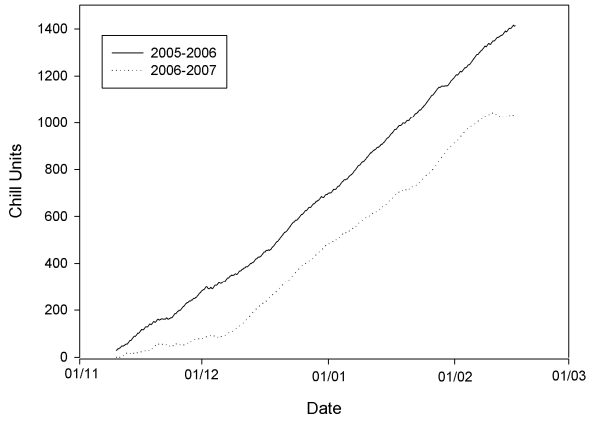


Figure 1. Chill Unit accumulation during 2005-06 and 2006-07, and flowering dates of cultivars in field conditions.

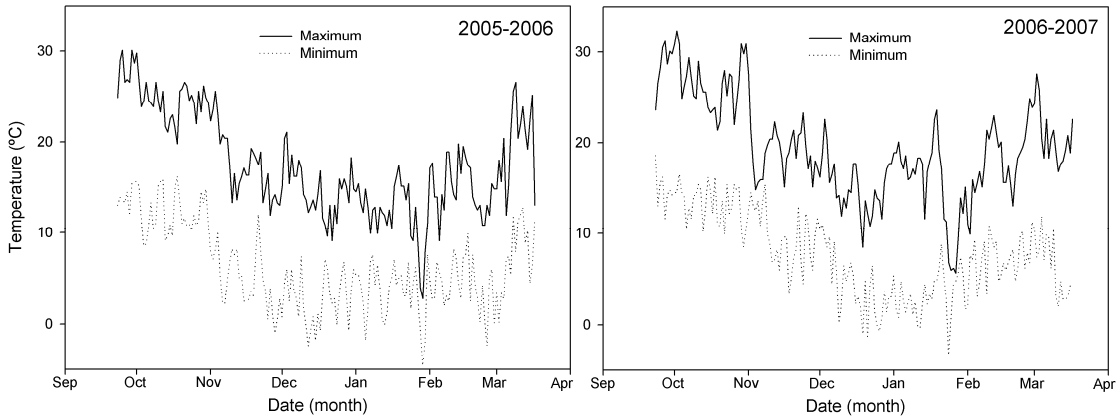


Figure 2. Maximum and minimum daily temperatures in 2005-06 and 2006-07 periods.

2.2.2. MTB for lateral vegetative buds

Seasonal progression of MTB in lateral vegetative buds followed an oscillating pattern during the two studied periods (Figure 3). During 2005-06 an important increase in MTB was reached in autumn, around late October, which was followed by a general decrease in late autumn (end of November-first of December) before reaching the maximum MTB at the beginning of winter (in late December in 'S 405/17' and in the beginning of January in 'Rojo Pasión' and 'Murciana') (Figure 3). The minimum MTB was reached by mid winter (February) just before flowering occurred. Dormancy release was considered when a continued and sharp decrease in MTB took place after chill accumulation. During 2006-07 a similar pattern was observed, although

the most important increase in MTB (maximum value for 'Murciana' and 'Rojo Pasión') occurred in autumn, at beginning of October, in all the cultivars. A sharp decrease in MTB was found in November, which coincided with unusual warm temperatures (30 ºC) (Figure 2). Thereafter, a progressive increase in MTB was observed in early winter (maximum value for 'S 405/17'). As in the previous year, the minimum values of MTB were recorded just before flowering in field conditions. The pattern of decrease in MTB of each cultivar was consequent with the time of bud burst in the field.

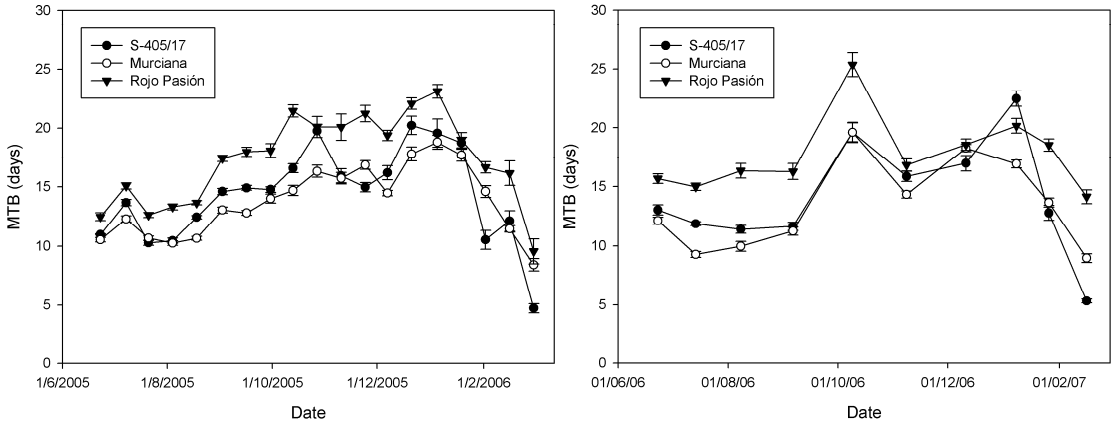


Figure 3. Progression of lateral vegetative bud dormancy in 2005-06 (left) and 2006-07 (right). Data show means of different orientations and position in the shoot.

Significant differences in MTB were found between cultivars as the season progressed and significant interaction Date*Cultivar was found in the two studied periods (Table 1 and Figure 1). This shows a strong influence of genotype over growth inhibition. There were no significant differences between shoot orientations during the two periods studied (Tables 1 and 2). On the other hand, the significant differences found in the interaction Date*Orientation did not follow any reasonable pattern. During 2005-06, significant differences in MTB were observed among bud positions along the shoot (Table 1 and 2). The MTB of the two uppermost lateral buds were significantly lower than the three subtending basal ones (Table 1 and 2).

2.2.3. MTB for terminal vegetative buds

MTB of terminal vegetative buds followed a similar pattern to MTB of lateral vegetative buds during 2006-07 (Figure 4). At the beginning of the sampling period MTB was similar for the three cultivars. As the season progressed, the MTB of 'Rojo Pasión' increased gradually until September, while MTB of 'S 405/17' and 'Murciana' slowly decreased. In October MTB increased

significantly in all cultivars, showing the highest dormancy intensity of the season. As in lateral vegetative buds, dormancy entry occurred even before any chill accumulation took place. 'Rojo Pasión' did not burst after 50 days. In November an important reduction of MTB took place, probably accentuated by the warm temperatures (Figure 2), but higher values than in September were achieved. In December 'Murciana' and 'Rojo Pasión' slightly increased their MTB. After that, a continued decrease of MTB took place as dormancy release. On the other hand, the MTB of 'S 405/17' remained constant in December, but increased in the beginning of January and diminished rapidly in the last two sampling dates (Figure 4).

Table 1. *F*-values obtained in the ANOVA for the studied variables and interactions for the MTB of lateral vegetative buds.

Variable	*DF*	*MS*	*F-value*	*P*
	2005-2006			
Cultivar	2	1548.893	258.263	0.000*
Orientation	2	17.066	2.846	0.058
Position on the shoot	4	24.427	4.073	0.003*
Date * Cultivar	36	45.595	7.603	0,000*
Date * Orientation	36	11.531	1.923	0.001*
Date * Position on the shoot	71	6.233	1.039	0.39
	2006-2007			
Cultivar	2	1679.387	184.462	0.000*
Orientation	2	9.155	1.006	0.366
Position on the shoot	3	8.182	0.899	0.441
Date * Cultivar	17	99.365	10.914	0.000*
Date * Orientation	18	12.39	1.361	0.143
Date * Position on the shoot	27	8.024	0.881	0.641

Table 2. Mean value of MTB in lateral vegetative buds depending on cultivar, orientation and position in the shoot. Different letters show significant differences among categories at 5% level, according to Tukey-b test. Mean values for each category do not consider the variation of the others categories.

Variable	*DF*	*MS*	*F-value*	*P*
	2005-2006			
Cultivar	2	1548.893	258.263	0.000*
Orientation	2	17.066	2.846	0.058
Position on the shoot	4	24,427	4.073	0.003*
Date * Cultivar	36	45.595	7.603	0.000*
Date * Orientation	36	11,531	1.923	0.001*
Date * Position on the shoot	71	6,233	1.039	0.39
	2006-2007			
Cultivar	2	1679.387	184.462	0.000*
Orientation	2	9.155	1.006	0.366
Position on the shoot	3	8.182	0.899	0.441
Date * Cultivar	17	99.365	10.914	0.000*
Date * Orientation	18	12,39	1.361	0.143
Date * Position on the shoot	27	8,024	0.881	0.641

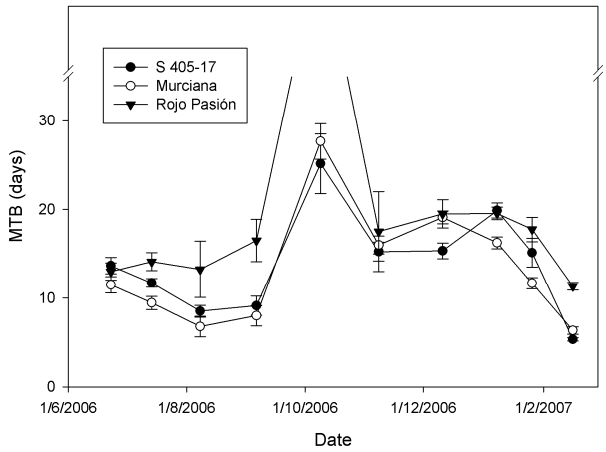


Figure 4. Progression of terminal vegetative bud dormancy in 2006-07. Terminal buds of 'Rojo Pasión' did not sprout on October 9 after 50 days in forcing conditions (shown as a break). Data show means of different orientations and position in the shoot.

The comparison of seasonal progression of MTB of terminal and lateral vegetative buds is shown in Figure 5. During the first two sampling dates no significant differences were found between lateral and terminal vegetative buds. From August to September terminal MTB acutely decreased compared to lateral MTB. From the beginning of August the MTB of terminal vegetative

buds sharply decreased reaching the maximum negative difference with regard to lateral buds. By the beginning of October, terminal vegetative buds sharply increased its MTB up to more than 25 days, reaching the maximum positive difference compared to lateral buds. After this a strong decrease of MTB was observed in both terminal and lateral buds, even though it was more accentuated in terminal buds. From November to January, MTB values followed a similar pattern in both types of buds. However, from mid January on, MTB of terminal vegetative buds decreased more acutely than lateral vegetative buds (Figure 5).

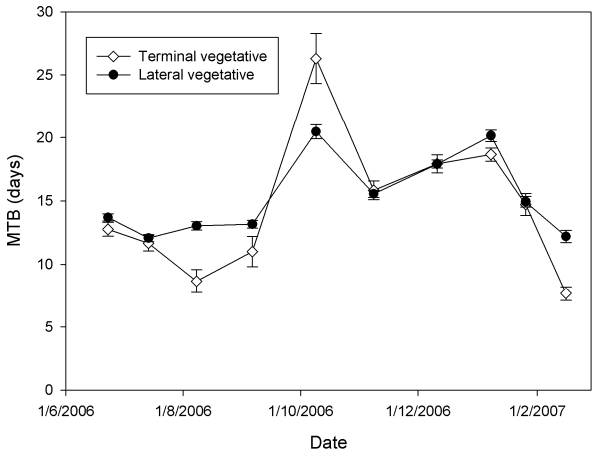


Figure 5. Comparison of MTB progression of terminal and lateral vegetative buds in 2006-07. Data show means of different orientations and position in the shoot.

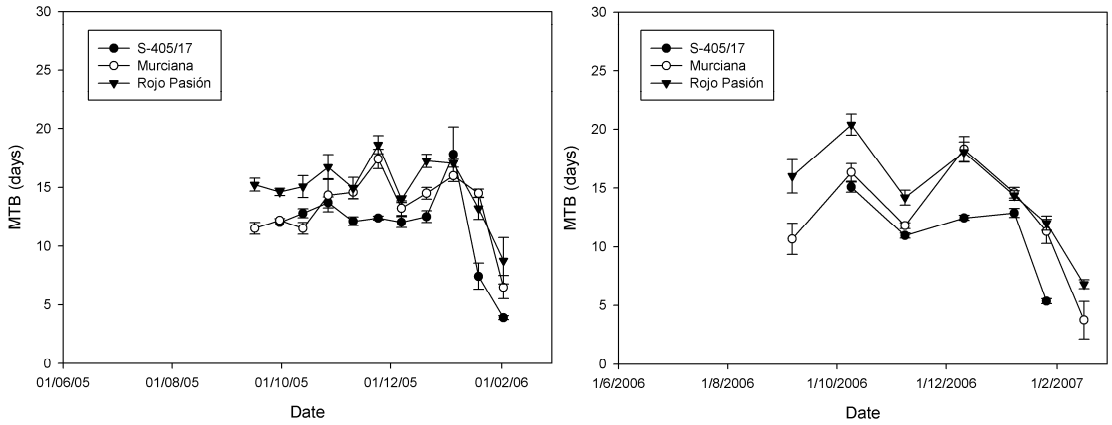


Figure 6. Reproductive bud dormancy progression in 2005-06 (left) and 2006-07 (right). Only MTB of differentiated bud flowers is shown. Data are means of different orientations and position in the shoot.

2.2.4. MTB for reproductive buds

Progression of MTB for reproductive buds followed a similar pattern than vegetative buds during the two studied periods, although it was even more oscillating than in the case of vegetative buds (Figure 6). During both 2005-06 and 2006-07, a gradual, but oscillating, increase in MTB was reached after floral whorls were differentiated in late September (Figure 7). The differentiation of floral whorls was more advanced in 'Rojo Pasión', which produced, after forcing, the first flowers readily distinguishable at the beginning of September. Floral whorl differentiation in 'Murciana' and 'S 405-17' took place in the middle of September and at the end of September, respectively. This showed that floral whorl differentiation was not completely simultaneous in different cultivars within the same species. During 2005-06 maximum MTB was reached by the end of November in 'Rojo Pasión' and 'Murciana', and in January in 'S 405/17'. From January on, MTB values began to decrease which is associated to the onset of dormancy release. Minimum MTB values were registered in the last sampling date, just before flowering in field conditions. In 2006-07 period, reproductive bud dormancy progression was considerably similar to vegetative buds. Thus, an important increase at beginning of October took place, which represented the maximum MTB values for 'S 405/17' and 'Rojo Pasión'. Later on, a sharp decrease in November and an increase in early winter (maximum value for 'Murciana') took place. MTB began to decrease when CR were partially satisfied, and the minimum MTB was achieved after bud dormancy breaking just before flowering in field conditions, as in 2005.

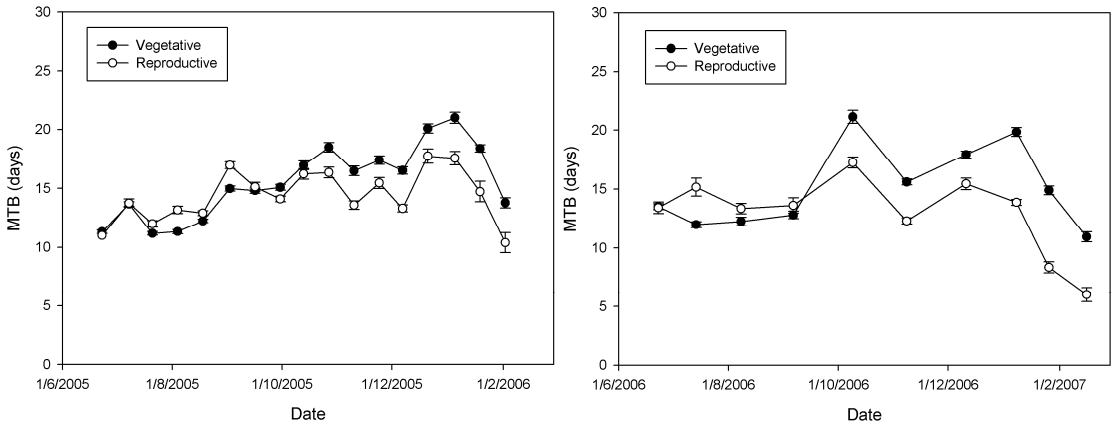


Figure 7. Comparison of MTB progression in vegetative and reproductive buds in 2005-06 (left) and 2006-07 (right). Data show means of different orientations, cultivars and position in the shoot.

Significant differences in MTB found in reproductive buds were very similar to those found in vegetative buds. Thus, significant differences were found between cultivars, positions along the shoot and in the interaction Date*cultivar in the two periods studied (Tables 3 and 4). This demonstrates the strong influence of genotype over growth inhibition progression. No significant differences were caused by orientation during the two years (Table 3 and 4). No significant differences were found either in the interactions Date*Orientation or Date*Position on the shoot (Table 3).

Table 3. *F*-values obtained in the ANOVA for the studied variables and interactions for the MTB of reproductive buds.

Variable	*DF*	*MS*	*F-value*	*P*
	2005-2006			
Cultivar	2	206.98	21.025	0.000*
Orientation	2	9.187	0.933	0.394
Position on the shoot	4	5.519	0.561	0.036*
Date * Cultivar	20	35.189	3.575	0.000*
Date * Orientation	21	15.049	1.529	0.064
Date * Position on the shoot	40	13.961	1.418	0.052
	2006-2007			
Cultivar	2	407.33	49.318	0.000*
Orientation	2	3.06	0.371	0.691
Position on the shoot	4	7.472	0.905	0.461
Date * Cultivar	17	35.342	4.279	0.000*
Date * Orientation	18	5.732	0.694	0.818
Date * Position on the shoot	35	9.241	1.119	0.297

Table 4. Mean value of MTB in reproductive buds depending on cultivar, orientation and position in the shoot. Different letters show significant differences among categories at 5% level, according to Tukey-b test. Mean values for each category do not consider the variation of the others categories.

Variable	Category	2005-2006 MTB (days)	2006-2007 MTB (days)
Cultivar	S 405/17	12.04 a	11.32 a
	Murciana	15.32 b	13.23 b
	Rojo Pasión	16.00 b	15.60 c
Orientation	South	13.36 a	13.34 a
	North-east	14.93 a	13.58 a
	North-west	14.63 a	13.60 a
Position in the shoot	1	13.69 a	13.08 a
	2	14.10 a	13.09 a
	3	13.76 ab	13.68 a
	4	15.00 bc	13.69 a
	5	16.00 c	13.99 a

The comparison of the seasonal progression of MTB for vegetative and reproductive buds is shown in Figure 7. During the floral differentiation period, from early summer to the first appearance of flowers (ca. September under our field conditions), no clear differences were found between MTB of vegetative and reproductive buds (Figure 7). However, after forming the first flowers –ca. September-, dormancy intensity in vegetative buds was higher than in reproductive buds in both years. Besides, dormancy release was earlier in reproductive buds than in vegetative buds. In spite of the lower temperatures and higher chill accumulation in 2005-06, it was not observed deeper dormancy intensity in reproductive buds in relation to 2006-07 period.

2.3. Discussion

The oscillating pattern of the seasonal progression of MTB in lateral vegetative buds (Figure 2) support the accepted idea that buds have the ability to enter into and exit from dormancy even at warm temperatures (>15 ºC) (Mauget and Rageau, 1988; Crabbé, 1994). According to Balandier *et al*. (1993a), the fluctuation in MTB values during the sampling period reflects similar changes in the bud intrinsic growth inability. At the beginning of the sampling period MTB was higher than in the latter part of the trial, showing an already imposed dormancy status as previous studies had reported (Crabbé and Barnola, 1996; Hauagge and Cummins, 1991b). This observation supports the idea that the dormancy induction is a continuum which, in some plants, begins as early as budbreak in the spring (Arora *et al.*, 2003). Crabbé and Barnola (1996) stated that the very act of bud formation is evidence of the establishment of dormancy, i.e. a result of potentially diverse processes that lead to a common result of suppressed growth.

The pattern of MTB progression obtained during autumn and winter is similar to the results obtained in Pecan by Amling and Amling (1980) and in apple by Cook and Jacobs (2000), where the onset of rest occurred prior to the advent of chilling accumulation. Williams *et al*. (1978) also found a similar pattern in decapitated 'Granny Smith' apple seedling. However, Hauagge and Cummins (1991b) working on apples, found that significant increases in dormancy started to occur only after the first frost and the beginning of chill accumulation. The MTB patterns observed in Spain coincided more closely with those documented by Balandier *et al*. (1993a, 1993b) for 'Armking' and 'Flordared' peach cultivars, cultivated on Reunion Island, in a tropical climate and those obtained by Cook and Jacobs (2000) in apple in mild winter climates in South Africa, than

those found by Rageau (1987) for 'Redhaven' peach cultivar in a temperate climate. Balandier *et al*. (1993b) questioned whether endodormancy really occurred in these cultivars in tropical conditions considering its low MTB values. Under the climatic conditions of the trials conducted in Spain, low MTB values indicated that the intensity of bud growth inhibition was never very high during the dormant period. The oscillating pattern of MTB along the winter could raise doubts as to whether endodormancy really does take place. However, the higher CR of the cultivars, compared to 'Armking' and 'Flordared' peach cultivars, and the higher chill accumulation during winter compared to that accumulated on Réunion Island, could nullify this hypothesis.

Differences between years regarding MTB values could be related to the different temperatures registered. Autumn and early winter temperatures seem to play a crucial role over dormancy intensity and dormancy progression. Thus, year 2005-06 was characterized by a cold November and December (Figure 2), which led to a progressive increase of dormancy during this period, when CR had not been still satisfied (Figure 3). Similar patterns of dormancy progression have been found in apple (Amling and Amling, 1980, Hauagge and Cummins, 1991b). Jacobs *et al*. (2002) and Heide and Prestrud (2005) have reported that the chilling period influences the progression of apple bud dormancy. On the other hand, the acute decrease in MTB observed in November 2006 could have been caused by the abnormal warm temperatures (above 30 ºC) registered in the last days of October (Figure 2). Chandler (1960) and Tamura *et al*. (1993) have reported dormancy release after the application of high temperatures. As in 2005-06, the low temperatures registered in late autumn and early winter induced a progressive increase in dormancy intensity. Only when CR were partially satisfied a sharp decrease of MTB was observed in both years, which coincided with dormancy release.

The higher chilling accumulation during 2005-06 (Figure 1) did not result in higher maximum values of MTB compared to 2006-07 (Figure 3). Nonetheless, the growth inhibition was more prolonged throughout the winter. This phenomenon could be associated with the higher chill accumulation during the end of autumn 2005 compared to the end of autumn 2006.

Our results have shown significant differences in MTB among cultivars, positions of the bud on the shoot and years (Table 1). A high variability also has been found among MTB values using single node cutting among cultivars, years and sites (Rageau 1987; Balandier *et al*., 1993a). Thus, in 2005-06 significant differences were found in the MTB depending on the bud position along the shoot, which indicated the existence of certain acrotony. The gradual increase in MTB in a basipetal

direction could suggest a gradient of increasingly deep dormancy from shoot apex to base. This gradient was established before the shoot was cut. During 2006-07, no significant differences in MTB were found between the lateral positions of the cuttings in the shoot. Considering the low chill accumulation during 2006 (Figure 1), these results could be consistent with the findings of Cook and Jacobs (1999) who stated that suboptimal winter chilling impedes development of acrotony in apple shoots.

Comparing lateral and terminal vegetative buds, the pattern of MTB progression was similar in both types of buds. However, terminal buds achieved a deeper dormancy but released from dormancy more easily than axillary buds, which is in concordance with previous works (Williams *et al*., 1978; Champagnat 1983, 1992, Crabbé and Barnola, 1996; Mauget and Rageau, 1988). Naor *et al*. (2003) stated that in apple the CR necessary for lateral vegetative buds were much higher than that needed for terminal vegetative and flower buds. Crabbé (1987) and Hauagge and Cummins (1991b) stated that during dormancy, lateral buds appeared less endodormant than terminal buds, tough this tendency was not static. It appears that, on entrance into dormancy, a distal shoot-forming or acrotonic tendency exists. This trend shifts to a proximal shoot-forming or basitonic tendency in early winter and then becomes more acrotonic as spring approaches (Barnola and Crabbé, 1991; Cook *et al*., 1998). These findings are consequent with our results.

As for reproductive buds, a similar pattern of MTB progression was found compared to vegetative buds. The differentiation of flower buds exerted an important role over dormancy intensity of vegetative and reproductive buds. After the differentiation of floral whorls, dormancy depth was always lower in reproductive buds compared to vegetative ones (Figure 7). Besides, the formation of complete flowers was concomitant to a generalised increase in the MTB in both vegetative and reproductive buds. This is in concordance with a recent work in apricot, where it was found that floral differentiation occurs before dormancy onset (Julian 2008). In both years the increment of MTB was achieved before any chill accumulation had been registered (Figure 6), which indicates reproductive bud dormancy was already established before chilling period, as it was observed in both lateral and terminal vegetative buds.

The lower depth of dormancy and the earlier dormancy release of flower buds compared to vegetative buds, found in this trial, are in agreement with Erez (2000), who stated that floral buds generally have lower CR. On the other hand, Guerriero *et al*. (1987) suggested that the differences between reproductive and vegetative buds 'times to burst' could be associated to either higher heat

requirements of vegetative buds than reproductive buds, or higher sink potential for nutrient of reproductive buds. According to our results, no significant differences in MTB in reproductive buds were found among different orientations. This is contrary to the findings of Viti *et al*. (2003) in 'San Castrese' and 'Orange Red' apricot cultivars, who found that the west orientation of the canopy and the medium-apical portion of the shoot were the best positions to obtain an earlier active growth of reproductive buds. The use of different cultivars and the different pedoclimatic conditions could have influenced this difference.

2.4. Conclusions

A shallow endodormancy state was observed in both vegetative and reproductive buds in apricot in the climatic conditions of the South-East of Spain. An oscillating pattern on dormancy progression was observed. This pattern was markedly influenced by autumn and early winter temperatures whereas the onset of dormancy occurred prior to the advent of chilling accumulation. After the differentiation of floral whorls, a higher increase in depth of dormancy was found in vegetative buds compared to reproductive buds. A deeper dormancy but an earlier release of dormancy was found in terminal vegetative buds compared to lateral vegetative buds. An effect of suboptimal chilling over acrotony was found.

3. INDUCTION AND RELEASE OF ENDODORMANCY IN APRICOT. EFFECT OF DIFFERENT CLIMATIC CONDITIONS.

3.1. Material and methdos

3.1.1. Plant material

Due to the unavailability of germplasm collections to carry out the experiments in every location studied, commercial orchards were used for the collection of plant material, except for the shoots collected in the experimental orchard of the CEBAS-CSIC in Cieza. Therefore, the availability of plant material was conditioned by the suitability of each cultivar to each area studied. The repetition of the same cultivar in different areas was frequently impossible due to the inexistence of commercial orchards. Apricot cultivars evaluated in each location and country (in Spain and South Africa) are summarized in Table 1. No rest breaking agent was applied during the periods studied.

Table 1. Classification of the cultivars studied by country, location, year and chill requirements.

	SOUTH AFRICA					
CR	Villiersdorp		Ladismith		Ceres	
	2007	2008	2007	2008	2007	2008
Very low[1,2]	Supergold		Supergold	Supergold		
Low[1,2]	Charisma	Charisma	Charisma	Charisma		
Low[1,2]	Palsteyn	Palsteyn	Palsteyn	Palsteyn		
Low[3]				Suapriseven		
Low, Medium[2]			Canino	Canino		
High[2,4]					Orange Red	Orange Red
	SPAIN					
CR	Campotéjar		Cieza		Barranda	
Low[2,4]			Currot			
Low-Medium[2,4]	Rójo Pasión		Rojo Pasión			
Medium[2,4]	Búlida de Arques*		Búlida		Búlida	
Medium[2,4]			Dorada			
Medium[2,4]			Murciana			
High[2,4]			Orange Red			

[1] Infruitec-ARC South Africa
[2] Chapter 2
[3] United States Patent PP10165
[4] Ruiz *et al*., 2007
* 'Búlida de Arques' is a cultivar slightly earlier than 'Búlida'

Spain: The plant material comprised apricot cultivars spanning the range of CR and flowering time in the apricot species in Spain. The cultivars were 'Currot', 'Rojo Pasión', 'Dorada`, 'Murciana', 'Búlida', 'Búlida de Arques' and 'Orange Red'. Cultivars were grafted onto the traditional apricot rootstocks.

South Africa: The plant material comprised 5 apricot cultivars spanning the range of CR and flowering time in the apricot species in South Africa. The cultivars were 'Supergold', 'Suapriseven', 'Palsteyn', 'Charisma', 'Canino' and 'Orange Red'. Cultivars were grafted onto the traditional apricot rootstocks.

3.1.2. Experimental design

In Spain, the experiments were conducted during 2007-2008. The cultivars studied were distributed in three different areas of the Murcia region: Campotéjar (mild area, altitude 142m, lat. 1º13'W, long. 38º8'N); Cieza (moderately cold area, altitude 241 m, lat. 38º16'N, long. 1º16'W); and Barranda (cold area, altitude 866m, lat. 38º2'N, long. 1º58'W) (Table 1). Apricot cultivars sampled in each location are shown in Table 1. Shoots were cut and put in forcing conditions at CEBAS-CSIC in the same day.

In South Africa, the experiments were conducted in 2007 and 2008. The cultivars studied (Table 1) were distributed in three locations in the Western Cape: Villiersdorp (mild area, altitude 466 m, lat. 33°58'S, long. 19°16'E); Ceres (cold area, altitude 980 m, 33°22'S, 19°0'E); and Ladismith (very mild area, altitude 550 m, lat. 33°28'S, long. 21º15'E). Shoots were delivered to the laboratories in Stellenbosch University by overnight courier.

Hourly temperatures were collected in each location with automatic data-loggers Escort Junior (Escort Data Logging Systems) in Spain and Tiny tag (Gemini Data Loggers UK) in South Africa.

In these field conditions, the initial date for chilling accumulation was considered to be when a consistent chilling accumulation occurred and the temperatures producing a negative effect (chilling negation) were scarce (Richardson *et al*., 1974; Erez *et al*., 1979b; Guerriero *et al*., 2002). Chill accumulated was assessed by chill hours (Hours below 7 ºC) (Weinberger, 1950); Chill Units of the Utah Model (Richardson *et al*., 1974); and Portions of the Dynamic Model (Fishman *et al*., 1987a, 1987b).

Unbranched one-year-old apricot shoots with a length of 30 cm were sampled ca. each 15 days from mid-May to August in South Africa in 2007, and from mid-September 2007 to February 2008 in Spain. Given the results obtained the first year, the period studied in 2008 was lengthened, and the shoots were collected from the end of January to August in South Africa.

The shoots were bundled in groups of 10 shoots per cultivar, location and date, and placed in 5 L plastic buckets containing 1 L of water and forced, in order to determine the depth of dormancy. The shoots were forced in a growth chamber with constant illumination (ca. 200 μmol m-2 s-1 photosynthetically active radiation) at a constant 25°C (Jacobs *et al*., 2002). The water was changed every two to three days, and the distal shoot end was dipped in 0.25% (v/v) sodium hypochlorite solution (3.5%) for ca. ten minutes. Approximately 1 cm of the bottom of the shoot was cut off weekly. Both vegetative and reproductive budburst was recorded every two to three days. The time to budburst (TB) was calculated as the time in days needed for budburst to occur on three shoots per bundle, i.e. days to 33% budburst.

3.2. Results

3.2.1. Maximum and minimum daily temperatures

Maximum and minimum daily temperatures during the periods studied in Spain and South Africa are shown in Figure 1 and Figure 2, respectively. In Spain, the 2007-2008 period registered temperatures similar to an average year. Barranda was the coldest area studied in Spain, with many days of minimum temperatures below 0 ºC from November to the first fortnight of February and maximum temperatures lower than those registered either in Campotéjar or Cieza. In Campotéjar, the minimum temperatures were fairly higher than in Barranda, which is consistent with the lower altitude of this location. In Cieza, the values were intermediate between those registered in Barranda and Campotéjar (Figure 1).

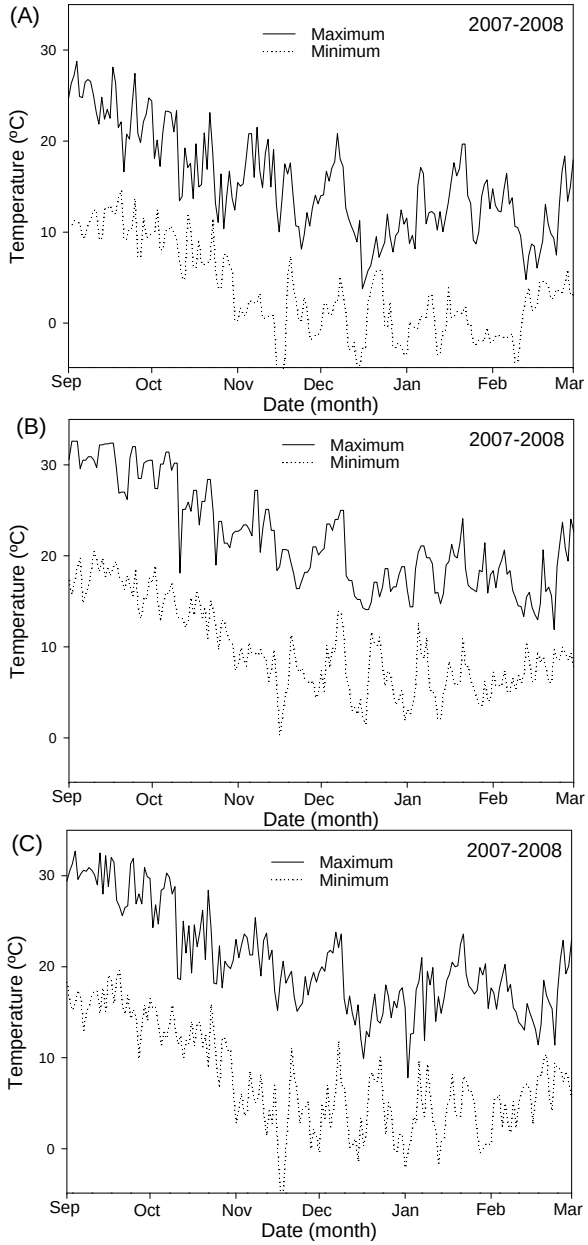


Figure 1. Maximum and minimum daily temperatures registered in 2007-2008 in different locations studied in Spain: Barranda (A), Campotéjar (B), Cieza (C).

In South Africa, 2007 was characterized by an earlier and colder winter compared to 2008. Ceres was the coldest area studied in South Africa (Figure 2), even though the minimum temperatures were higher than those registered in Barranda (Spain). In Ladismith, high daily thermal amplitude was observed, which is characteristic of a semi-desert inland area. In Villiersdorp, the area closest to the ocean, minimum daily temperatures were higher than in the other areas studied (Figure 2).

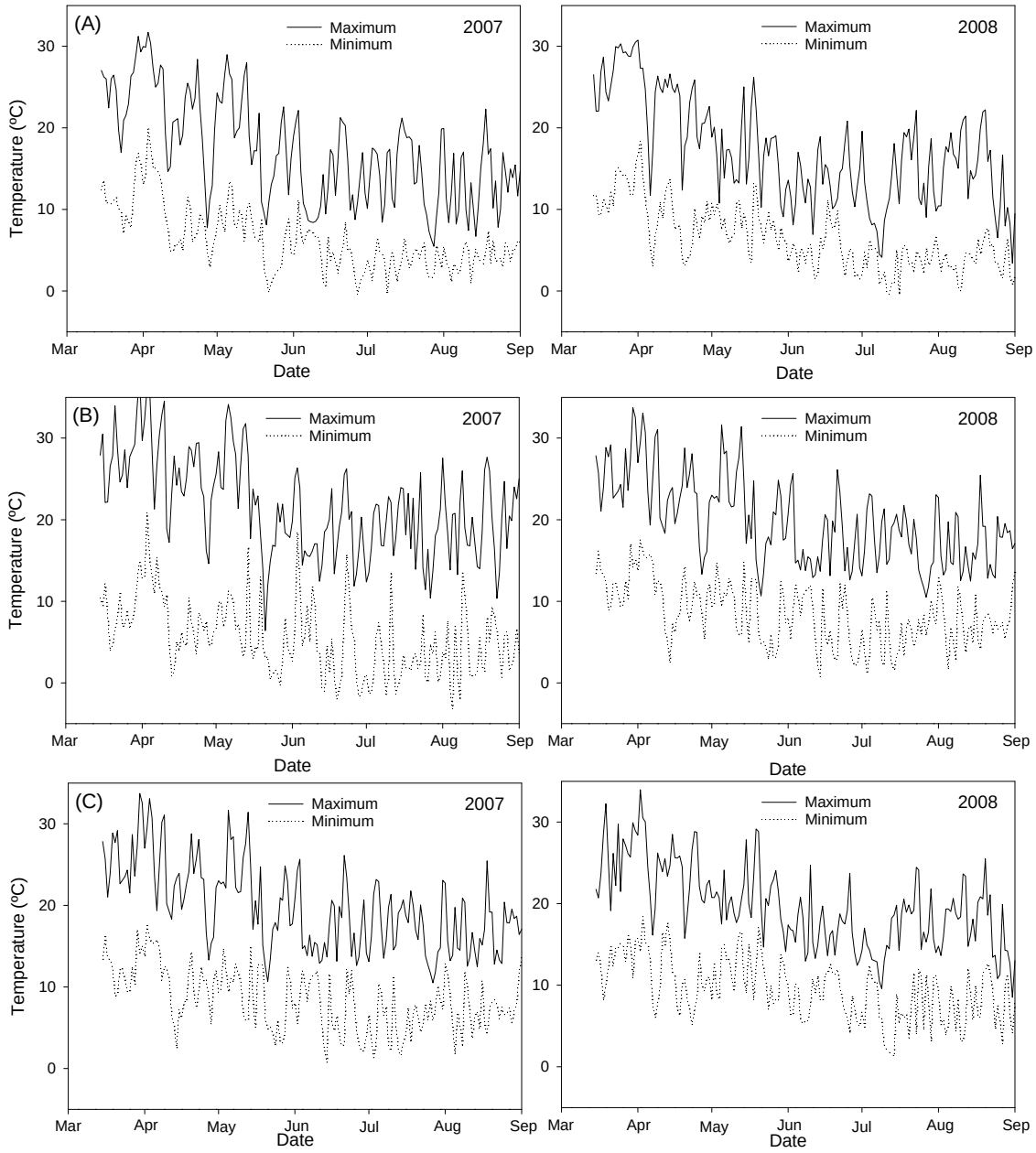


Figure 2. Maximum and minimum daily temperatures registered in 2007 and 2008 in different locations studied in South Africa: Ceres (A), Ladismith (B), and Villiersdorp (C).

3.2.2. Chilling accumulation in the different locations studied

Figure 3 shows the chilling accumulation in Spain between ca. November 1 and February 28 in Barranda, Campotéjar and Cieza, measured by 'Chill Units' (CU) (Utah Model), 'Portions' (Dynamic Model) and 'Hours below 7 ºC'. In Barranda, the chilling period began earlier than in Cieza and Campotéjar, and the accumulation was fairly higher than in the other locations. In

Barranda, ca. 1800 CU were accumulated, a value considerably higher than the accumulation in Cieza (ca. 1200 CU) and Campotéjar (ca. 900 CU) (Figure 3).

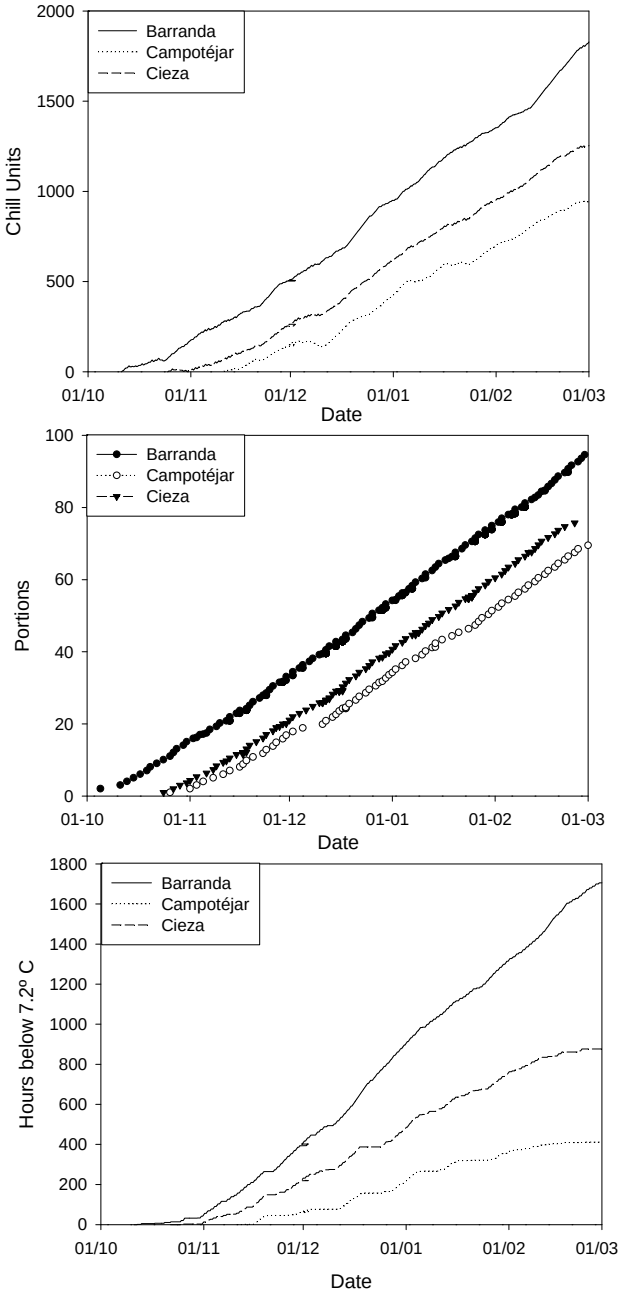


Figure 3. Chill accumulated in 2007-2008 in the different areas studied: Barranda, Campotéjar and Cieza. Chill Units, Portions and Hours below 7 ºC.

In both countries, the chilling accumulation during November (May in South Africa –SA-) was, in general, rather low, compared to December (June in SA) and January (July in SA). No accumulation was registered in October, except in Barranda.

In South Africa, the onset of chilling accumulation depended considerably on the area studied (Figure 4). In Ceres, the coldest area, the chill accumulation began earlier (second fortnight of April) than in Villiersdorp (medium area) and Ladismith (warm area) (second fortnight of May). The total chill accumulation in Ceres was fairly higher than in Villiersdorp and Ladismith (Figure 4), and was even higher than in Cieza,Spain. However, in Villiersdorp and Ladismth, the chill accumulation was very low (ca. 650 and 550 CU, respectively) compared to the values registered in Spain.

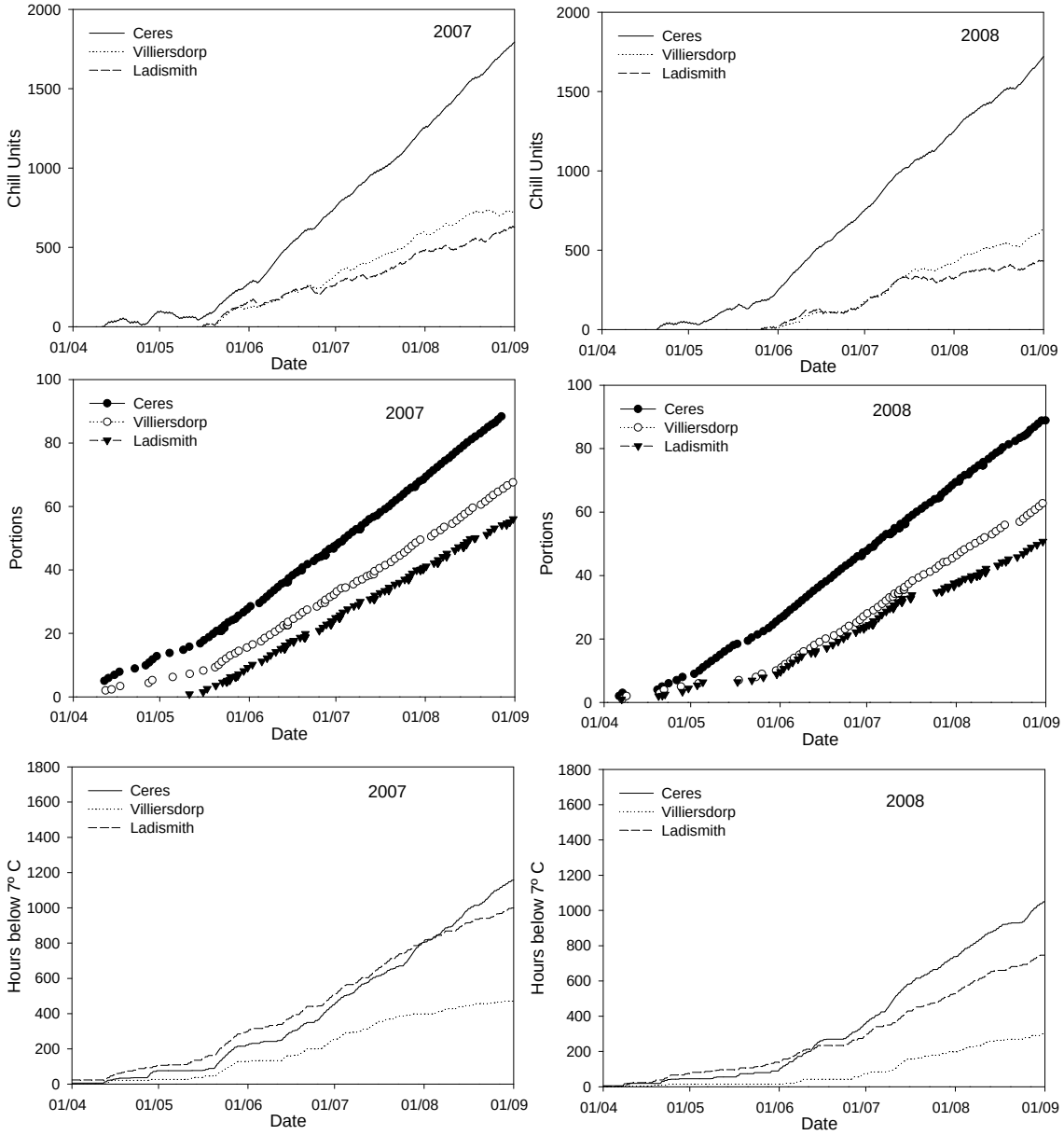


Figure 4. Chill accumulation in 2007 and 2008 in the different areas studied in South Africa: Ceres, Villiersdorp and Ladishmith. Chill Units, Portions and Hours below 7 ºC.

3.2.3. Dormancy progression in Spain

Values of time to budbreak in the different locations and cultivars studied in Spain are shown in Figure 5. In Campotéjar, the location with lowest altitude and mildest winter, the first data of time to budburst (TB) in vegetative buds were recorded in mid-September. By that date, 'Rojo Pasión' and 'Búlida de Arques' showed a shallow dormant state, which rapidly increased on the following sampling dates. The deepest dormancy was achieved in the beginning of October for 'Rojo Pasión'. At this time, a missing value was registered for 'Búlida de Arques', which achieved the deepest dormancy at the end of October. From that date on, a continuous decrease was experienced, except for a minor increase in the first days of January. Dormancy was released completely by the end of January (Figure 5). As for reproductive buds, a high percentage of missing values were registered until November. However, a similar trend to that of vegetative buds was found from November on, which suggests that this period coincides with the satisfaction of CR for breaking of dormancy. In 'Rojo Pasión', endodormancy of reproductive buds at the beginning of November was clearly deeper than that of vegetative buds.

In Cieza, a similar dormancy pattern was found in both vegetative and reproductive buds (Figure 5). In vegetative buds, on the first sampling date (September 14), 'Búlida' showed the shallowest dormancy, whereas 'Murciana' and 'Orange Red' already showed an intermediate value. On the next sampling date, an important increase was found in these three cultivars, whereas the other cultivars did not show budburst. By the end of October, all cultivars showed a deep dormancy, even though this value was lower than on the previous sampling date in the case of 'Murciana' and 'Búlida'(Figure 5). In the second fortnight of November, all cultivars registered an acute decrease in the days to budburst. By that time, less than 200 CU had been accumulated in field conditions (Figure 3). In December, all cultivars maintained or even increased depth of dormancy, except for 'Currot'. For instance, 'Dorada' and the high-chill cultivar 'Orange Red' had a TB of above 20 days. The low-chill cultivar 'Currot' decreased its TB along December and by the first week of January; 'Currot' had decreased its TB to below 10 days, reaching dormancy release. From December on, 'Currot' clearly showed the lowest endodormancy, whereas 'Orange Red' showed the highest endodormancy state, which is concomitant with the CR calculated for those cultivars in the same climatic conditions (Ruiz *et al*., 2007). From January until the last sampling date, in mid-February, a generalized decrease of the depth of dormancy was observed. As for reproductive buds, the pattern of dormancy progression found in Cieza in 'Dorada', 'Murciana' and 'Búlida' differed

with regard to low-chill cultivars. Thus, 'Currot' and 'Rojo Pasión' showed a gradual but constant decrease in TB from November to February, without a dormancy release pause in December.

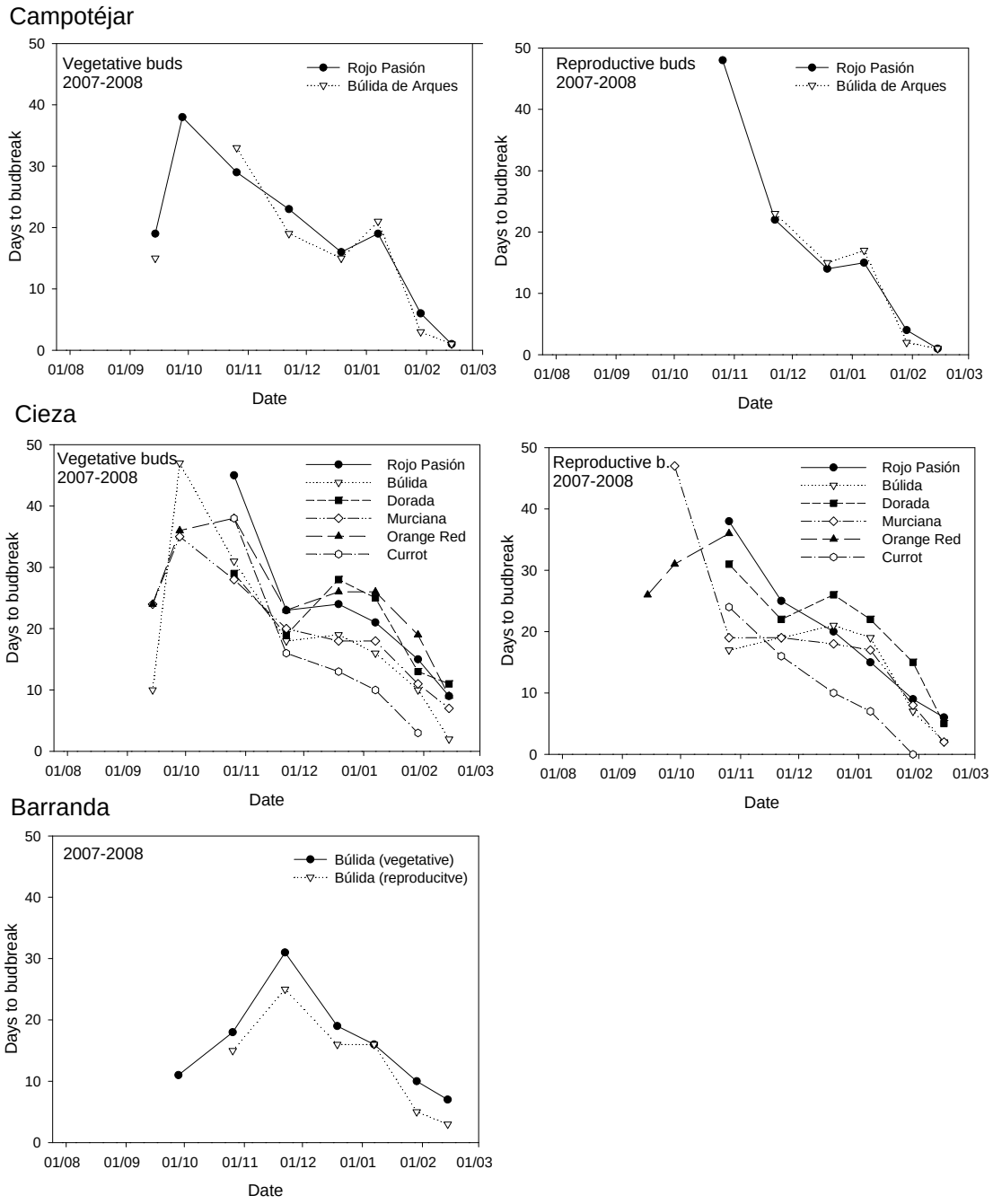


Figure 5. Time to budbreak in the different areas studied in the period 2007-2008 in Spain.

In Barranda, 'Búlida' showed a different dormancy progression than in Cieza (Figure 5). The state of dormancy continued to deepen along October and November, reaching its maximum

TB in the second fortnight of November, two months later than in Cieza, and when ca. 400 CU had accumulated. At that time, the TB in all cultivars in Cieza and Campotéjar had decreased considerably. Thereafter, a continuous decrease was observed until mid-February, which coincided with the dormancy release (Figure 5).

3.2.4. Dormancy progression in South Africa

Figure 6 shows the dormancy progression curves in 2007 and 2008 of the different cultivars studied in three different areas of the Western Cape in South Africa.

In Ladismith in 2007, a generalized decrease in TB was observed from late May (November in Spain) to late July (January in Spain), although 'Palsteyn' and 'Supergold' showed a pause in the phase of dormancy release in both vegetative and reproductive buds. 'Canino' reached maximum dormancy on the second sampling date (beginning of June – December in Spain) in both reproductive and vegetative buds. In 2008, TB data were available from the end of January (July in Spain); the results show an already imposed dormancy in all cultivars. A generalized decrease of dormancy was observed in the first days of February in all cultivars. 'Suapriseven' was the first cultivar entering into maximum dormancy (March 1), which was followed by an acute decrease, a phase of pause in the dormancy breaking and a subsequent dormancy release. 'Palsteyn' showed the same pattern as 'Suapriseven', but two months later, achieving maximum dormancy state at beginning of May. 'Charisma', 'Canino' and 'Supergold' reached maximum dormancy at the end of May, and released dormancy thereafter without pauses. As for reproductive buds, results were similar to 2007, although a deeper dormancy was observed (Figure 5).

In Villiersdorp, in 2007, a decrease in dormancy was found from late May to August, with a pause in the releasing of dormancy in the second fortnight of June. For reproductive buds, although only a few TB values were obtained on evaluated cultivars, a similar trend was found, but with a pause phase in the second fortnight of July. In 2008, buds reached dormancy in early March and maintained dormancy until a slight increase was observed in late May, which was followed by a decrease in dormancy coinciding with dormancy release. In reproductive buds, TB values showed a continuous decrease in dormancy intensity from the first of May (Figure 6).

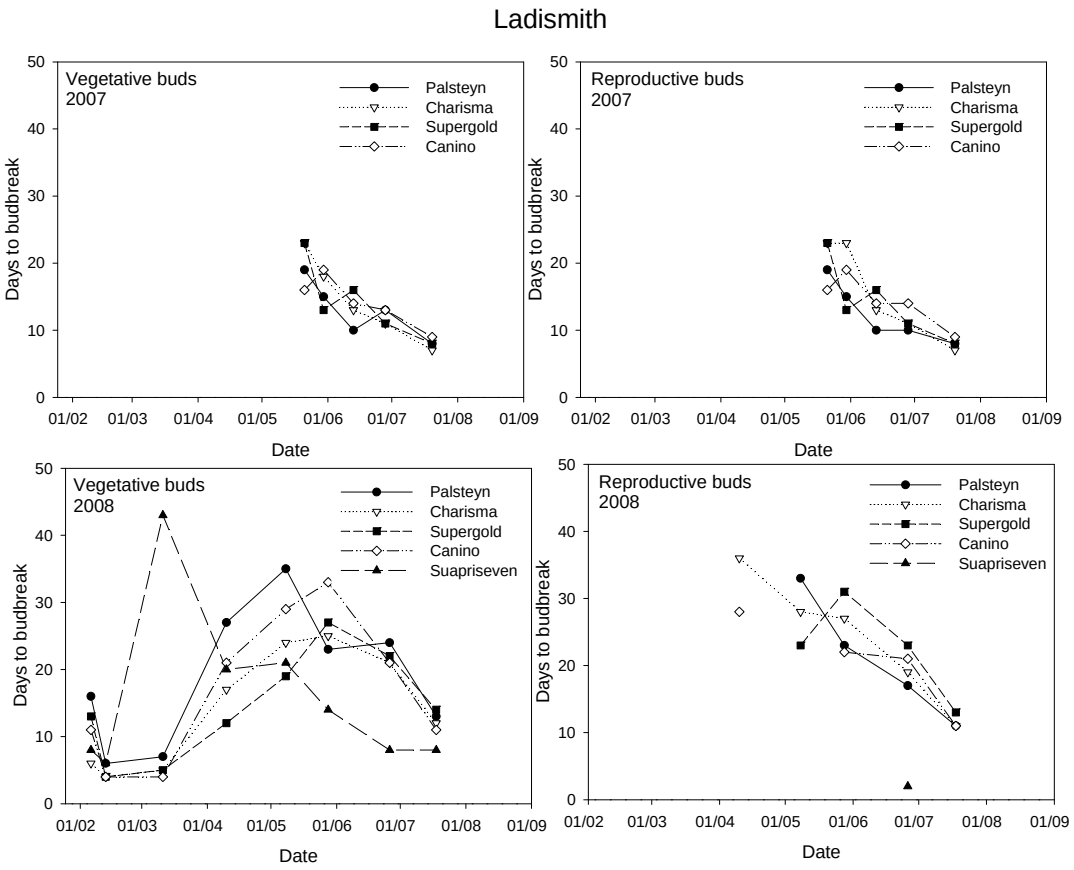


Figure 6. Time to budbreak in the different areas studied in South Africa.

In Ceres, the coldest area in South Africa, a decrease in TB in 'Orange Red' from mid May to July of 2007 was found, followed by a slight increase in dormancy intensity during July and an acute decrease in early August. In 2008, maximum depth of dormancy was achieved in the beginning of April, and dormancy intensity was maintained until the first of July. Thereafter, TB values decreased, coinciding with dormancy release by end of July. With regard to reproductive buds, a high percentage of missing values was registered. Nonetheless, the data observed suggested a decrease in TB from May to August (Figure 6).

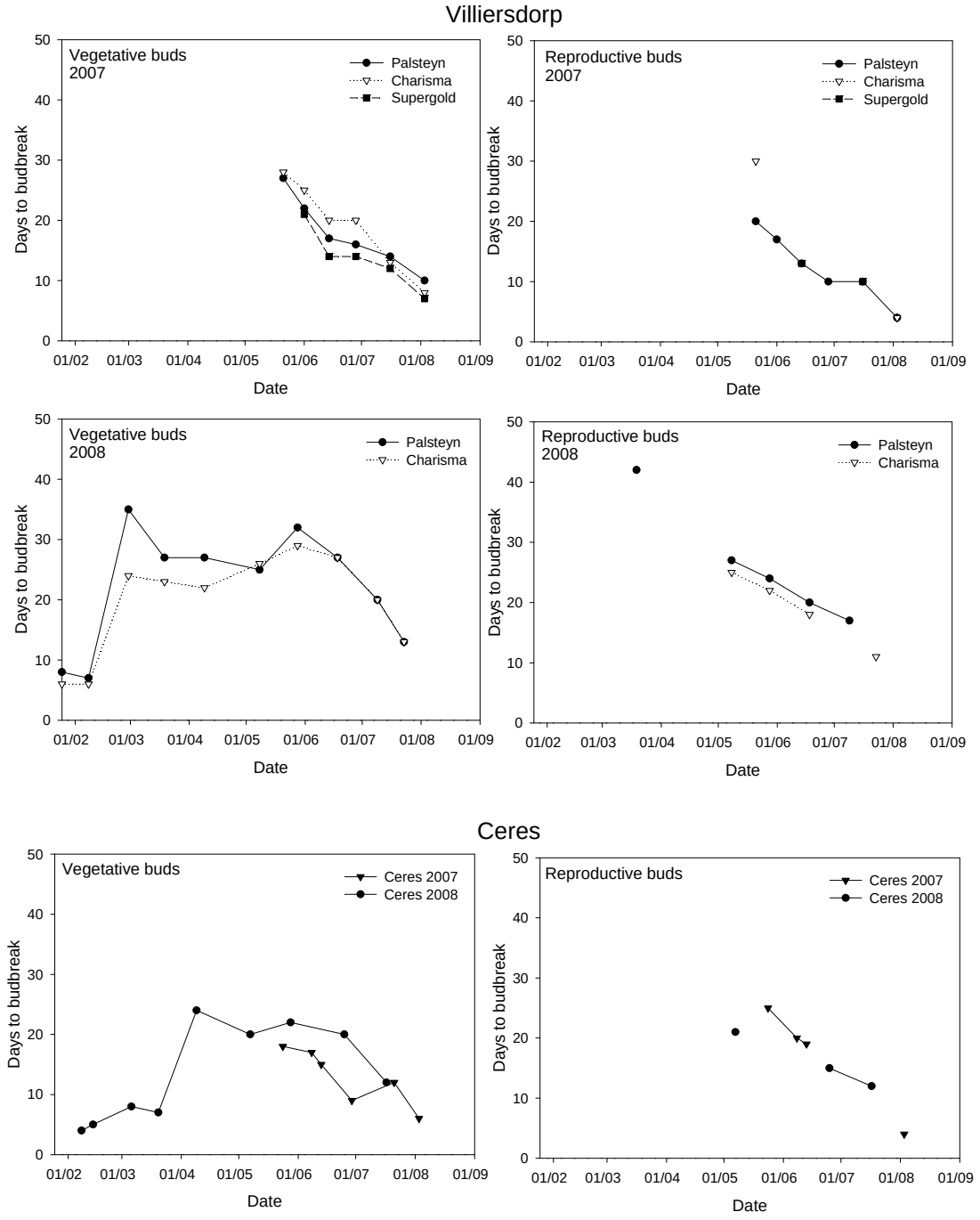


Figure 6. (Continuation). Time to budbreak in the different areas studied in South Africa.

3.3. Discussion

3.3.1. Maximum and minimum daily temperatures

The variability of temperatures registered in the three locations evaluated in Spain is consistent with the altitude variation of the locations. The proximity of the areas and the similar climate conditions make altitude the most important factor in the variability of temperatures among areas in Spain. In general, the locations studied in South Africa were warmer than in Spain. The higher variability registered among consecutive days of both maximum and minimum temperatures in South Africa should be remarked. This is consistent with the changeable winters of the Western Cape area. Besides, the differences of temperature registered in South Africa were associated with both altitude and geographical location. Thus, a high thermal amplitude was found in Ladismith, which is situated more inland in a sub-desert area.

3.3.2. Chilling accumulation in the different locations studied

In Spain, according to the chill accumulated in each area, the locations could be classified as a cold area (Barranda), moderately cold area (Cieza) and warm area (Campotéjar). Similarly, in South Africa, Ceres could be classified as a cold area, whereas Villiersdorp and Ladismith could be classified as very warm areas. However, this classification highly depends on the model used for assessing chill accumulation. In Ladismith, which is characterized by a semi-arid climate and high daily thermal amplitude, Hours below 7 ºC were similar to those registered in Ceres in 2007 and Cieza, Spain, although the CU accumulation was considerably lower. Thus, Ladismith and Villiersdorp registered a similar value of CU, but more than two times the Hours below 7 ºC were registered in Ladismith. According to the Hours-below-7 ºC-Model, Ladismith would be a moderately cold area, whereas Villiersdorp would be a warm area.

In Spain, the differences in chill accumulated among locations were considerably higher when the Hours-below-7 ºC and Utah Models were used. The Dynamic Model minimized the differences among the locations. In South Africa, the Dynamic Model also minimized the differences between locations as well as between years. This can be explained because this model considers the synergic effect of moderate temperatures with low temperatures for breaking dormancy (Fishman *et al*., 1987a; 1987b); this is the factor responsible for homogenising the total chill accumulation between years. However, the Dynamic Model also reduced considerably the

variability between locations in Spain and South Africa. Regarding the variability between years in South Africa, the Dynamic Model showed the lowest variability (Figure 4). A high variability was found in the Hours-below-7 ºC-Model. These results agree with those previously found by Ruiz *et al*. (2007) in Spain. In both cases, the Dynamic Model and Utah Model showed the lower variation among years, and the Hours-below-7 ºC-Model registered a high variability. It should be remarked that the Dynamic Model showed a very low variability in spite of the markedly different temperatures registered in the different locations and years (Figure 1, Figure 2). This indicates a high conservancy of the Dynamic Model with regard to showing differences between patently different years and locations. The use of chill accumulated by the Dynamic Model under these climatic conditions could mask the negative effect of an insufficient chilling accumulation in a mild winter area.

It is worthwhile to mention that the temperature values were registered in a meteorological station as usual. The temperature values in the apricot buds during daylight hours were always higher than the values registered in the meteorological station. Therefore, the chill accumulation calculated is higher than if it would be calculated using the bud temperatures.

3.3.3. Dormancy progression in Spain

The pattern of dormancy progression in Cieza was very similar to that observed in Campotéjar. However, the chill accumulation in Cieza was higher than in Campotéjar (200 CU or 400 Hours below 7 ºC more in Cieza). In both areas, the deepest dormancy was reached in all cultivars before any chill had been accumulated. This result contrasts with the common belief that buds reach dormancy with the onset of chilling, closer to winter (Crabbé, 1994). Hauagge and Cummins (1991b), working on apples, found that significant increases in dormancy started to occur only after the first frost and the beginning of chill accumulation. However, Cook *et al*. (2000) found that the apple cultivars ‘Granny Smith’ and ‘Golden Delicious’, low and high chill cultivars respectively, reached maximum dormancy before any considerable chill had been accumulated in a moderately cold area (1350 CU of mean chill accumulation). Amling and Amling (1980), working on Pecan, found that the onset of rest occurred prior to the advent of chilling accumulation. Cook *et al*. (1998) found apple rootstocks already dormant in the beginning of October in Belgium. Besides, Tromp *et al.* (2005) stated that winter dormancy usually starts in summer and may have already terminated by mid-winter. Heide (2008) indicated that the photoperiodic response of the *Prunus* species is highly temperature dependent. The advent of lower minimum temperatures in September

(compared to summer) and the rapidly shortening photoperiod around fall equinox could trigger the induction of dormancy. This could explain the early dormancy entrance. However, the reason for variation among locations would remain unclear. In Barranda, the coldest location studied in Spain, although dormancy begins also in late summer, the maximum endodormancy is not reached until the end of November (Figure 5).

On the other hand, the data obtained in Cieza are, in general, in accordance with the dormancy release dates of flower buds found for the same year, cultivars and location (Chapter 3). However, some differences should be remarked, for example, the cultivars 'Rojo Pasión', 'Dorada' and 'Orange Red' had similar values of TB by mid-February. These are low-medium, medium and high chill cultivars, respectively. Thus, with this methodology it was not possible to accurately differentiate the dormancy release among cultivars with manifestly different CR. Besides, the low-chill cultivar 'Currot' and the high-chill cultivar 'Orange Red' had the same maximum TB value, although no previous budburst was found in 'Currot'. Regarding the different patterns between cultivars found in reproductive buds, the lower CR of 'Currot' and 'Rojo Pasión' showed a more continuous decrease in dormancy intensity compared to the other cultivars in Cieza (Figure 5).

In Barranda, the dormancy state of 'Búlida' began to deepen in late September with the advent of low temperatures and a decreasing photoperiod, which agrees with the findings of Heide (2008). However, 'Búlida' reached its deepest dormancy only when ca. 400 Chill Units had been accumulated, which coincided with leaf fall. Hauagge and Cummins (1991b) stated that apple bud dormancy starts to intensify soon after bud formation and reaches maximum intensity by the time of leaf fall/senescence. In Cieza and Campotéjar, with higher minimum temperatures in late summer, the deepest dormancy was achieved nearly two months earlier, when no Chill Units had been accumulated. These results contradict the results found by Cook *et al*. (2000) in the apple cultivars 'Granny Smith' and 'Golden Delicious'. These cultivars reached maximum dormancy before any considerable chill had been accumulated in a moderately cold area (1350 CU of mean chill accumulation), whereas they needed ca. 600 CU to reach maximum dormancy in a warm area.

To summarize, dormancy increased more rapidly in warm areas than in cold areas, and maximum depth of dormancy was achieved earlier. As photoperiod conditions were quite similar among these areas, the climatic conditions imposed by different altitudes may explain these differences. Thus, the warmer the area, the earlier deep dormancy is achieved. The depth of dormancy (maximum TB value) was not related to the CR of the cultivars. However, during

dormancy release, low CR cultivars had, in general, lower values of TB in both vegetative and reproductive buds, whereas the high CR cultivars also showed higher endodormancy values in this period.

3.3.4. Dormancy progression in South Africa

The time of the onset of dormancy was dissimilar in the different locations studied in South Africa. In Ladismith and Ceres, all cultivars except 'Suapriseven', entered into dormancy at the beginning of April, which is in accordance to the low minimum temperatures registered at the end of summer. In Ladismith, the location with the lowest chil unit accumulation (Figure 4), an already established dormancy was found in the middle of summer in 2008. This agrees with the findings of Crabbé and Barnola (1996), who stated that the very act of bud formation is evidence of the establishment of dormancy. In Villiersdorp, where Chill Units and hours-below 7 ºC are very low, a high level of dormancy was already achieved by March. In Ceres, maximum dormancy was already achieved in April. This result is in agreement with the dormancy progression found by Cook *et al*. (1998) in apple in a nearby area. Thus, dormancy was reached earlier in the areas with higher minimum temperatures or warmer areas. This result disagrees with those found by Cook *et al*. (2000) in apple, where maximum dormancy was achieved earlier in the colder area. As for the dormancy pattern, most of the cultivars had reached maximum depth of dormancy by late autumn (during May in South Africa), which was followed by a decrease in TB, a pause period (when dormancy depth remained constant or even increased), and subsequent dormancy overcoming. The exceptions were the cultivars 'Canino', 'Charisma' and 'Supergold', studied in Ladismith, which in 2008 reached maximum dormancy at the beginning of June and released dormancy thereafter with no pause period. The different maximum depths of dormancy found among these cultivars with different CR agrees with previous results in several species such as apple (Hauagge and Cummins, 1991a; Cook, 2000); Pecan (Amling and Amling, 1980); cherry (Kapp and Cook, personal communication); and peach (Balandier, 1993b). Maximum depth of dormancy was also similar among the different locations studied in both countries, which could contrast with the different mean times to bud break found by Balandier *et al*. (1993a) on Reunion Island (21º5' S, 55º E) and in Clermont-Ferrand, France (46ºN, 3ºE). The greater similarity between climatic conditions studied in our experiment compared to those in the study of Balandier *et al*. (1993a) could explain the divergence of results.

The generalized earlier dormancy release of reproductive buds compared to vegetative buds is in agreement with the established concept of lower CR in reproductive buds (Erez, 2000).

Within each country, the cultivars in locations with higher minimum temperatures in late summer reached dormancy earlier than those in colder locations. Comparing areas with similar minimal temperatures, dormancy onset was generally earlier in South Africa than in Spain. The dormancy patterns found in South Africa were similar to those found in Spain. However, some differences should be remarked. 'Suapriseven', for example, showed the same pattern found in 'Búlida' in Cieza (Spain), but maximum dormancy and dormancy release occurred ca. one month earlier, considering the sixth month lag, in 'Suapriseven' in Ladismith.

'Búlida' released from dormancy on similar dates in the different areas, whereas the chill accumulated was significantly different. Besides, 'Canino' showed considerably lower CR in Ladismith than when grown in Cieza, where has ca. 780 CU. These results agree with those found by Balandier *et al*. (1993a) in markedly different climatic conditions. This lack of homogeneity in the CR to overcome dormancy raises serious doubts about the reliability of the models used. Consequently, several hypotheses and questions may arise. For example, can we use these linear models along the endodormant period to characterize dormancy progression through the effect of the variable of temperature alone? Might there be an uneven effect of temperature and other variables along the endodormant period? And are the CR of a cultivar a constant, or are they an extremely variable parameter depending on the environment? Variables such as photoperiod (Heide, 2008), time of temperature application and combination of cold and warmth should be included in the models. Another question that should be taken into consideration is whether or not these models really describe the physiology of the trees. It is possible that the previous success of the models used in the areas where they were developed is a result of the fact that the models correspond with the real physiological process that guides dormancy behaviour. Yet once the models are used in different areas, a missing link with the physiology soon arises, calling into question whether the models are really measuring the signals that control dormancy behaviour in the plant.

Moreover, the dormancy curves described in the six locations studied reflect a shift towards precocity compared to previous results in colder areas. What is more, within all the areas studied, the warmer areas presented an earlier dormancy onset and earlier dormancy release than the colder areas; within countries with no significant latitude variation, endodormancy was achieved earlier in the warmer locations. Similarly, the critical photoperiod for growth cessation (first stage before

dormancy) clinally decreased in northern tree ecotypes according to variation in latitude or altitude (Olsen 2003; Olsen *et al*., 2004). A clinal variation in light quality requirements to maintain growth has also been demonstrated in woody species, at least for northern populations (Olsen, 2006).

Consequently, and taking into account our results, a clinal variation in dormancy progression under warm temperatures could be hypothesized for apricot cultivars in warm-winter areas. Thus, the earlier resumption of growth due to the earlier, favorable temperatures from late winter to spring could be related to the subsequent precocity to reach endodormancy. This would be in accordance with the clinal variation between cold and warm areas regarding flowering, growth capacity after grafting, and even ripening, observed in apricot in the climatic conditions studied.

This hypothesis would be in accordance with the fact that in warmer climatic conditions, the accuracy of the models, generally developed in colder areas, has been questioned (Ruiz *et al*., 2007; Linsley-Noakes, 1994; Balandier *et al*., 1993). Some adaptations of the models to warm conditions could also be interpreted as a way to reduce the effect of warm temperatures, either negating the effect of very warm days (Positive Chill Units Method, 1995); considering the properly documented, positive effect of moderate temperatures (Dynamic Model, Fishman, 1987a); or minimizing the variability through the election of the most suitable temperature threshold (Cesaraccio, 2004).

3.4. Conclusions

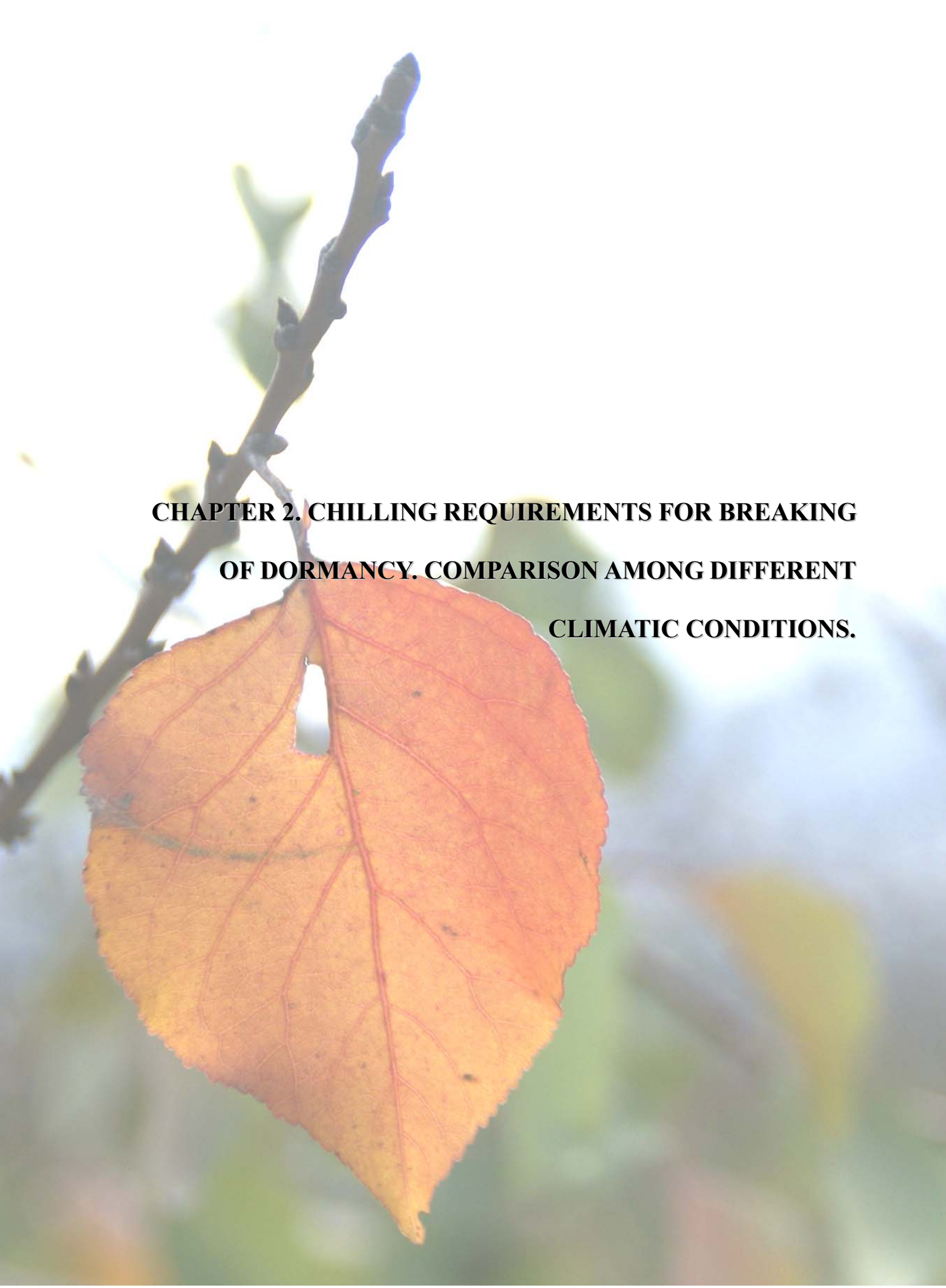
In spite of the different chill accumulations, dormancy patterns and maximum depth of dormancy found in South Africa were similar to those found in Spain, but with certain phase differences depending on the area. An earlier maximum depth of dormancy was found in those areas with HIGHER minimum temperatures at the end of summer. Similar results were obtained among cultivars with different CR, even though dormancy release tended to be earlier in low CR cultivars. Comparing vegetative and reproductive buds, the dormancy patterns and maximum depth of dormancy were similar, but dormancy release was earlier in reproductive buds.

Dormancy induction took place in late summer, prior to the advent of chilling temperatures. The decrease of minimum temperatures in late summer, coinciding with the decreasing photoperiod, could to trigger the onset of dormancy. Nonetheless, an interaction with other factors, such as altitude, might be implied, as maximum dormancy took place earlier in warmer areas.

The assumption that dormancy induction is a result of chilling accumulation seems erroneous in the warm areas studied in both countries. Considerable decrease was found in dormancy depth before the advent of chilling accumulation in warm areas, suggesting that dormancy release could be influenced by other factors. These factors appear to be more related to the previous growing season than to the winter itself. The chill accumulated when dormancy release took place for the same cultivars in different locations was considerably different.

Through the improvement of the physiological knowledge of the basis of dormancy, especially regarding the external signalling related to dormancy, the development of more multifaceted and reliable models could be possible.

Finally, a clinal variation in dormancy progression under warm temperatures for apricot cultivars in warm-winter areas is suggested. The wide range of plant material studied, with parental ascendance from quite diverse climatic provenances, and consequently, with different adaptations, could have contributed to accentuating the complexity of the responses.



CHAPTER 2. CHILLING REQUIREMENTS FOR BREAKING OF DORMANCY. COMPARISON AMONG DIFFERENT CLIMATIC CONDITIONS.

1. INTRODUCTION

Apricot culture is greatly restricted by climatic conditions, especially related to chill accumulation in several growing areas, with a significant influence on productivity (Quamme *et al*., 1982; Guerriero and Bartolini, 1991). The difficulty that a number of apricot cultivars have in adapting to environmental conditions different from those of their origin is well known (Bassi *et al*., 2006), and it is due mainly to the need for an adequate satisfaction of their CR for breaking of dormancy. Knowing the chilling requirement of a cultivar has a significant practical and economic impact on the control, maintenance and production of woody plants (Fennell, 1999), and it is necessary for crop management of apricot cultivars in their most suitable areas. In this manner, incomplete dormancy release affects tree behaviour in three main ways: late bud break, a low level of flower bud break and a lack of uniformity of leafing and bloom, resulting in a higher flower bud drop (Legave *et al*., 1982; Viti and Monteleone 1991, 1995; Erez, 2000). On the other hand, in the case of cultivars with low CR (i.e. early-flowering cultivars) growing in cold winter areas, blooming happens too early because CR are quickly satisfied. In this case, the low temperatures can damage the swelling buds in their more susceptible phenological stages (Bartolini *et al*., 2006b), resulting in the appearance of floral anomalies (Clanet and Salles, 1972), and frost can induce an important loss of yield (Scorza and Okie, 1990).

In mild winter areas, where early-ripening temperate fruits are usually grown, CR are usually not adequately satisfied. The knowledge of CR of the cultivars is crucial to the correct application of chemical breaking agents. Thus, chemical breaking agents are usually applied when about two-thirds of the CR has been satisfied (Erez, 2000). This application is essential to guarantee a proper dormancy release and to bring forward the date of dormancy breaking, aimed at obtaining an adequate level of productivity and early flowering and, therefore, an early harvest (Erez, 1987a).

In apricot, to establish with precision the flower bud chilling and heat requirements is of crucial importance, and it could allow the adaptability of a cultivar to be more accurately predicted. Different models have been developed to explain the relationships between dormancy breaking and temperature, which is the most decisive climatic factor. Hutchins suggested in a 1932 unpublished oral report the use of Hours below 7 ºC as a measure of the amount of cold received during winter (Sharpe and Sherman, 1990). Later on, Weinberger (1950) used this method to estimate CR in peach. The establishment of the Utah Model by Richardson *et al*. (1974) supposed an important advance. The Utah Model is the one most widely used to assess CR; it assigns chill unit values to

different temperature ranges, and thus weights the efficiency of different temperatures for CR fulfilment. This model performed well under temperate conditions but failed to predict the end of dormancy under subtropical conditions (Linsley-Noakes and Allan, 1994; Erez, 2000). Subsequently, models adjusted with regard to the Utah Model were developed, such as the Low-Chilling Model (Gilreath and Buchanan, 1981b); North-Carolina Model (Shaltout and Unrath, 1983); Positive Chill Units (PCU) (Linsley-Noakes *et al*., 1994); and others. Couvillon and Erez (1985b), Erez and Couvillon (1987) and Erez *et al*. (1979a) reinforced the hypothesis that the dormancy-breaking process could be explained as a two-stage reaction. The first is reversible by high temperatures; the latter stage is irreversible. Fishman *et al*. (1987a, 1987b) proposed the Dynamic Model, which improved some difficulties of the Utah Model, especially in mild-winter climates (Erez *et al*., 1990). More recently, Cesaraccio *et al*. (2004) developed the Chill Days Model, which related the beginning of chill accumulation to a phonological stage (i.e. leaf fall or harvest) and calculates the chill and anti-chill days and the chilling requirement by trial and error to minimize the root mean square error of predicted and observed bud-burst dates.

Problems related to the inaccurate selection of cultivars with unsuitable chilling requirements occur, affecting apricot production, especially in mild winter climates. Depth of rest, and consequently the chilling requirement, is a specific parameter of each cultivar. Therefore, important differences between cultivars have been reported (Saure, 1985; Erez and Fishman, 1998; Egea *et al*., 2003; Guerriero *et al*., 2006; Viti *et al*., 2006; Ruiz *et al*., 2007). Moreover, environmental conditions linked to different locations or other effects of the year seem to play an important role in relation to the chill requirements variability for each genotype. Previous work reported an important effect of the year in several apricot cultivars (Ruiz *et al*., 2007). The time of application of cold and heat during the dormancy period and the incidence of the plant physiology stage could be related to the chilling requirements variability (Weinberger, 1950; Thompson *et al*., 1975; Couvillon and Erez, 1985b; Young, 1992; Tehranifar *et al*., 1998).

The lack of a systematic calculation of chilling requirement for apricot cultivars and new selections, in addition to the scarce, often dissimilar units of CR -chill hours (Weinberger, 1950), Chill Units (Richardson *et al*., 1974) or Portions (Fishman *et al*., 1987a, 1987b), has entailed problems related to the inaccurate selection of cultivars with unsuitable CR, affecting apricot production, especially in mild winter climates.

The data available for the apricot species, which is the least-studied temperate fruit with regard to CR, show that the range of CR in most apricot cultivars is from 800 to 1,200 Chill Units, with extreme values ranging from 500 to above 1,400 (Guerriero *et al*., 2002; García *et al*., 1999; Tabuenca, 1964; Bailey *et al*., 1978, 1982; Ruiz *et al*., 2007). Besides, the CR is not a constant factor (Tromp *et al*., 2005). It is genetically determined, but other environmental factors such as latitude and elevation can affect its value (Lang, 1989).

On the other hand, heat requirements (HR) for flowering represent the thermal integral required for flowering after breaking of dormancy. It is still not clear whether cultivars have specific HR for flowering (Overcash, 1965; Gianfagna and Mehlenbacher, 1985; Rom and Arrington, 1966), or whether flowering date is determined basically by CR (Brown, 1957; Swartz and Powell, 1981; Couvillon and Erez, 1985a).

Even though the risks related to the lack of knowledge of the HR of apricot cultivars are scarce, knowing these values will provide us with more possibilities for the management of this crop. For example, a cultivar with low CR but high heat requirement could be cultivated in relatively cold areas (Citadin *et al*., 2001). Hence, methods for determining the HR of blooming have been developed (Richardson *et al*., 1974; Anderson *et al*., 1986). These methods essentially consist of establishing the heat accumulation, above a threshold, to which a tree is exposed, from breaking of dormancy until flowering date.

The aim of the first part of this chapter, in two successive years in Spain and Italy, was to study the effect of different climatic conditions on the overcoming of dormancy in several apricot cultivars growing in two different environmental areas: Murcia (Spain) and Tuscany (Italy). The effects of location and year were evaluated as well as the accuracy of the Utah Model in assessing the CRs in a Mediterranean climate.

The second part of the chapter, is aimed at the calculation of the CR for breaking of dormancy and the heat requirement for flowering of a group of apricot cultivars which cover the full range of flowering time in this species in the two hemispheres, as well as the comparison of some of the same cultivars in the two climatic conditions. Moreover, comparison and analysis of the different evaluation models of CR will be accomplished, as well as the study of relationships between chilling, HR and flowering dates. The work was carried out during four successive years in Spain and two consecutive years in South Africa

Hence, the information obtained will provide a better understanding of the apricot species regarding chill, HR and flowering dates, which will be very useful for improving apricot cultivation. Besides, the comparison of the behaviour of the same cultivars in markedly different climatic conditions will be very helpful to the understanding of the possibilities of adaptation of apricot and the portability of the chill requirement units calculated with Chill Units, Portions or Hours-below-7 ºC-Model in different climatic conditions.

2. CHILLING REQUIREMENTS FOR BREAKING OF DORMANCY IN MURCIA (SPAIN) AND TUSCANY (ITALY).

2.1. Materials and methods

2.1.1. Plant material

During two consecutive seasons (2006-2007 and 2007-2008), trials were carried out on mature apricot trees (*Prunus armeniaca* L.) of cultivars 'Currot', 'Goldrich', 'Orange Red', 'San Castrese' and 'Stark Early Orange' (SEO), which were chosen on the basis of their supposed different flower bud CR, from low to medium and high CR (Ruiz *et al*., 2007). The cultivars were evaluated in two different experimental fields located in representative crop areas of Italy and Spain, under a typical Mediterranean climate. The environmental conditions were the following: Italy, Tuscan coastal area (Venturina-Livorno, altitude 6 m, lat. 43°02' N, long. 10°36' E) with mild winter conditions; and Spain, Murcia (Cieza-Calasparra, altitude 241 m, lat. 38º16' N, long. 1º16' W), a dry and hot region.

2.1.2. Methods

Hourly temperatures were recorded by automatic data-loggers: Tinytag Plus ® (Gemini Data Loggers, West Sussex, UK, 2003) in Italy and Escort ® (Datalogging Systems, Buchanan, Virginia, USA, 2002) in Spain. The amount of cold received by the plants was quantified in terms of Chill Units (CU) calculated according to the Utah Model (Richardson *et al*., 1974). In field conditions, the initial date for chilling accumulation was considered to be when a consistent chilling accumulation occurred and the temperatures producing a negative effect (chilling negation) (Richardson *et al*., 1974; Erez *et al*., 1979a; Guerriero *et al*., 2002) were scarce.

In both locations, the chilling requirements for each genotype were estimated using a common protocol. From the beginning of the chilling accumulation in the orchard, for each cultivar, three one-year-old fruiting shoots from the west and top part of the canopy, as suggested by Viti *et al*. (2003), were collected every 7 days to determine the overcoming of endo- and ecodormancy. Endodormancy was established by the 'forcing test', a validated method proposed for apricot (Guerriero *et al*., 2000). The fruiting shoots were placed in a 5%-sucrose solution and maintained

for 7 days in a growth chamber under the following environmental conditions: temperature 23°C, relative humidity 60% and photoperiod 12 hours at 300-400 μE m^{-2} s^{-1}.

Both physical and physiological parameters were used to determine the date of breaking of endo- and ecodormancy (Faust *et al*., 1995; Guerriero *et al*., 2002). From sampled shoots, before and after forcing, flower buds (3 replicates of 15 buds/cultivar) were sampled and weighed fresh to establish the dormancy release. The date of breaking of endodormancy was established when, after 7 days in the growth chamber, 30% of the flower buds were in Baggiolini´s stage B – C (Baggiolini, 1952), and there was a difference of at least 25-30% between the weights of unforced and forced buds. For establishing the end of ecodormancy, unforced flower buds were examined, and the reactivation phase (eco-dormancy ended) was considered when a fresh weight increase of at least 25-30% occurred between two successive bud samplings in the field (Guerriero *et al*., 2000) and 30% of the flower buds were in Baggiolini´s stage B – C. The chilling requirements for breaking endo- and ecodormancy coincided with the chill accumulated until the date of dormancy release in both cases.

The heat requirements of the evaluated cultivars from breaking of endodormancy to ecodormancy release were calculated as the Growing Degree Hours (GDH) accumulated in the experimental orchard, following the model proposed by Richardson *et al*. (1974).

Under field conditions, the flowering date was recorded when 50% of flowering (F_{50}) occurred. Periodical checks (every 2-3 days) were carried out on the trees for this purpose. Observations were carried out on 10 one-year-old fruiting shoots (about 500 flower buds). The fertility index (flower buds/cm, for one-year-old mixed twigs), blooming (flowers/flower buds) and fruit-set (fruits/flowers at 40 days after full blooming) were evaluated. The flowering and fruit-set data were analysed statistically using SPSS ® 13.0 software for Windows (Lead Technologies Inc., Chicago, IL). ANOVA was carried out and means were separated by the LSD test ($P \leq 0.05$).

2.2. Results

2.2.1. Climatic conditions

The winter climatic conditions showed considerable variations between the twoyears and the two geographical areas studied. In Venturina, Livorno (Italy), the year 2006-2007 was characterised by relatively high average daily temperatures in the late autumn and in the early winter (December

and January) (Figure 1A), while the year 2007-2008 was characterised by low temperatures in early winter (Figure 1A), as usually occurs in the Tuscan coastal area (Bartolini *et al*., 2006a, 2006b). Mean values of maximum and minimum temperatures from November 3 to January 20 were 2 ºC higher in 2006-07 than in 2007-08. Consequently, the CU accumulations differed. In the first year, the 1000-CU threshold occurred very late, on March 7 (Figure 2A) while 1200 CU were never achieved In the second year, temperatures were favourable for a good chilling accumulation: 1000 CU occurred on January 28 and 1200 CU on February 13 (Figure 2B).

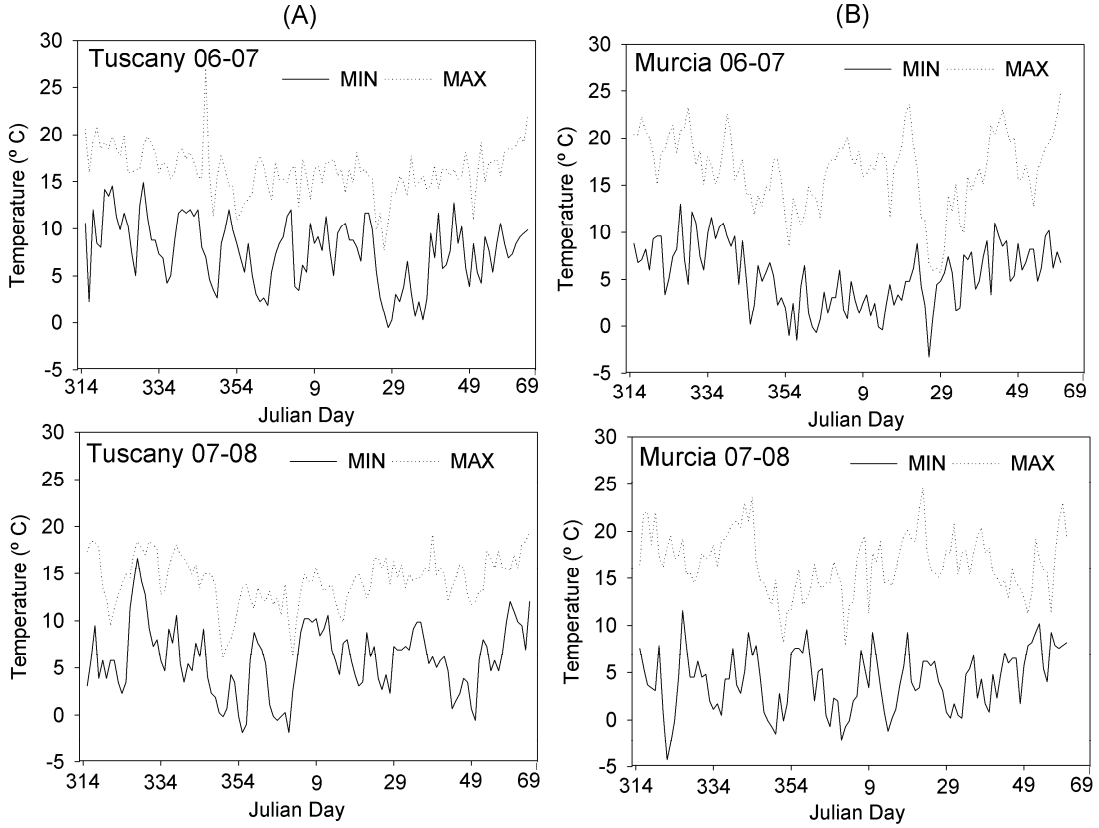


Figure 1. Daily (minimum and maximum) temperatures recorded during the winters 2006-07 and 2007-08 in Tuscany, Italy (A) and Murcia, Spain (B). Minimum and maximum mean temperatures from 345 (December 11) to 20 (January 20) are reported in Julian days

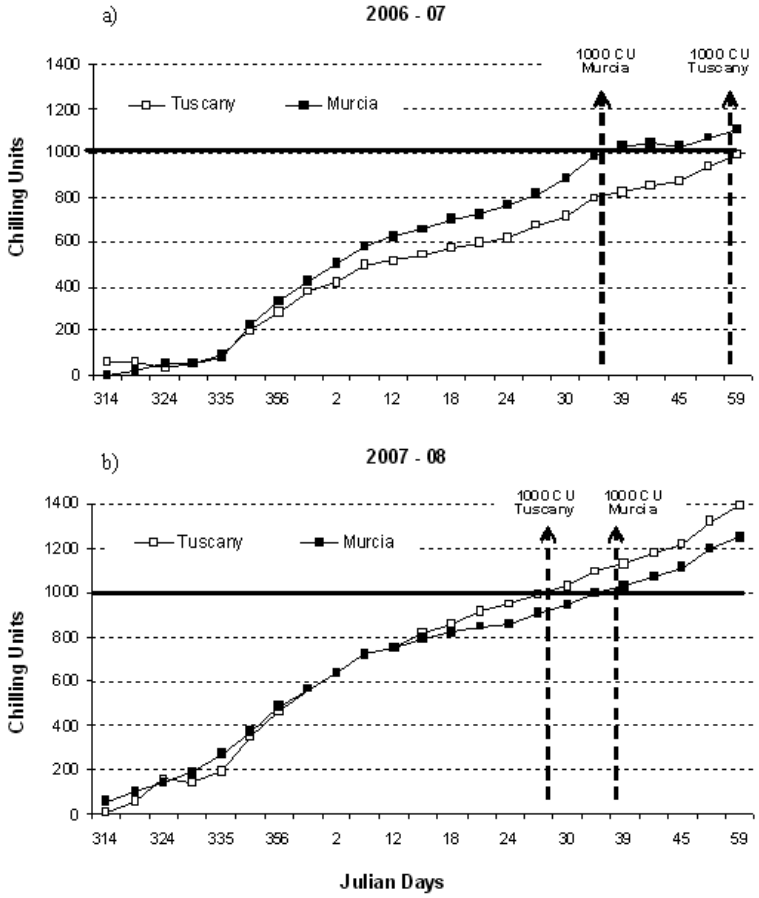


Figure 2. The chilling units (CU) accumulation for 2006-07 (a) and 2007-08 (b) in Tuscany, Italy and Murcia, Spain.

In Spain, the late autumn and early winter of 2006-2007 were also characterised by relatively high temperatures, especially in November (Figure 1B). A delay in the onset of chill accumulation of 9 days, compared to 2007-2008, was observed and only a normal accumulation began late, at the beginning of December: 1000 CU were accumulated on February 6 (Figure 2a) and 1200 CU were never achieved. In 2007-2008, 1000 CU were achieved on February 4 (Figure 2b) and 1200 CU occurred on February 22. In spite of the differences in autumn temperatures between years, a difference of only 150 CU was recorded at the end of February.

Differences between the two geographical areas regarding chill accumulation were observed, especially in the winter of 2006-2007 which was significantly colder in Murcia (Spain) than in Tuscany (Italy) (Figure 2A). On the contrary, chill accumulation in the winter of 2007-2008 was quite similar in the two locations (Figure 2B).

2.2.2. Flower bud dormancy release

The chilling requirements for breaking of dormancy, in both locations and years, are shown in Table 1. The cultivars showed variable chilling requirements in relation to the geographical area and the climatic conditions of the year, according to the Utah Model.

Table 1. Chilling requirements (CU) for breaking endo- and ecodormancy, Julian Days (JD) passed and heat requirements (GDH) from the breaking date of endodormancy to that of ecodormancy.

	Tuscany (Italy)											
	2007						2008					
Cultivars	Endo		Eco		Δ JD[a]	GDH	Endo		Eco		Δ JD[a]	GDH
	CU	JD	CU	JD			CU	JD	CU	JD		
Currot	517	12	638	25	13	2083	725	9	932	23	14	1611
San Castrese	827	39	993	59	20	3326	932	23	1093	36	13	1615
Orange Red	871	45	996	65	20	3398	932	23	1206	44	21	2421
Goldrich	961	54	993	59	5	913	1206	44	1319	51	8	485
SEO	-	-	-	-	-	-	1411	62	-	-	-	-
	Murcia (Spain)											
	2007						2008					
Cultivars	Endo		Eco		Δ JD[a]	GDH	Endo		Eco		Δ JD[a]	GDH
	CU	JD	CU	JD			CU	JD	CU	JD		
Currot	620	12	1003	37	25	2114	647	3	920	28	25	3168
San Castrese	1017	38	1039	47	9	2116	945	31	1226	55	24	2967
Orange Red	1105	54	1087	61	7	1505	1186	50	1251	58	8	1443
Goldrich	1063	50	1097	60	10	2067	920	28	1239	56	28	3431
SEO	-	-	-	-	-	-	-	-	-	-	-	-

[a] ΔJD is the difference between the breaking of JD-endodormancy and that of the JD-ecodormancy in Julian days

2.2.2.1. Tuscany, Italy

In both years, the cultivars broke their endodormancy in accordance with their known CR classification: low, medium and high CR (Guerriero *et al*., 2006; Viti *et al*., 2006). Generally, in the unusual warm-winter year (2006-2007), all cultivars showed lower CU requirements with respect to the regular year (2007-2008) (Table 1). The early-blooming cultivar 'Currot' showed the lowest chilling requirements in both years (517 CU in 06-07 and 725 CU in 07-08), the end of endodormancy corresponding to the first days of January; 'San Castrese' and 'Orange Red' had, in both years, low-medium CR, about 850 CU in 06-07 and 930 CU in 07-08. 'Goldrich' showed

medium–high CR, ranging from 961 in 06-07 to 1206 CU in 07-08. During the first, warm year, it was not possible to assess the end of endodormancy in 'SEO', which is characterised by very high CR, while in 07-08 'SEO' showed endodormancy breakage at 1411 CU.

As regards to the date (Julian days) of endodormancy breaking, a wide difference between years was observed (Table 1). In the first year (06-07), the end of endodormancy was always than in 07-08, ranging from 3 days in the case of the early-blooming cultivar 'Currot' to 22 days for 'Orange Red'. Over the two years, the breaking of ecodormancy was recorded for each cultivar with a similar delta Julian Days (Δ JD), representing the number of days from the endodormancy breaking time to the corresponding overcoming of ecodormancy. However, in this Δ JD period, the GDH for ecodormancy breaking were, for all cultivars, noticeably higher in the warmer year (2006-2007).

2.2.2.2. Murcia, Spain

Regarding the breaking of endodormancy, the cultivars showed different chilling requirements (Table 1): low, medium and high. 'Currot' showed low CR (around 630 CU, averaged over the two years), 'San Castrese' medium (around 980 CU) and 'Goldrich' showed a moderate value (around 990 CU) but with a high variation between years. 'Orange Red' exhibited high CR (around 1145 CU). The chilling requirements for 'Currot' and 'Orange Red' were similar to those reported previously in the same climatic conditions (Ruiz *et al*., 2007). In 'SEO', the assessment of the end of both endodormancy and ecodormacy was not possible, probably because the chill accumulation was not enough to fulfil its chilling requirements: only a few buds reacted to the forcing test, showing its lack of adaptation to Murcia's climatic conditions. Overall, the chilling requirements were quite similar in both years for all cultivars. All cultivars broke endodormancy later in 2006-2007 (Table 1) than in 2007-2008, with differences ranging from 4 days in 'Orange Red' up to 22 in 'Goldrich'. All the cultivars, with the exception of 'San Castrese', were released from ecodormancy later in 2006-2007. The number of days from endodormancy breakage to the corresponding overcoming of ecodormancy (Δ value) was different among cultivars as well as between years. The low-CR cultivar 'Currot' showed the highest delta value (25 JD), while the high-CR cultivar 'Orange Red' showed the lowest (around 8 JD). In both cultivars, the results were practically the same over the two consecutive years. In contrast, 'San Castrese' and 'Goldrich' showed very different JD values between years (Table 1). In Murcia's climatic conditions, these results indicate that the CR is inversely correlated both to the length of the ecodormancy period, as

well as the heat requirement (GDH) necessary for overcoming ecodormancy. This result agrees with the findings of Ruiz *et al*. (2007), where the apricot cultivars with lower chilling requirements showed higher heat requirements. Pawasut *et al*. (2004) found a similar relationship in ornamental peaches.

Table 2. Chilling requirements (CU) for breaking endodormancy, depending on location and year.

Cultivars	Tuscany (Italy)				Murcia (Spain)				Total	
	2007	2008	Mean	*sd*(%)	2007	2008	Mean	*sd*(%)	Mean	*sd*(%)
Currot	517	725	621	24	620	647	634	3	627	13
San Castrese	827	932	880	8	1017	945	981	5	930	8
Orange Red	871	932	902	5	1105	1186	1146	5	1024	12
Goldrich	961	1206	1084	16	1063	920	992	10	1038	14
SEO	-	1411	-	-	-	-	-	-	-	-

The chilling requirements variability for each cultivar in the two locations and years is shown in Table 2. Variability was more marked in Italy between years, for all cultivars, reaching values of 16% for 'Goldrich' and 24% for 'Currot'. In Murcia, variability between years ranged from 3% ('Currot') to 10% ('Goldrich'). The total variability, considering locations and years, ranged from 8% for 'San Castrese' to 14% for 'Goldrich' (Table 2).

2.2.3. Blooming and fruit set

2.2.3.1. Tuscany, Italy

In 2006-07, 'SEO' did not bloom, all its flower buds dropped. 'San Castrese' and 'Goldrich' showed a delayed blooming time with respect to 2007-08, while 'Currot', with very low CR, flowered about one month earlier (Figure 3). As a consequence, in the warm year (2006-2007), the range of blooming dates was wider: from 25 JD ('Currot') to 80 JD ('Goldrich') in 2007 and from 54 JD ('Currot') to 79 JD ('Orange Red') in 2008.

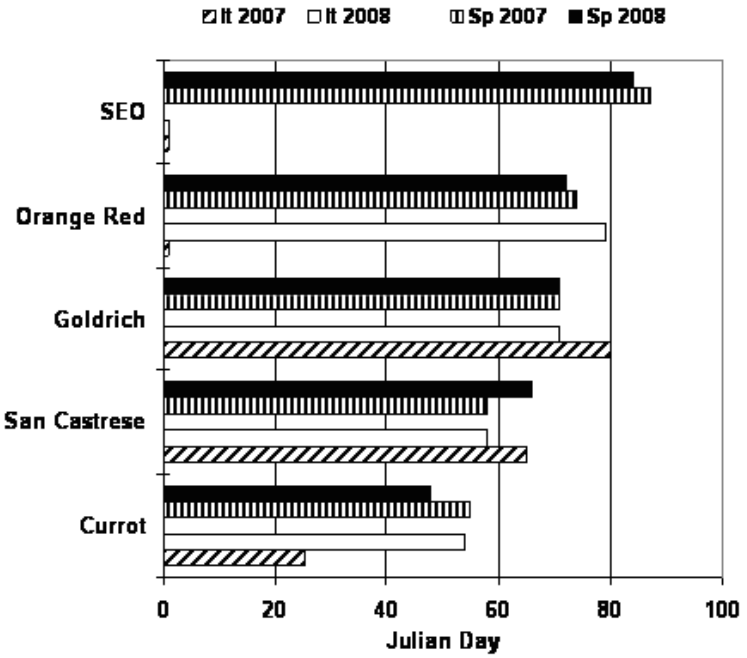


Figure 3. The flowering dates (F50) recorded in 2007 and 2008 in Tuscany (Italy) and Murcia (Spain).

The cultivars showed medium-high fertility index values, which were similar in the two years: from 1.3 in 'Currot' to 1.9 in 'Goldrich' (Table 3). In 2006-2007, the cultivars showed very low blooming and fruit-set percentages; the cultivar 'SEO', characterised by very high chilling requirements, did not bloom as a possible consequence of an insufficient chilling accumulation. In 2007-2008, the cultivars showed very good bloom and relatively good fruit set, 'Currot' and 'San Castrese'being the highest. Despite the high chilling accumulation, 'SEO' confirmed its erratic behaviour in relation to bloom and fruit set.

Table 3. Fertility index (flower buds/cm on one-year-old fruiting shoots) and percentage of flowering and fruit set in Italy (It) and Spain (Sp) Different letters in each column show significant differences at P ≤ 0.05 according to the LSD test.

2007						
Cultivars	Fertility index (buds/cm)		Bloom (%)		Fruit set (%)	
	It	Sp	It	Sp	It	Sp
Currot	1.31 b	1.32 ab	16.31 ab	11.65 b	9.37 a	17.67 a
San Castrese	1.42 b	0.88 b	22.0 a	21.06 a	6.81 b	19.44 a
Orange Red	1.79 a	1.93 a	8.13 b	10.28 b	2.90 b	7.86 a
Goldrich	1.93 a	1.11 b	5.40 b	6.80 b	2.63 b	0.00 a
SEO	1.60 ab	0.84 b	0.00 b	0.00 b	0.30 b	0.00 a
2008						
Cultivars	Fertility index (buds/cm)		Bloom (%)		Fruit set (%)	
	It	Sp	It	Sp	It	Sp
Currot	1.29 bc	1.49 ab	49.64 b	60.06 a	10.45 a	18.44 b
San Castrese	1.41 bc	0.93 c	61.72 a	29.01 b	8.63 a	28.33 a
Orange Red	1.76 a	1.84 a	44.69 b	58.96 a	1.19 c	6.41 c
Goldrich	1.89 a	1.24 bc	24.03 c	36.07 b	5.90 b	3.42 c
SEO	1.67 ab	0.82 c	2.68 d	0.00 c	0.30 c	0.00 c

2.2.3.2. Murcia, Spain

Flowering was earlier in 2008 than in 2007 for 'Currot' and was also 2-3 days earlier for 'Orange Red' and 'SEO', whereas for 'Goldrich' it occurred on the same date. By contrast, flowering was more earlier in 2007 than in 2008 for 'San Castrese' (Figure 3). These results are concomitant with the ecodormancy release dates. The bloom dates ranged from 55 JD ('Currot') to 87 ('SEO') in 2006-2007 and from 48 ('Currot') to 84 ('SEO') in 2007-2008.

The cultivars showed differences in behaviour between Tuscany (Italy) and Murcia (Spain). In Murica, the cultivars showed medium-high values of fertility index, especially 'Currot' and 'Orange Red', whose values were the highest. In 2007, blooming percentages ranged from 0% ('SEO') to 21.06% ('San Castrese'). Overall, in 2007 all cultivars showed lower blooming and fruit set than in 2008, associated with the lower chill accumulation (Table 3). Besides, in both years, the cultivars with lower CR ('Currot' and 'San Castrese') had higher fruit set compared to those with higher CR ('Orange Red', 'Goldrich' and 'SEO'). In 2008, bloom amount ranged from 0% ('SEO') to 61.72% ('San Castrese'), fruit set: from 0% ('SEO') to 28.33% ('San Castrese') (Table 3).

In relation to fertility index, 'Currot' and 'Orange Red' showed, in general, higher values in Murcia than in Tuscany, whereas values were higher in Tuscany for 'San Castrese', 'Goldrich' and 'SEO' (Table 3). Regarding blooming, 'San Castrese' showed a better behaviour in Tuscany, whereas values were higher in Murcia for 'Orange Red' and 'Goldrich'. The behaviour of 'Currot' differed between years (Table 3). Concerning fruit set, higher values were obtained in Murcia, in both years, for 'Currot', 'San Castrese' and 'Orange Red' and only 'Goldrich' showed a better fruit-set in Tuscany (Table 3).

2.3. Discussion

The climatic data showed noteworthy differences between years and locations. The 2007-2008 winter could be considered a standar year in relation to chilling accumulation, while 2006-07 was a warm one, particularly in Tuscany (Italy) - where the winter was constantly mild. A very warm late autumn occurred in both locations, which led to a lower and delayed chilling accumulation. In 2006-07, an accumulation threshold of 1000 CU was achieved very late in Italy (March 7), while in Murcia, 1000 CU were recorded on February 6, similar to what occurred in 2007-2008. Moreover, in both locations, 1200 CU, considered the effective threshold for endodormancy to be overcome in most apricot cultivars (Guerriero *et al*., 2006; Ruiz *et al*., 2007), were never achieved.

In these different climatic conditions, a comparison of the two years shows that the dates of endodormancy breaking of the tested cultivars occurred at different CU amounts. In both locations, in the colder year (2007-08), all cultivars satisfied the chilling requirement in accordance with previous observations recorded during several years (Guerriero *et al*., 2006; Viti *et al*., 2006; Ruiz *et al*., 2007). In 2008, even 'SEO' broke its endodormancy in Italy. This was an exceptional event, considering that this cultivar is often affected by an irregular ontogenetic process in Mediterranean areas (Bartolini and Viti, 1999). In Spain, however, no response to the forcing test nor flowering in field conditions was achieved in 'SEO'. This result confirms the lack of adaptation of this cultivar to warm climatic conditions, like those of Murcia.

During the warmest year (2006-07), all cultivars except 'SEO' showed a surprising behaviour. Dormancy breakage was achieved with relatively lower CU accumulation in Tuscany (Italy) and a considerable delay of endodormancy release was experienced in both locations compared to 2007-08. For 2006-07, the Pisa-Murcia comparison shows that the breakage of flower

bud endodormancy occurred at similar Julian Days for the evaluated cultivars, although much greater chilling requirements were recorded in Murcia for all cultivars.

The effect of the year regarding chilling requirements for breaking of dormancy was highly substantial in Tuscany (Italy), while variability between years was less important in Murcia (Spain). The total variability of the chilling requirements for each cultivar, considering both locations and years, ranged from 8 % ('San Castrese') to 14 % ('Goldrich'). Therefore, the variability of the results shows that the Utah Model was not completely accurate with regard to establishing the chilling requirements for dormancy release under a Mediterranean climate. This method resulted particularly appropriate under cool environments and was adaptable also to temperate regions (Seeley, 1996). However, several studies have reported that the Utah Model did not accurately predict dormancy completion under warm climatic conditions (Buchanan *et al*., 1977; Gilreath and Buchanan, 1979; Erez *et al*., 1990; Linsley-Noakes and Allan, 1994).

As regards the transition between endodormancy and ecodormancy breaking, expressed by the Δ JD, a low variation between the years was observed, particularly in Italy where the same Δ JD occurred, with the exception of 'San Castrese' where the ecodormancy breaking was delayed (by 8 JD) in the warmer year. In Spain, the time from endo- to ecodormancy was also constant for 'Currot' and 'Orange Red' between years, while 'San Castrese' and 'Goldrich' showed an erratic behaviour, but with a delay in the 'regular' year.

In Murcia, the amount of CR, in particular considering 'Currot' (lowest CR) and 'Orange Red' (highest CR), are inversely proportional to the length of the ecodormant period, which is in accordance with results reported by Ruiz *et al*. (2007) who observed that cultivars with high CR showed a lower heat requirement to reach bloom. However, in Tuscany, this relationship was not confirmed because the same cultivars showed a different behaviour. This feature suggests that factors other than temperature regimes related to the location and climatic conditions could have an effect on the regulation of the dormancy release.

As regards the GDH to reach blooming, an important variation was observed between the two years in both locations. The different heat requirement recorded in most cultivars could be due to interferences of other climatic factors, affecting bud development (Garcia *et al*., 1999). These results suggest that the GDH accumulation, starting just after the endodormancy release, could not affect the ecodormancy breaking date and, subsequently, the blooming time. Particularly in Tuscany (Italy), compensation seems to occur between CU accumulation and GDH requirements, a low CU

amount pairing with high GDH, as in 2006-2007 in Italy. The results obtained suggest that higher temperatures could partially compensate for the low amount of chilling (Garcia *et al*., 1999).

Throughout the two years, a wide range in the flowering time (F50) was observed. This feature was more evident in the warmer year (2006-07), when differences of about 32 and 55 days were recorded in Spain and Italy, respectively. This wider range of F_{50} could be associated with the higher temperatures recorded that could have determined an earlier blooming of the early cultivars and a delay of the late ones.

In both areas, a reduced bloom set and consequent low fruit set occurred during the warmer year (2006-07), in comparison with the 'normal year' (2007-08). This was particularly marked in Italy, where the climatic conditions in 2006-07 were very unusual. In particular, the high-chilling-requirement cultivars, considered together, showed the lowest blooming and fruit-set, as observed also in the same environmental conditions by Guerriero *et al*. (2006). It is likely that these cultivars were unable, in the warmer year, to satisfy completely the chilling requirement of a high percentage of buds. The resulting low fruit set could be the result of an altered flower bud development, determined by a lack of synchronisation of the dormancy cycle, xylem differentiation and microsporogenesis processes (Bartolini *et al*., 2006a, 2006c), as well as inadequate mobilisation of stored metabolites (Rodrigo *et al*., 2000). Both in Murcia and in Tuscany, cultivar 'SEO' was always characterised by an absence of flower bud growth, with a consequent lack of blooming. Moreover, a late and heavy flower bud drop happened when the vegetative buds began to swell. This cultivar confirmed its very high CR: only in Italy during the regular year (2007-08) was it possible to establish the endodormancy release, although this was followed by a low bloom. This result confirms the findings of previous studies, suggesting that in 'SEO' other factors, such as physiological and/or hormonal stimuli or signals, could be responsible for the normal phenological development of buds (Guerriero *et al*., 2000).

2.4. Conclusions

The observations carried out in two different environmental areas under a Mediterranean climate and two different years showed an important variability regarding the chilling requirements of apricot cultivars. Overcoming of flower bud dormancy is affected by several endogenous and environmental factors that may interact differently on a particular genotype. During the dormant season, the measurement of only one temperature parameter, although considering the minimum

and maximum daily values or hourly temperatures, was not sufficient to explain the differing behaviours of cultivars in the two locations,. The assessment of chilling requirements by the Utah Model, considering only hourly temperatures, seems not to be completely accurate under mild winter climates. It is probable that, rather than the sole action of the temperature in terms of chilling, other climatic parameters, such as temperature fluctuations between day and night and the time of chilling release (late autumn, early winter or mid-winter), could play a very important role in regulating the breaking of dormancy. The improvement of this model, taking into account these other climatic factors that can interfere with the physiological state of a genotype, is recommended.

On the other hand, the complex process of dormancy seems regulated by more intrinsic factors that switch on specific targets, causing a transduction of signals via a cascade of biochemical, physiological and anatomical events, involving the restoration of bud meristematic activity and thus leading to the overcoming of dormancy (Bartolini *et al*., 2004; Viti *et al*., 2008). Therefore, additional studies, in order to ascertain in-depth the mechanisms involved in the dormancy process, are necessary for improving the models developed for assessment of chilling requirements.

The blooming and fruit set also were influenced by the environmental conditions occurring in the two years. They were lower in the warmer winter (2006-07). This could have been due, among other factors, to an irregular or insufficient CR fulfillment.

3. CHILLING REQUIREMENTS FOR BREAKING OF DORMANCY IN MURCIA (SPAIN) AND THE WESTERN CAPE (SOUTH AFRICA).

3.1 Material and methods

3.1.1. Plant material

Spain: The plant material comprised 10 apricot cultivars spanning the range of flowering time in the apricot species in Spain. The cultivars were 'Currot', 'Búlida', 'Bergeron' and 'Orange Red', which are international references. The new cultivars 'Rojo Pasión', 'Selene', 'Murciana' and 'Dorada', as well as two advanced selections ('S 405/17' and 'Z 111/61') from the CEBAS-CSIC apricot breeding programme, were also included in this study.

South Africa: The plant material comprised 5 apricot cultivars spanning the range of flowering time in the apricot species in South Africa. The cultivars were 'Supergold', 'Palsteyn', 'Charisma', 'Canino' and 'Orange Red'.

3.1.2. Experimental design

In Spain, the experiments were conducted during 2006, 2007, 2008 and 2009 on mature apricot cultivars grown in the germplasm collection situated in an experimental orchard of CEBAS-CSIC situated in Cieza-Calasparra (South East of Spain, altitude 241 m, lat. 38º16'N, long. 1º16'W).

In South Africa, the experiments were conducted in 2007 and 2008. Due to the unavailability of a germplasm collection to carry out the experiments, three commercial orchards of the region of the Western Cape in South Africa were used. The locations were Villiersdorp (altitude 466 m, lat. 33° 58'S, long. 19° 16'E); Ceres (altitude 980 m, 33° 22'S, 19° 0'E); and Ladismith (altitude 550 m , lat. 33° 28' S, long. 21º15'E). 'Palsteyn', 'Charisma' and 'Supergold' (in 2007), were collected in Villiersdorp; 'Canino' and 'Supergold' (2008) were collected in Ladismith; whereas 'Orange Red' was collected in Ceres. Shoots were delivered to the laboratories in Stellenbosch University by overnight courier.

Hourly temperatures were collected with the automatic data-loggers Escort Junior (Escort Data Logging Systems) in Spain and Tinytag (Gemini Data Loggers UK) in South Africa.

In these field conditions, the initial date for chilling accumulation was considered to be when a consistent chilling accumulation occurred and the temperatures producing a negative effect (chilling negation) (Richardson *et al*., 1974; Erez *et al*., 1979b; Guerriero *et al*., 2002) were scarce.

From the beginning of the chilling accumulation in the orchard, for each cultivar, three branches (with lengths of around 40 cm and diameters of 8-10 mm) were picked every 3-4 days from trees in the field and placed in a growth chamber, in controlled conditions. The apricot branches were placed in a 5% sucrose solution, making a fresh cut in the base of the branches. The branches were maintained at 25±1 ºC under white fluorescent tubes (55 µmol m^{-2} s^{-1}) during a photoperiod of 16 h and at 18±1 ºC during a dark period of 8 h, with a constant relative humidity of 65%. After 5 days, the sucrose solution was changed and basal branch cuts were refreshed. The branches were maintained for 10 days in the forcing growth chamber.

3.1.3. Determination of chilling requirements (CR)

Both physical and physiological parameters were used to determine the date of breaking of dormancy (Faust *et al*., 1997; Guerriero *et al*., 2002; Ruiz *et al*., 2007)

After 10 days in the growth chamber, the development stage of the flower buds was tested. The date of breaking of dormancy was established when, after 10 days in the growth chamber, 30% of the flower buds were in Baggiolini´s stage B – C (Baggiolini, 1952), and there was a 30% weight increase in the flower buds compared with the relatively constant previous weights. The CR coincided with the chill accumulated until the date of dormancy release. Quantification of CR was by chill hours (Hours below 7 ºC) (Weinberger, 1950), Chill Units of the Utah Model (Richardson *et al*., 1974) and Portions of the Dynamic Model (Fishman *et al*., 1987a; 1987b).

3.1.4. Determination of heat requirements (HR)

The HR of the evaluated cultivars were calculated as the Growing Degree Hours (GDH) accumulated from breaking of dormancy to the F_{50} (50% of opened flowers) date in the experimental orchard, following the models proposed by Richardson *et al*. (1974, 1975) and Anderson *et al*. (1986). The values of GDH and flowering time in South Africa were not included since trees were sprayed in the first fortnight of August to obtain a correct dormancy release. Therefore, the values obtained in these conditions are influenced by the treatment.

3.1.5. Statistical analysis

Statistical analyses were performed using SPSS 15.0 for Windows (Chicago, IL). Differences between groups were determined by Tukey´s b Test. Coefficient of variation ($c_v = \frac{\sigma}{\mu} * 100$), were calculated to assess the variability of CR among cultivars and years using the different models. Correlation coefficients were determined as the coefficient of Pearson. Statistical analyses were performed using SPSS 14.0 for Windows (Chicago, IL).

3.2. Results

3.2.1. Maximum and minimum daily temperatures.

Maximum and minimum daily temperatures during the period studied in Spain and South Africa are shown in Figure 1 and Figure 2, respectively. In Spain, 2005-2006 and 2008-2009 were characterized by relatively cold winters for the studied area, whereas in 2006-2007 and 2007-2008, temperatures were higher, especially in the last months of autumn 2006 (Figure 1).

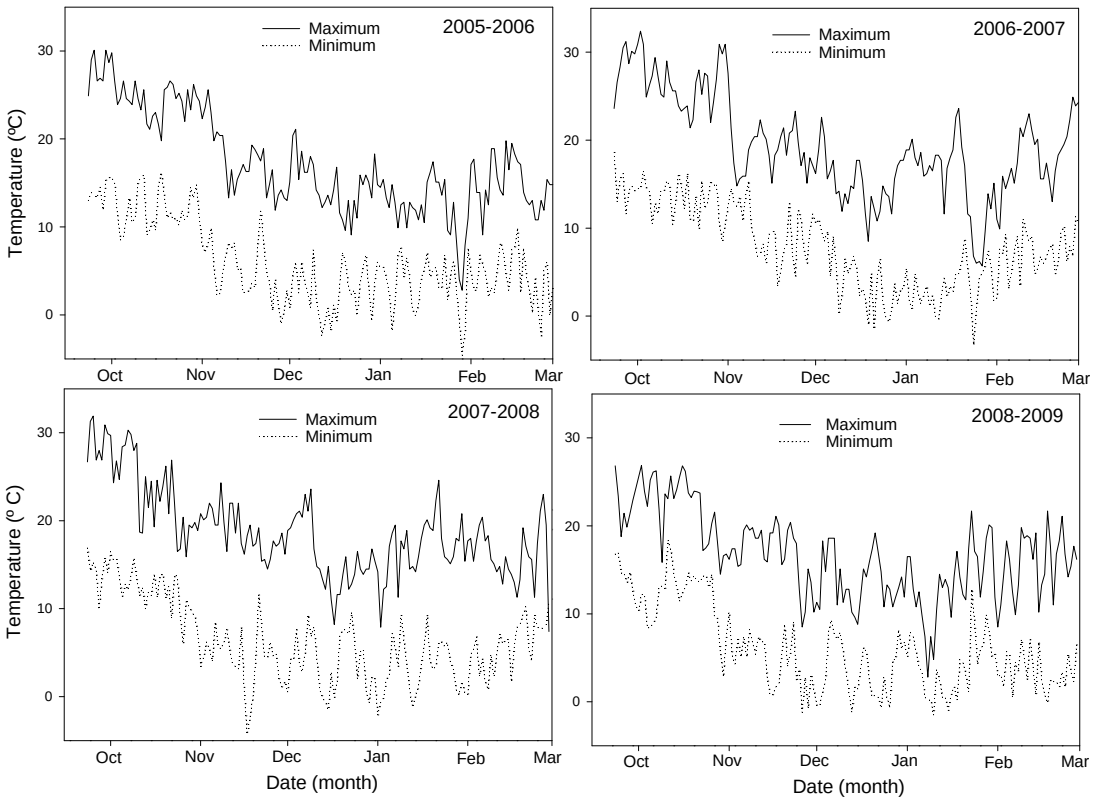


Figure 1. Maximum and minimum daily temperatures registered in the period October-March from 2005 to 2009 in Cieza,Spain.

In South Africa, 2007 was characterized by an earlier and colder winter compared to 2008 (Figure 2). Ceres was the coldest area studied there. In Ladismith, high daily thermal amplitude was observed, which is characteristic of a semi-desert inland area. In Villiersdorp, the area closest to the ocean and with the lowest altitude, minimum daily temperatures were higher than in the other studied areas.

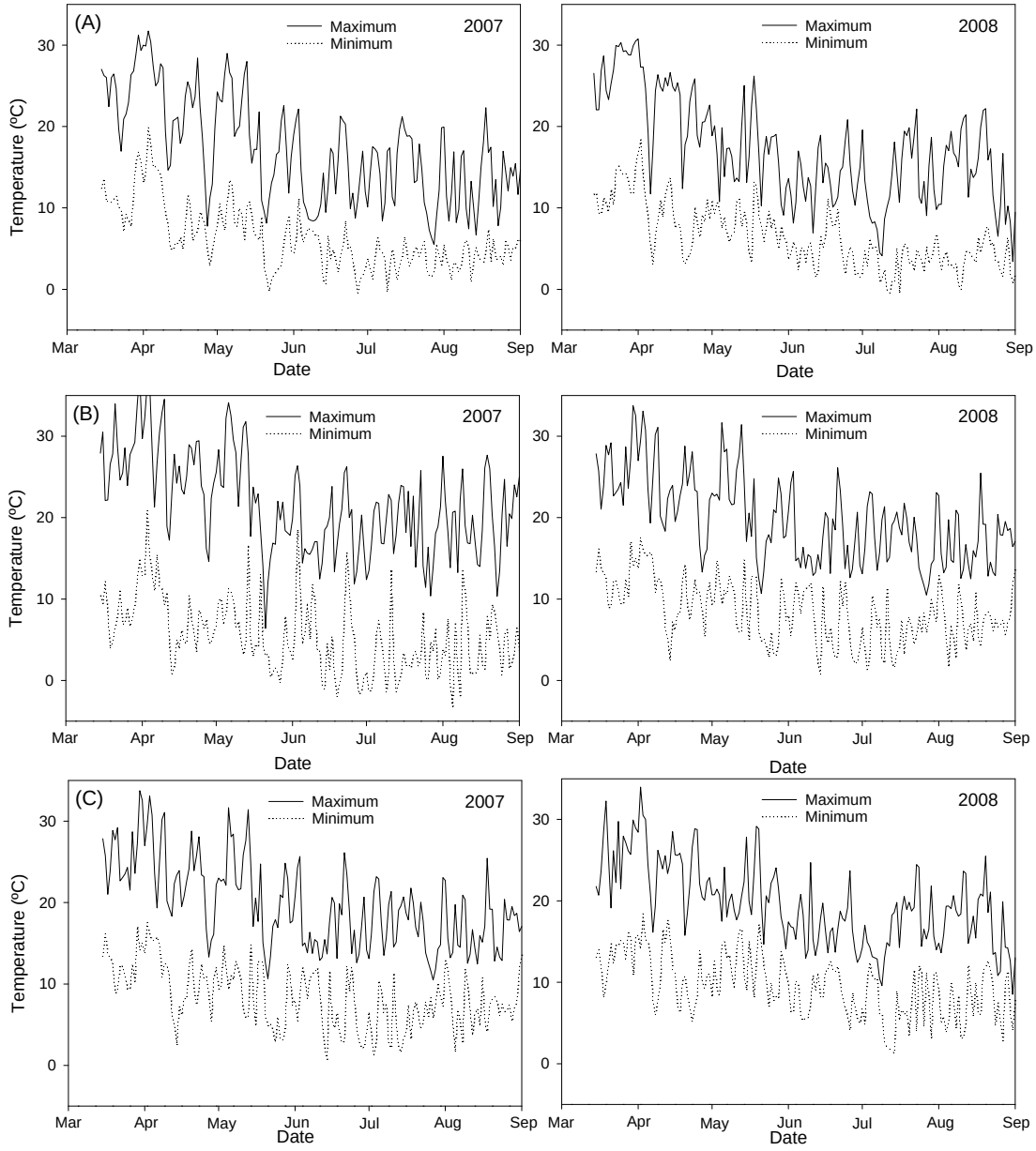


Figure 2. Maximum and minimum daily temperatures registered in the period March-September in 2007 and 2008 in different locations studied in South Africa: Ceres (A), Ladishmith (B), and Villiersdorp (C).

3.2.2. Chilling accumulation in field conditions

Figure 3 shows the chilling accumulation in Spain between ca. November 1 and February 28 in four consecutive years: 2005-2006, 2006-2007, 2007-2008 and 2008-2009, measured by 'Chill Units' (CU) (Utah Model), 'Portions' (Dynamic Model) and 'Hours below 7 ºC'.

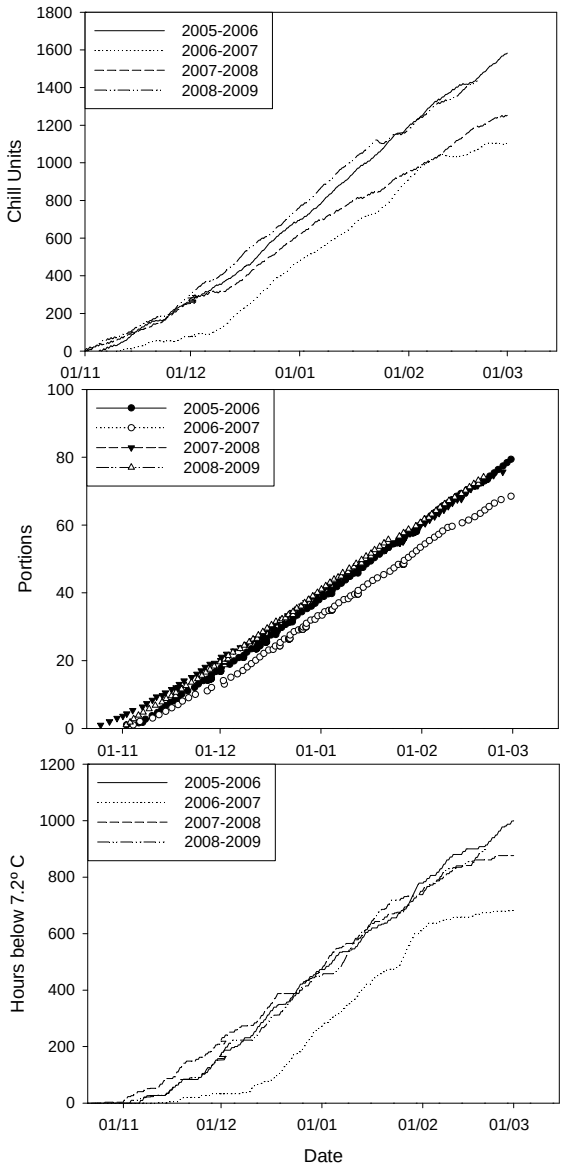


Figure 3. Progression of chill accumulation in the period November –March in Cieza(Spain) between 2005 and 2009. Results are expressed in Chill Units (Utah Model) (above), Portions (Dynamic Model) (centre) and Hours below 7 ºC (Hours-below-7 ºC-Model) (below).

Temperature values were registered in a meteorological station as usual. Temperature values in the apricot branches during daylight hours are higher than those registered in the meteorological

station. On the other hand, in these field conditions, the chilling accumulation during November is, in general, rather low compared to December and January. The first fortnight of February also contributes considerably in some years (Figure 3).

Regarding chill accumulation in Murcia (Spain), excepting for the period 2006-2007, more homogeneous results between years until February 28 were obtained with the Dynamic and Hours-below-7 ºC Models than with the Utah Model (Figure 3).

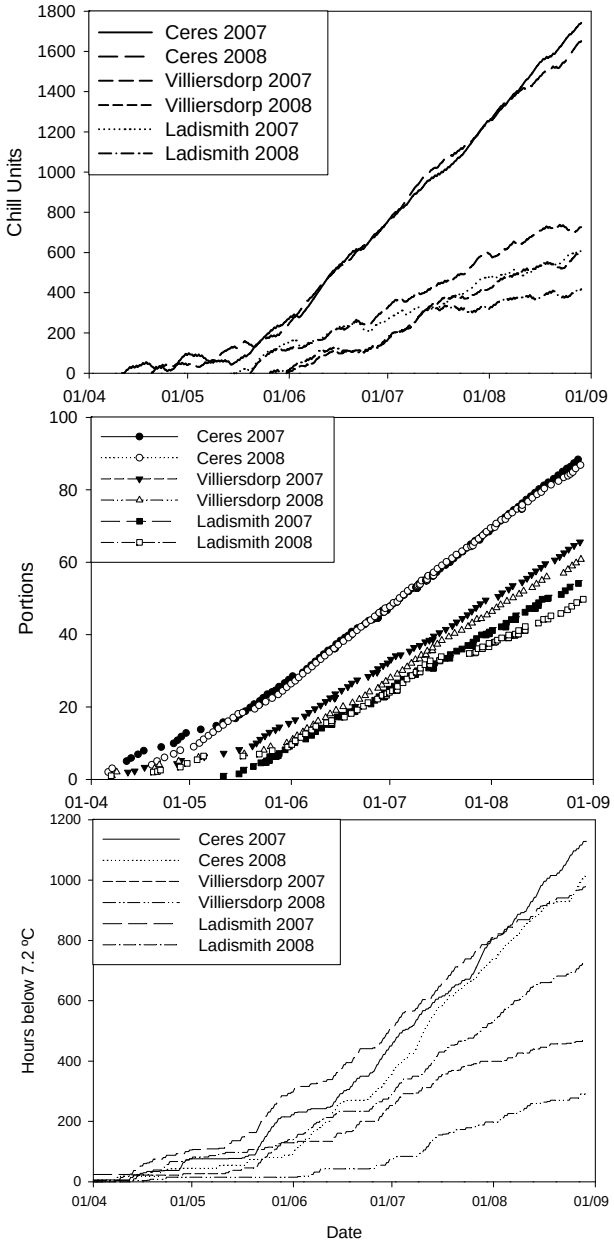


Figure 4. Progression of chill accumulation in Ceres, Villiersdorp and Ladismith, (South Africa) in the period April-September during 2007 and 2008. Results are expressed in Chill Units (Utah Model), Portions (Dynamic Model) and Chill hours , (Hours-below-7 ºC-Model).

Figure 4 shows the chilling accumulation in South Africa between ca. April 1 (November 1 in the northern Hemisphere) and August 28 (February 28 in the northern Hemisphere) in two consecutive years, 2007 and 2008, measured by Chill Units (CU) (Utah Model), Portions (Dynamic Model) and Hours below 7 ºC. Differences among locations and years were considerably dependant on the model used.

3.2.3. Chilling Requirements for breaking of dormancy of apricot cultivars

Table 1 shows the CR for apricot cultivars in four consecutive years in Spain. 'Currot' was the apricot cultivar with the lowest CR (642 CU). The apricot selection 'S 405/17' finished its dormancy after the accumulation of 755 CU. Both 'Currot' and 'S 405/17' can be considered cultivars with low CR. Apricot cultivars 'Rojo Pasión', 'Búlida', 'Z 111/61', 'Dorada', 'Murciana' and 'Selene' showed intermediate CR, with values of 874, 1048, 1046, 1069, 1030 and 1057 CU, respectively. Finally, 'Bergeron' and 'Orange Red' were the apricot cultivars with the highest CR in our study, finishing dormancy after the accumulation of 1134 and 1172 CU, respectively. According to Table 2, the cultivars could be classified, with some overlapping, by their CR, either as low chill ('Currot' and 'S 405/17'; medium chill ('Rojo Pasión', 'Murciana', 'Z 111/61', 'Búlida', 'Selene', 'Dorada'); or high chill ('Bergerón' and 'Orange Red').

The CR of the five apricot cultivars studied in South Africa, situated in three different locations and calculated in two consecutive years, are shown in Table 3. In spite of the fact that the cultivars chosen in South Africa covered the range of CR for the apricot species, as in Spain, the values obtained were notably lower than those found in Spain over a period of four consecutive years. These results were consistent with the three models considered.

Table 1. Chilling requirements for breaking of dormancy in Spain.

Cultivar	Year	Dormancy Breaking	Chill Units			Portions			Hours below 7 ºC			Flowering
			Value	Mean	*cv* (%)	Value	Mean	*cv* (%)	Value	Mean	*cv* (%)	
Currot	2006	02-ene	716	642	15.5	38.9	37.7	7.2	484	409	17	26-feb
	2007	12-ene	621			40.7			323			24-feb
	2008	24-dic	511			34.3			388			17-feb
	2009	28-dic	722			37.1			442			12-feb
S 405/17	2006	09-ene	833	755	7.9	43.8	42.4	6.1	542	504	11.7	05-mar
	2007	17-ene	693			43.6			447			25-feb
	2008	10-ene	731			43.6			566			25-feb
	2009	31-dic	763			38.5			460			14-feb
Rojo Pasión	2006	27-ene	1135	874	20.7	56.6	48.2	11.9	702	566	19.8	09-mar
	2007	22-ene	733			46.4			474			02-mar
	2008	14-ene	775			46.2			613			03-mar
	2009	04-ene	853			43.7			474			28-feb
Búlida	2006	31-ene	1184	1048	14	59.7	56.4	5.2	779	708	8.3	10-mar
	2007	06-feb	1005			57.3			647			01-mar
	2008	24-ene	860			52.7			676			03-mar
	2009	25-ene	1144			55.9			731			08-mar
Z 111/61	2006	31-ene	1184	1046	14.4	59.7	56.5	5.1	779	708	8.9	09-mar
	2007	05-feb	989			56.4			637			03-mar
	2008	24-ene	860			52.7			676			10-mar
	2009	26-ene	1151			57.2			738			09-mar
Murciana	2006	31-ene	1184	1030	12.9	59.7	55.6	8.1	779	690	8.8	08-mar
	2007	09-feb	1037			59.3			650			02-mar
	2008	24-ene	860			52.7			676			03-mar
	2009	16-ene	1041			50.8			653			08-mar
Dorada	2006	31-ene	1184	1069	11	59.7	57.7	4.9	779	720	6.8	12-mar
	2007	14-feb	1032			60.6			659			16-mar
	2008	28-ene	921			55.2			714			10-mar
	2009	23-ene	1139			55.4			727			08-mar
Selene	2006	01-feb	1205	1057	11	60.7	56.9	6.7	779	705	8	07-mar
	2007	09-feb	1037			59.3			650			01-mar
	2008	28-ene	921			55.2			714			04-mar
	2009	18-ene	1066			52.3			675			22-feb
Bergeron	2006	10-feb	1348	1134	15.7	67.5	61.7	6.9	880	762	11.5	10-mar
	2007	16-feb	1040			61.4			668			12-mar
	2008	31-ene	946			57.5			750			10-mar
	2009	31-ene	1203			60.2			751			09-mar
Orange Red	2006	10-feb	1348	1172	10	67.5	64.3	9.9	880	777	12.7	09-mar
	2007	26-feb	1107			67.5			681			15-mar
	2008	15-feb	1126			67.5			841			12-mar
	2009	21-ene	1107			54.8			705			11-mar

Table 2. Mean values of CR calculated by years and genotypes for each model in Spain. Different lower case letters in the same column and group show significant differences. Different capital letters show significant differences in the *CV* of the models.

Variable	Category	Chill Units		Portions		Hours below 7 ºC	
Year	2006	1132	a	57.4	a	738	a
	2007	929	ab	55.3	a	584	b
	2008	851	b	51.8	a	661	ab
	2009	1019	ab	50.6	a	636	ab
Genotype	Currot	642	c	37.8	d	409	d
	S 405/17	755	bc	42.4	cd	504	cd
	Rojo Pasión	874	abc	48.2	bc	566	bc
	Murciana	1030	ab	55.6	ab	690	ab
	Z 111/61	1046	ab	56.5	ab	708	ab
	Búlida	1048	ab	56.4	ab	708	ab
	Selene	1057	ab	56.9	ab	705	ab
	Dorada	1069	a	57.7	a	720	ab
	Bergeron	1134	a	61.7	a	762	a
	Orange Red	1172	a	64.3	a	777	a
Mean *CV*	Model	13.31	A	7.20	B	11.36	A

Table 3. CR for breaking of dormancy in South Africa.

Location/ Cultivar	Year	Dormancy breaking	CR (Utah Model)	Mean	*cv (%)*	CR (Dynamic Model)	Mean	*cv (%)*	CR H<7 ºC	Mean	*cv (%)*
Villiersdorp											
Palsteyn	2007	1-7-07	335	274	31.7	33.2	31.6	7.2	257	171	71.7
Palsteyn	2008	5-7-08	212.2			30.0			84		
Villiersdorp											
Charisma	2007	4-7-07	375	290	41.3	34.4	31.7	11.9	292	188	78.8
Charisma	2008	3-7-08	205.5			29.1			83		
Ladysmith											
Canino	2007	3-7-07	290	304	6.6	26.6	29.8	14.8	545	488	16.7
Canino	2008	16-7-08	318.5			32.9			430		
Ceres											
Orange Red	2007	9-7-07	892	957	9.6	53.6	55.4	4.5	553	568	3.6
Orange Red	2008	15-7-08	1022			57.2			582		
Villiersdorp											
Supergold	2007	2-7-07	350	237	67.2	33.2	27.2	31.2	267	260	4.1
Ladismith											
Supergold	2008	25-6-08	124.5			21.2			252		

Figure 5 show the relationship between the different methods of estimating the CR in Spain. In our field conditions, there is a high correlation coefficient (R = 0.89) between Chill Units and Portions, that is to say between the Utah and Dynamic Models. The relationship between Hours below 7 ºC and Chill Units is high (R = 0.90), as well as between Hours below 7 ºC and Portions (R = 0.90). This model (Hours below 7 ºC) is useful for the growers due to its simplicity, and it proves an indicative value of the dormancy situation. It should be also remarked the high correlation between the date of dormancy breaking and the CR calculated by the different models (Table 6).

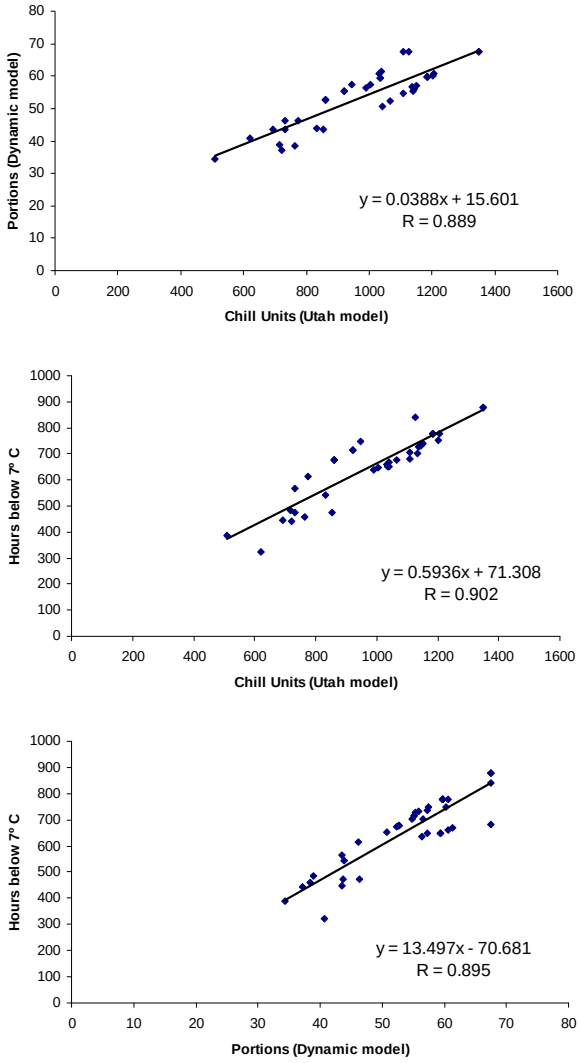


Figure 5. Correlations among the Utah Model, Dynamic Model and Hours-below-7 ºC-Model estimating CR for breaking of dormancy in Spain.

Our results indicate a high positive correlation between CR and flowering date, with a correlation coefficient ca. R = 0.75 in the three models of chilling estimation (Table 6).

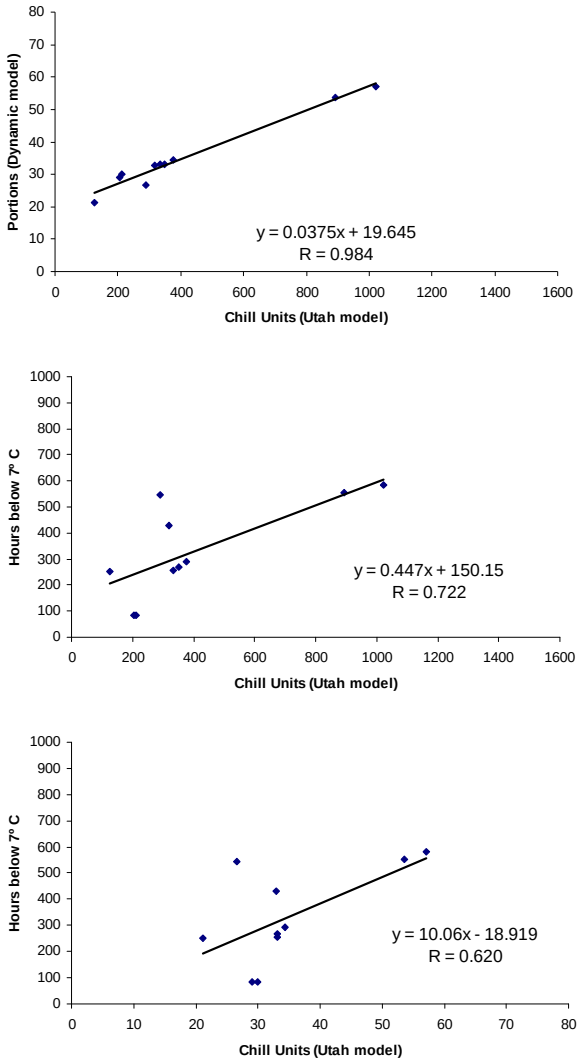


Figure 6. Correlations among the Utah Model, Dynamic Model and Hours-below-7 ºC-Model estimating CR for breaking of dormancy in the three locations studied in South Africa.

In South Africa, the correlations among models showed different results to those found in Spain (Figure 6). Even though three different locations were also included in these regressions, a high correlation coefficient (R = 0.98) between Chill Units and Portions was obtained. Therefore, both models could be used in the determination of CR in the same way. The relationship between Hours below 7 ºC and Chill Units is very low (R = 0.52), as well as between Hours below 7 ºC and

Portions (R = 0.38). Therefore, the accuracy of this model (Hours below 7 ºC) in these climatic conditions is questionable.

3.2.4. Heat requirements for flowering of apricot cultivars

The heat requirements calculated in Spain are shown in Table 4. The range was between 4604 GDH, in the case of 'Selene', and 6189 GDH, for 'Dorada', according to the Richardson Model (Table 4). Some cultivars showed similar values in the four studied years, such as 'Dorada' or 'S 405/17', whereas important differences were observed in other cultivars. No significant differences among cultivars were found in the HR calculated with the Richardson Model or with the Anderson model (Table 5), however, significant differences were found among cultivars with regard to the ΔJD. The medium and high CR cultivars showed significantly lower ΔJD. The mean values of the ΔJD ranged from 49.8 in 'Currot' to '29.5' in 'Orange Red'. The CV among years concerning the HR for flowering for all the evaluated cultivars considered together was very similar with the two methods used, being 16.41% with the Richardson method and 17.65% with the Anderson method. Due to the similarity of values and CV, only Richardson values were used for the correlations. The relationship between CR for breaking of dormancy and HR for flowering is negative (Table 6), but the correlation coefficient was not very high and ranged from R = -0.35 in Portions to R = -0.51 in Chill Units. These correlations were higher when ΔJD (difference in days from dormancy breaking date to F50 date, usually expressed as growing degree days) were used, ranging from R = -0.48 in Hours-below-7 ºC to R = -0.75 in Portions.

No relationship between HR and flowering date was observed (R = -0.02) (Table 6), which indicates that flowering date is not influenced by HR. Besides, no significant differences in the HR (Table 5) were found among cultivars with strong differences in F_{50} (Table 4).

Table 4. Heat requirements for flowering (Growing Degree Hours). ΔJD represents the days from dormancy breaking to F50.

Cultivar	Year	Dormancy Breaking	F50	GDH (Richardson)			GDH (Anderson)			ΔJD		
				Value	Mean	cv	Value	Mean	cv	Value	Mean	cv
Currot	2005-2006	02-jan	26-feb	5415	5773.6	12.3	4373	5080.5	17.2	55	49.8	12.4
	2006-2007	12-jan	24-feb	5892			5421			43		
	2007-2008	24-dic	17-feb	6710			6157			55		
	2008-2009	28-dic	12-feb	5077.2			4371			46		
S 405/17	2005-2006	09-jan	05-mar	5619	5626.1	9.3	4653	4999.6	12.2	55	46.3	14.3
	2006-2007	17-jan	25-feb	5630			5211			39		
	2007-2008	10-jan	25-feb	6265.9			5757			46		
	2008-2009	31-dic	14-feb	4989.4			4378			45		
Rojo Pasión	2005-2006	27-jan	09-mar	4777	6077.7	17.2	4193	5564	19.6	41	45.8	15.9
	2006-2007	22-jan	02-mar	6086			5759			39		
	2007-2008	14-jan	03-mar	7338			6848			48		
	2008-2009	04-jan	28-feb	6109.7			5457			55		
Búlida	2005-2006	31-jan	10-mar	5009.4	5294.1	10.7	4477	4912.5	8.9	38	35.3	23.8
	2006-2007	06-feb	01-mar	4637			4596			23		
	2007-2008	24-jan	03-mar	5737			5279			38		
	2008-2009	25-jan	08-mar	5793.1			5298			42		
Z 111/61	2005-2006	31-jan	09-mar	4695	5720.4	17.2	4152	5341.9	18.3	37	37.5	22.3
	2006-2007	05-feb	03-mar	5317			5290			26		
	2007-2008	24-jan	10-mar	7006			6543			45		
	2008-2009	26-jan	09-mar	5863.5			5382			42		
Murciana	2005-2006	31-jan	08-mar	4386.2	5391.6	22.7	3834	5008.8	22.2	36	36.5	33.7
	2006-2007	09-feb	02-mar	4467			4502			21		
	2007-2008	24-jan	03-mar	5737			5279			38		
	2008-2009	16-jan	08-mar	6976			6421			51		
Dorada	2005-2006	31-jan	12-mar	5575.2	6188.9	7.3	5101	5856.2	9.4	40	38.8	15.7
	2006-2007	14-feb	16-mar	6257			6307			30		
	2007-2008	28-jan	10-mar	6666			6227			41		
	2008-2009	23-jan	08-mar	6257.3			5790			44		
Selene	2005-2006	01-feb	07-mar	4023.7	4604.9	15.7	3481	4276.7	16.7	34	31	23.7
	2006-2007	09-feb	01-mar	4167			4181			20		
	2007-2008	28-jan	04-mar	5620			5221			35		
	2008-2009	18-jan	22-feb	4609			4224			35		
Bergeron	2005-2006	10-feb	10-mar	3999	5150.5	18.6	3639	4855.9	20.1	28	31.8	21.6
	2006-2007	16-feb	12-mar	5305			5356			24		
	2007-2008	31-ene	10-mar	6317			5874			38		
	2008-2009	31-ene	09-mar	4980.8			4555			37		
Orange Red	2005-2006	10-feb	09-mar	3684	4916	33.1	3314	4698	31.9	27	29.5	46.4
	2006-2007	26-feb	15-mar	3880			4011			17		
	2007-2008	15-feb	12-mar	4875			4687			25		
	2008-2009	21-ene	11-mar	7225			6780			49		

Table 5. Mean values of HR and ΔJD calculated by years and genotypes for each model in Spain. Different lower case letters in the same column and group show significant differences. Different capital letters show significant differences in the CV of the models.

Variable	Category	Richardson		Anderson		ΔJD		F50	
Year	2006	4718	c	4122	b	39.1	a	66.7	a
	2007	5164	bc	5063	a	28.2	b	63.5	a
	2008	6227	a	5787	a	40.9	a	63.1	a
	2009	5788	ab	5265	a	44.6	a	60.7	a
Genotype	Currot	5774	a	5080	a	49.8	a	50.8	c
	S 405/17	5626	a	4999	a	46.3	a	55.3	bc
	Rojo Pasión	6078	a	5564	a	45.8	a	62.5	ab
	Búlida	5294	a	4912	a	35.3	b	64.5	ab
	Z 111/61	5720	a	5341	a	37.5	b	66.8	a
	Murciana	5392	a	5008	a	36.5	b	64.3	ab
	Dorada	6189	a	5856	a	38.8	b	70.5	a
	Selene	4605	a	4276	a	31.0	b	60.5	ab
	Bergeron	5150	a	4855	a	31.8	b	69.3	a
	Orange Red	4916	a	4698	a	29.5	b	70.8	a
Model	CV	16.41	A	17.65	A	22.98	A		

Table 6. Relationship between CR for breaking of dormancy (Chill Units, Portions and Hours below 7 ºC), HR for flowering (Growing Degree Hours and ΔJD-days from endodormancy breaking to F50), flowering date (F50) and date of dormancy release in Spain.

Variables correlated		R[b]	Significance
ΔJD	GDH[a]	0.66	0.000
ΔJD	Chill Units	-0.51	0.001
ΔJD	Portions	-0.75	0.000
ΔJD	Hours below 7 ºC	-0.48	0.002
ΔJD	F50	-0.36	0.025
ΔJD	Date of dormancy breaking	-0.88	0.000
GDH	Chill Units	-0.51	0.001
GDH	Portions	-0.49	0.001
GDH	Hours below 7 ºC	-0.35	0.028
GDH	F50	-0.02	0.880
GDH	Date of dormancy breaking	-0.48	0.002
Chill Units	Portions	0.89	0.000
Chill Units	Hours below 7 ºC	0.90	0.000
Chill Units	F50	0.70	0.000
Chill Units	Date of dormancy breaking	0.71	0.000
Portions	Hours below 7 ºC	0.90	0.000
Portions	F50	0.81	0.000
Portions	Date of dormancy breaking	0.94	0.000
Hours below 7 ºC	F50	0.75	0.000
Hours below 7 ºC	Date of dormancy breaking	0.72	0.000
F50	Date of dormancy breaking	0.76	0.000

[a]GDH values were values calculated by Richardson Model (1974).
[b]R: correlation coefficient

3.2.5. Flowering date of apricot cultivars

The flowering dates of the evaluated cultivars in Spain over four years are shown in Table 1. Results showed important differences between some of the cultivars. The earliest flowering cultivar was 'Currot' with a flowering date between February 12 and February 26, from 11 to 27 days before the latest flowering cultivar 'Orange Red'. The selection 'S 405/17' also displayed an early flowering date, between February 14 and March 5. These two cultivars, 'Currot' and 'S 405/17', could be grouped in the early-flowering cultivar according to Table 5. The group of cultivars 'Rojo Pasión', 'Búlida' and 'Murciana' can be classified as middle-flowering, because their flowering dates are in the first days of March. The latest flowering cultivars were 'Dorada', 'Z 111/61', 'Bergeron' and 'Orange Red', with flowering dates around mid-March, and therefore these cultivars could be very interesting for regions with a frost risk. Our results indicated a high positive correlation between CR and flowering date, with a correlation coefficient ca. R = 0.75 in the three models of chilling estimation (Table 6).

3.3. Discussion

Weinberger (1950) and Brown (1957) suggested February 15 as the limit date to accumulate useful chilling for breaking dormancy. In accordance with our results obtained in Spain, chilling accumulation until February 15 is sufficient for satisfying CR and breaking dormancy in most apricot cultivars (Guerriero *et al*., 2002; Ruiz *et al*., 2007) except in the unusual warm autumn-winter of 2006-2007 when only 1000 CU were accumulated.

As for the variability of chill accumulation, it was very high with the Utah Model, both in Spain and in South Africa. The Dynamic Model considers the synergic effect of moderate temperatures with low temperatures for breaking dormancy (Fishman *et al*., 1987a; 1987b); this is probably the factor responsible for homogenising the chill accumulation between years. These results partially agree with those previously found by Ruiz *et al*. (2007) in the southeast of Spain. In both cases, the Dynamic Model showed the lowest variation among years. However, the results differ regarding the variability of the Chill unit and Hours-below-7 ºC Models, showing the need for study during a long series of years. This lack of variability of the Dynamic Model among years, considering the different temperatures (Figure 1), can be interpreted as an inability of the model to really represent the widely different climatic conditions registered in different years (Figure 1).

In areas with a very mild climate, important differences between the Utah and Dynamic Models have been reported (Erez *et al*., 1990; Erez, 2000). In these regions, the Utah Model is inadvisable because it was developed in a cooler area. In spite of the fact that southeast Spain is a region with a mild climate, our results show a relatively-high chilling accumulation in two out of the four studied years. Besides, the variability between years is similar with both the Utah and the Dynamic Model, and the correlation between the two models is very high (Figure 5). In our opinion, this is because our experimental orchard is located in an interior region, which is cooler than other areas in the South East of Spain, and thus the Utah Model can be useful. Besides, in South Africa, the onset of chilling accumulation depended considerably on the area studied. Thus, in Ceres CU accumulation began on the second fortnight of April, whereas in Villiersdorp and Ladismith it began on the second fortnight of May. Besides, the accumulation was markedly different in the three different areas (Figure 4). The highest chill values (ca. 1700 CU) were accumulated in Ceres, compared to the other locations studied, and were even higher than those in Cieza, Spain. However, in Villiersdorp and Ladismth, the chill accumulation was very low (ca. 650 and 550 CU, respectively) compared to that registered in Spain. These results show the high variability of the areas studied in South Africa. It must be remarked that in Ladismith, characterized by a semi-arid climate and high daily thermal amplitude, Hours below 7ºC were similar to those registered in Cieza, Spain, although the CU accumulation was fairly lower because of the chill negation of the high daily temperatures. The Dynamic Model not only reduced considerably the variability between years in Spain, but also significantly reduced the differences between markedly different climatic conditions in the diverse areas studied in South Africa. Thus, the accuracy and universality of the model to determine the CR or to characterize the chill accumulation of one area can be questioned. Regarding the variability between years in South Africa, the Dynamic Model showed the lowest variability (Figure 4), which also occurred in Spain. A high variability was found in Chill Units and hours-below-7 ºC. These results agree partially with those previously found by Ruiz *et al*. (2007) in Spain. In both cases, the Dynamic Model and Utah Model showed the lower variation among years, while the Hours-below-7 ºC Model registered high variability. It should be remarked that the Dynamic Model showed very low variability in spite of the different temperatures registered (Figure 1). In 2006-2007 many cultivars showed erratic leafing and blooming conditioned by the marginal chill accumulation (personal observation). However, according to the Dynamic Model, during this year enough chill had been accumulated to satisfy the mean CR previously calculated by Ruiz *et al*. (2007). This could be interpreted as a certain inability of the

model to really represent the widely different climatic conditions registered among years in this area. Therefore, the use of chill accumulated by the Dynamic model under these climatic conditions could mask the negative effect of an insufficient chilling accumulation.

Regarding the CR calculated in Spain, the results were, in general, similar to those found by other authors (Guerriero *et al*., 2002; Ruiz *et al.,* 2007). The early-flowering cultivar 'Currot' showed CR 45% inferior to those of other cultivars such as 'Bergeron' or 'Orange Red'. Nevertheless, the CR of 'Currot' are significantly higher than those of some peach cultivars (Erez, 1995), which proves the difficulties of cultivating the apricot species in those areas with a very warm climate. The range of CR for the majority of the studied cultivars was between 850 and 1150 CU (Table 2). 'Orange Red' was the apricot cultivar with the highest CR, around 1200 CU. This value approaches, and in some years exceeds, the upper limit of chilling accumulation in the area where the experimental field is located; probably because of this, 'Orange Red' shows erratic productive behaviour in our region (Egea *et al*., 2004).

In New Jersey (USA), Bailey *et al*. (1982) studied the CR of three apricot cultivars for four years, and the results ranged between 873 and 1343 Chill Units according to the Utah Model. Tabuenca (1964), determined the CR for 'Currot' and 'Búlida'in Zaragoza (Spain), by the Hours below 7 ºC model, and the calculated values were rather higher than the results obtained in this study and by Ruiz *et al*. (2007). The different climatic conditions in which the study of Tabuenca (1964) was determined could have influenced this divergence of results.

The difference of temperature between real bud temperature and that of the meteorological station has an effect on the results concerning CR of apricot cultivars. In this way, the different CR calculated for an apricot cultivar in different locations may be due, among other reasons, to the different radiation level in the crop area. The knowledge of the CR calculated in the field conditions is very interesting, although presumably these CR will be lower than those calculated in growth chamber conditions (Ruiz *et al*., 2007).

Regarding the variability among years of the CR calculated by different models, the high variation of temperatures among the studied periods (Figure 1) entailed high values of the coefficient of variation (*cv*) in CR, calculated by the Utah Model and by the Hours-below-7 ºC-Model (Table 1). Significant differences among years in the CR were observed by the Utah and Hours-below-7 ºC Models (Table 2). These results partly disagree with those found by Ruiz *et al*. (2007), where the Utah Model and Dynamic Model showed a similar variability, but lower than that

showed by the Hours-below-7 ºC-Model. This indicates the importance of year-by-year variation in the testing of models, and, it could be suggested, the need to carry out this kind of experiment during a long series of years.

Moreover, the differences between years for the same cultivar were around 30%. Similar results were obtained by Valentini *et al*. (2004), who measured a CV of 27.7% after the study of five apricot cultivars during three consecutive years. García *et al*. (1999) obtained a similar variation after the study of several apricot cultivars during three consecutive years, but their values were slightly higher than ours (Table 2). Ruiz *et al*. (2007) obtained a CV of only 6.3% in the same climatic condition. Thus, the data obtained with the Utah Model in different areas and from different apricot cultivars registered a wide variation between years and genotypes. This variation should be taken into account before using the information obtained by this model. Regarding the Dynamic Model, in our study the CV among years for all the evaluated cultivars considered together was quite low (7%) and coincided with that obtained by Ruiz *et al*. (2007) in the same climatic conditions and with the same cultivars. The maximum variation for a genotype was obtained in 'Rojo Pasión' (11.9%), and the minimum in 'Dorada' (4.9%).

As for the CR calculated in South Africa, the ranges of chilling requirements of all the cultivars were completely different in each location, especially for Utah and Hours-below-7 ºC (Tables 1 and 3). This difference of ranges seems related to the fact that three different locations, which had different temperature regimes during winter, were chosen in South Africa. In CU, for example, the ranges were 124-1022 in South Africa and 621-1203 in Spain. In Portions, the ranges were 21-57 in South Africa and 34-64 in Spain, whereas in Hours below 7 ºC, the ranges were 84-582 in South Africa and 323-880 in Spain. 'Canino', cultivated in Ladismith, the most remarkable example, registered only 304 CU compared to the 780 obtained in Spain (data not shown). This would suppose a difference of more than 60% of the CR calculated in Spain. 'Orange Red', cultivated in Ceres, showed a lower variability between locations compared to the values calculated in Spain, with a variation that ranged from 14% (Dynamic Model) to 37% (Hours-below-7 ºC-Model). The similar chill accumulation between Ceres and Barranda may explain this low variation. The high daily thermal amplitude found in Ladismith leads to a chill negation during the day that may explain the higher variation for the Utah Model. Nonetheless, the variation found with the Dynamic Model, which takes into account the positive effect of moderate temperatures, was higher than 35%. This value is similar to the variation between 'Currot' and 'Orange Red' (41%) in the

same location in Spain. Even higher differences were found between the CR requirements of peach cultivars in Reunion Island (21º5' S, 55º E) and Clermont-Ferrand, France (46ºN, 3ºE) (Balandier *et al*., 1993a).This suggests that the variability of the climatic conditions can lead to variability in the CR calculation that can be even higher than the variation between different cultivars of the same species. This variability seems to be higher in mild winter conditions. Thus, for the correct interpretation of CR values, it should be taken into account the location where CR have been calculated. The existence of reference cultivars is also advisable for the correct interpretation of these data. The considerable inaccuracy of the models in different climatic conditions calls into question the reliability of modelling chill accumulation through temperatures to determine the endodormancy release of temperate fruits. An improvement in the knowledge of the physiological and genetic basis of the induction and release of dormancy, in addition to the consideration of factors other than temperature, such as photoperiod, nitrogen content, water status, pruning, juvenility, moment of application of temperatures, combinations of low and high temperatures, etc., and the interaction among them, could lead to an understanding of the complex process of dormancy.

On the other hand, considering the successful cultivation of apricot cultivars in marginal chill areas with appropriate chemical treatment in South Africa, the expansion of apricot growing areas to marginal chill areas in Spain could be a possibility to consider. This would be interesting especially for those growers working with early-flowering/ripening cultivars aimed at the early fresh market.

As for the correlations between the different methods of estimating the CR in Spain, the high correlation between Utah and Dynamic Model found was also reported in previous studies in field conditions with similar winter temperatures (Linsley-Noakes and Allan, 1994; Erez *et al*., 1990; Erez *et al*., 1998; Ruiz *et al*., 2007). Therefore, both models could be used in the determination of CR in the same way. However, a lower correlation was obtained by Ruiz *et al*. (2007) between Hours below 7 ºC and Portions in the same climatic conditions, which shows the influence of the year effect over this correlation. On the other hand, the high correlation between CR and flowering time found is concomitant with that found by others authors (Weinberger, 1944; Ruiz *et al*., 2007).

With regard to the heat requirements, the range of HR for flowering for the studied cultivars was similar to that found by Ruiz *et al*. (2007) in the same climatic conditions and cultivars.

However, important differences were found among years for some cultivars. For example, the CV among years in the case of 'Orange Red' was 33%. Guerriero *et al*. (2002) obtained similar HR for flowering in the case of 'Bergeron' and 'Orange Red' compared to those found in our study (Table 4).

The significant correlation found between CR and ΔJD seems to be related to the lower temperatures recorded when low CR cultivars release from dormancy, compared to those registered when high CR cultivars overcome dormancy. Couvillon and Erez (1985a) pointed out that cultivars with low CR cultivated together with cultivars that have high CR in the same field conditions must show lower HR. These authors indicated that excessive chill causes 90% of the heat requirement variations and, consequently, the cultivars have no specific HR for flowering, which contradicts studies carried out by other authors (Gianfagna and Mehlenbacher, 1985; Rom and Arrington, 1966). In this manner, Linsley-Noakes and Allan (1994) did not find differences concerning HR between three nectarine cultivars with different CR, and Egea *et al*. (2003) obtained similar HR for flowering in a group of almond cultivars with very different CR. Our results show that the variability among cultivars is fairly lower than the variability in HR that can be obtained during different years in the same location.

On the other hand, a similar correlation value was observed in the correlation of CR and HR in previous work carried out by Pawasut *et al*. (2004) in ornamental peaches, and in by Ruiz *et al*. (2007) in apricots. Nevertheless, these results are in disagreement with previous studies on other species (Couvillon and Erez, 1985a). Besides, results obtained in apricot cultivars by Guerriero *et al*. (2002) and Bailey *et al*. (1982) did not show correlation between these parameters. These contradictory results could be explained by the fact that the climatic conditions are very different, especially the temperature, and by the strong effect of the year-by-year variation. It could be hypothesized that the higher HR of cultivars with low CR could be because these cultivars finish their dormancy at a very cold time of year and, probably, the GDH accumulation is less effective than at later dates with higher temperatures.

Swartz and Powell (1981) observed in apple cultivars that the higher the CR, the higher were the HR for flowering. However, the results derived from this work (Swartz and Powell, 1981), as well as results obtained by other authors (Brown, 1957; Spiegel-Roy and Alston, 1979), indicate that this situation could be due to a residual effect of dormancy.

Long exposures to low temperatures below the threshold of heat accumulation reduce the HR considerably. A decrease of HR has been observed when a cultivar receives much more chill than its CR (Couvillon and Erez, 1985a; Citadin *et al*., 2001). However, some exceptions have been indicated concerning peach cultivars that have high HR in spite of receiving long cold periods (Citadin *et al*., 2001). Several studies have shown that the HR of cultivars can be modified by a continuous chilling accumulation after the breaking of dormancy (Couvillon and Herdershott, 1974; Spielgel-Roy and Alston, 1979; Swartz and Powell, 1981).

Considering the results shown, and especially, that no significant differences were found among cultivars' HR and that no significant correlation was found between HR and flowering time, it can be suggested that other factors determine flowering date, especially CR, which are related to the date of endodormancy breaking. However, temperatures after breaking release are also important. Spiegel-Roy and Alston (1979) reported that late-flowering cultivars result from the combination of high CR and high HR. Gianfagna and Mehlenbacher (1985) determined that high HR, together with a high minimum temperature for bud growth, produce late-flowering cultivars in apple, while high CR have no influence. However, no relationship between flowering date and HR was obtained in our study, which indicates that other factors are responsible for variation of the flowering date.

Finally, the classification of the cultivars obtained according to their flowering time, was similar to that obtained by Ruiz *et al*. (2007); even though flowering dates in the study of Ruiz *et al*. (2007) study were different than those collected during these four consecutive years, this may explain the different values obtained in several parameters in this study and is in accordance with the high year-by-year variation observed.

3.4. Conclusions

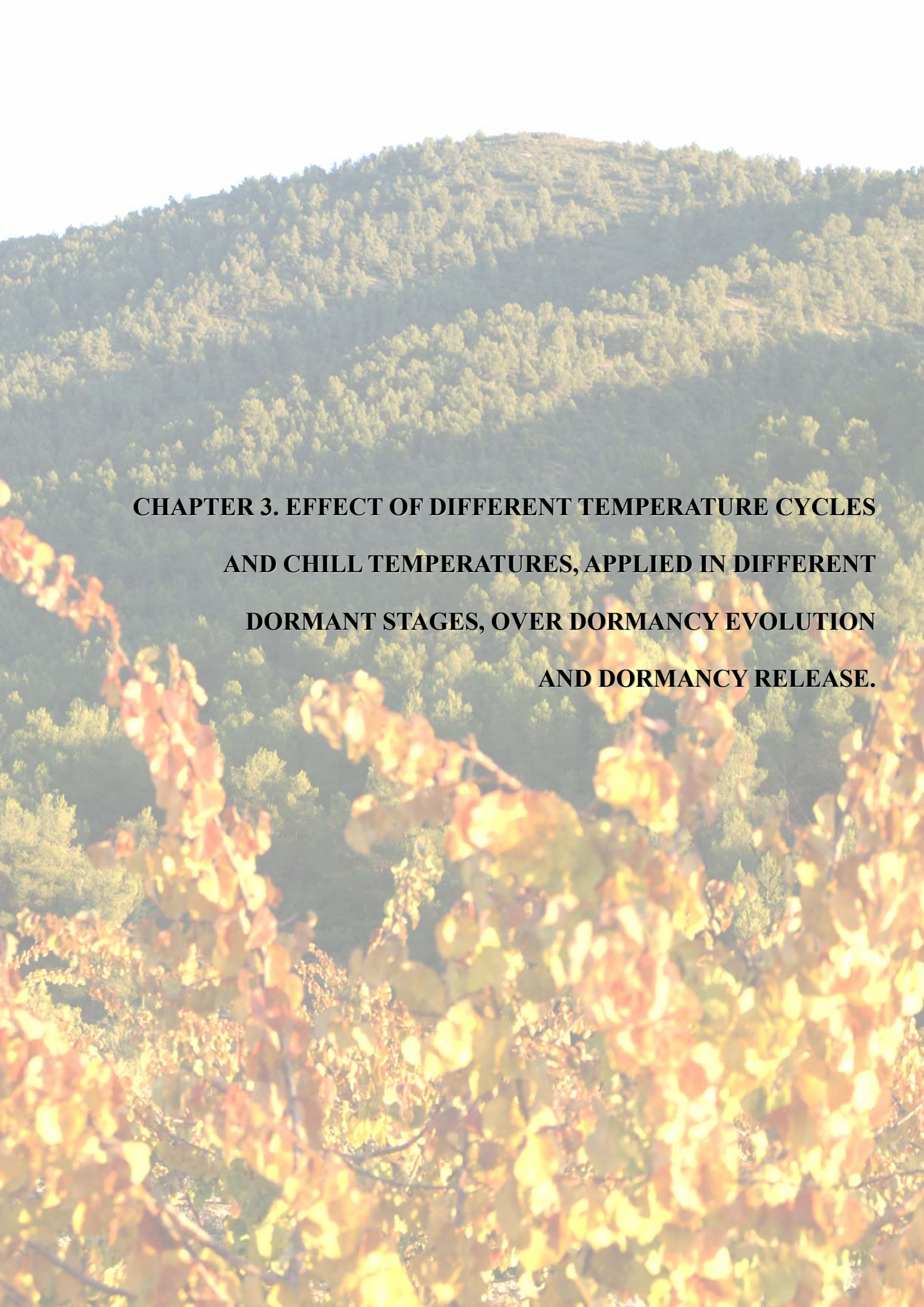
The CR of the evaluated cultivars in Spain and in South Africa were homogeneous regarding year-by-year variability according to the Dynamic Model. A higher variability was found for the Hours-below-7 ºC and Utah Models. The relationship among all models was close in Spain (R values ca. 80%), whereas in South Africa, only the Dynamic and Utah Models have a high correlation (R = 0.98).

The range of CR for of the group of cultivars studied was markedly different between countries and a high variability in the values of CR was obtained between years and cultivars. The

variability was higher when CR were calculated by Utah and Hours-below-7 ºC Models. Likewise, 'Orange Red' showed very different CR in both countries. 'Canino', cultivated in Ladismith, the most remarkable example, registered only 304 CU compared to the 780 obtained in Spain. The apricot cultivars showed a range of HR between 4604 GDH, in the case of 'Selene', and 6067 GDH, for 'Rojo Pasión'; however, no significant differences in HR were found among cultivars. Our results indicate that HR for flowering are not an intrinsic characteristic of the cultivar in the apricot species. A negative correlation was observed between CR for breaking of dormancy and HR for flowering, although the correlation coefficient was quite low. However, an important negative correlation was found between the period when HR are accumulated (days from endodormancy release to flowering) and the date of dormancy breaking. A high positive correlation between CR and flowering date, but no correlation between HR and flowering date, was found. It seems clear that in apricot, flowering date is mainly determined by the chilling requirement of the cultivars, being the period when HR are accumulated (ΔJD) higher for the low chill cultivars. This is in accordance with the usually lower temperatures registered when low chill cultivars release from endodormancy compared to high chill cultivars. The considerable higher ΔJD needed in low chill cultivars could be related to the higher need of HR previously found by others authors.

This work has been carried out for four years in Cieza, Spain and for two years in three different locations in the Western Cape of South Africa with wide groups of cultivars that spanned the range of flowering time in this species in both countries. Besides, three of the currently most used methods to estimate CR were applied. Therefore, the results obtained represent an advance with regard to the global knowledge of the apricot species, concerning CR for breaking of dormancy, HR for flowering, the variability of CR among locations and the method used to estimate CR.

Finally, the successful cultivation of apricot under marginal CR accumulation areas in South Africa suggests the possibility of expanding the cultivated area in Spain to marginal chill areas with an appropriate culture, especially using rest breaking agents. This could have an important effect on the production of apricot cultivars oriented to the early fresh market..



CHAPTER 3. EFFECT OF DIFFERENT TEMPERATURE CYCLES AND CHILL TEMPERATURES, APPLIED IN DIFFERENT DORMANT STAGES, OVER DORMANCY EVOLUTION AND DORMANCY RELEASE.

1. THE EFFECT OF DIFFERENT TEMPERATURE CYCLES, APPLIED IN DIFFERENT DORMANT STAGES, ON DORMANCY RELEASE IN APRICOT.

1.1. Introduction

Although considerable progress has been achieved in the last few decades, large functional gaps of knowledge still exist regarding bud-dormancy induction, maintenance, and release (Saure, 1985; Faust *et al*., 1997; Arora *et al*., 2003). Among the points that remain unclear are the similarities or differences between different species or cultivars (Crabbé and Barnola, 1996; Paiva and Robitaille, 1978; Gilreath and Buchanan, 1981a), the effect, sometimes uneven according to the literature, of different temperatures or combinations of temperatures (Erez *et al*., 1979a; Couvillon and Erez, 1985b; Rageau *et al*., 1998; Jacobs *et al*., 2002; Naor *et al*., 2003), the relationship of these diverse results with the dormancy status of the plant, and its effect on the physiological state of the plant (Weinberger, 1950; Thompson *et al*., 1975; Couvillon and Erez, 1985b; Young, 1992; Tehranifar *et al*., 1998). Another gap in our knowledge which remains unfilled is the efficiency of daily cycles which combine high temperatures – above 18-20 ºC - with efficient temperatures for dormancy breaking. So far, cycles of 16h of low temperatures and 8h of high temperatures have been frequently applied (Overcast and Campbell, 1955; Erez *et al*., 1979b; Guerriero *et al*., 1985b; Naor *et al*., 2003), even though there has been some indication of the higher efficiency of daily cycles with shorter high-temperature periods compared with the continued application of low temperatures (Couvillon and Erez, 1985b).

There is special interest also in the different CR for the overcoming of dormancy that exist among different types of buds (terminal, lateral vegetative, or reproductive buds) and their role in the processes of budburst and fruit set (Scalabrelli and Couvillon, 1986; Guerriero *et al*., 1987; Cook *et al*., 1998).

This work is aimed at clarifying some of these issues in apricot buds. Concretely, the effect over dormancy release of different daily cycles of temperatures, with a limited duration of the high temperatures, in different stages of the dormancy-release process has been studied. The study has been carried out considering both vegetative and reproductive buds during two consecutive years.

1.2. Material and methods

1.2.1. Plant material

One-year-old shoots of apricot cultivar 'Palsteyn' were used as plant material. This cultivar was obtained by the Fruit Technology Research Institute of Stellenbosch (South Africa) from the cross of "Blenhein" x "Canino" and is characterized by low CR for breaking of dormancy and by an early flowering date.

Plant material was collected randomly, in a commercial orchard situated in Villiersdorp (Western Cape, lat. 33º 39', long. 19º 17', South Africa), during two consecutive years, 2007 and 2008. Shoots were homogeneous in all cases and were sampled from similar height and different orientations in the trees. Trees were grafted onto an apricot rootstock, trained as vase with a planting distance of 5 x 2 m and drip-irrigated.

1.2.2. Experimental design, sampling dates, and temperature treatments

Three sampling dates were used on the basis of different chill accumulations: 1.- June 14 2007 (215 CU); 2.- April 22 2008 (0 CU), and 3.- June 18 2008 (107 CU). On each sampling date, 330 50-cm-long shoots of apricot cultivar 'Palsteyn' were cut and defoliated. Thirty shoots per treatment were bundled in three replicate bundles of ten shoots each. The bundles were then placed at random in 5-l buckets with their bases in ca. 1 l of tap water containing 5 ml/l household bleach (5% sodium hypochlorite). Four growth chambers were used to apply the temperature treatments, with the following temperature regimes: 1.- constant temperature of 5 ºC (chill pre-treatment); 2.- daily cycle of 19/5h at 5/25 ºC, respectively; 3.- daily cycle of 19/5h at 5/20 ºC, and 4.- daily cycle of 19/5h at 5/15 ºC. The different temperature cycles were applied for 60 days. Treatments were established as follows on each of the sampling dates: T1.- 60 days at continuous 5 ºC; T2.- 60 days at 5/15 ºC; T3.- 60 days at 5/20 ºC; T4.- 60 days at 5/25 ºC; T5.- 30 days at continuous 5 ºC followed by 30 days of 5/15 ºC; T6.- 30 days at continuous 5 ºC followed by 30 days of 5/20 ºC; T7.- 30 days at continuous 5 ºC followed by 30 days of 5/25 ºC; T8.- 45 days at continuous 5 ºC followed by 15 days of 5/15 ºC; T9.- 45 days at continuous 5 ºC followed by 15 days of 5/20 ºC; T10.- 45 days at continuous 5 ºC followed by 15 days of 5/25 ºC. The bottom 1 cm of each shoot was removed every 15 days during the chilling period.

1.2.3. Forcing conditions and budburst scoring

After the 60 days of the different treatments, all shoots were forced at 25 ºC with a light source of ca. 200 µmol m^{-2} s^{-1} of photosyntetically active radiation (93% "cool white" fluorescent light and 7% incandescent). Before the beginning of forcing, the shoots were cut to a length of 30 cm and during forcing the bottom 1 cm was removed weekly. During forcing, the shoots were checked three times a week in order to establish the mean time to budbreak (MTB) in each type of bud. Budbreak was considered as being when a green tip was visible among the bud scales, which corresponds to stage B-C of Baggiolini (1952). The time, in days, since the sampling dates for the occurrence of budburst (MTB) was established when at least one green tip per shoot on five shoots per bundle was observed (50% budburst). The types of bud considered were: terminal vegetative, lateral vegetative, vegetative (including lateral and terminal), and reproductive.

1.2.4. Chilling accumulation

Hourly mean temperatures during the autumn and winter of 2007 and 2008 were collected in the field by Tinytag sensors (Gemini Data Loggers, UK), and used to calculate chill accumulation by Chill Units (Richardson *et al*., 1974) and Portions (Fishman *et al*., 1987a; Fishman *et al*., 1987b). The chill accumulated at the sampling dates was calculated in both years, as well as the chilling requirement (CR) for breaking of dormancy of 'Palsteyn'. Both physical and physiological parameters were used to determine the date of breaking of dormancy (Guerriero *et al*., 2002).

1.2.5. Statistical analysis

Statistical analytical procedures were performed using the SPSS® 15.0 software for Windows (Chicago, IL). Differences in mean time to budbreak among treatments and sampling dates were analyzed by ANOVA for each type of bud, in order to determine the efficiency of the different temperature treatments applied at different stages of endodormancy, in the different kinds of bud evaluated.

1.3. Results

1.3.1. Chill accumulation and chilling requirements for breaking of dormancy

Chill accumulation, recorded in 2007 and 2008 in Villiersdorp and expressed in Chill Units (Utah Model) and Portions (Dynamic Model), is shown in Figures 1 and 2, respectively. The year 2007 corresponded to a year with high chill accumulation for the area studied, whereas 2008 is characterized as a year with low chill accumulation. Thus, the area where sampling was performed is characterized by low chill accumulation. Differences between years regarding chill accumulation were more marked using Chill Units (Figure 1) than Portions (Figure 2). Table 1 shows the dormancy release dates and chilling requirements calculated for 'Palsteyn' in 2007 and 2008. The dates of dormancy release were very similar, whereas the chilling requirements values varied greatly from year to year when Utah and Hours below 7 ºC Models were used. However, Dynamic Model showed low difference between years.

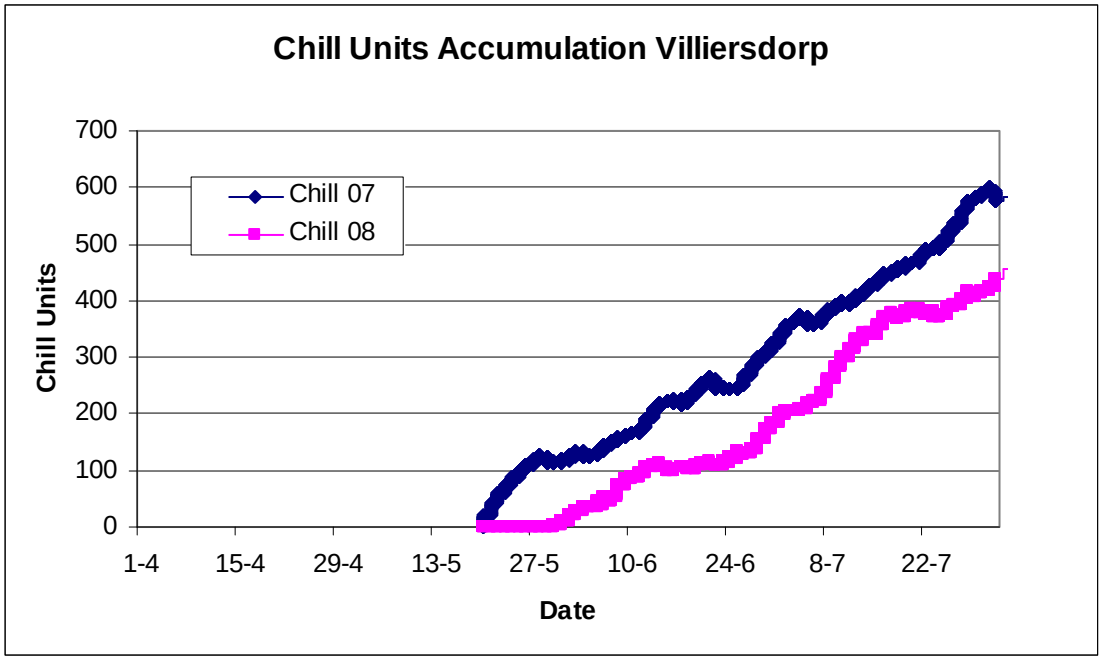


Figure 1. Chill Unit accumulation (Utah Model) in Villiersdorp in 2007 and 2008.

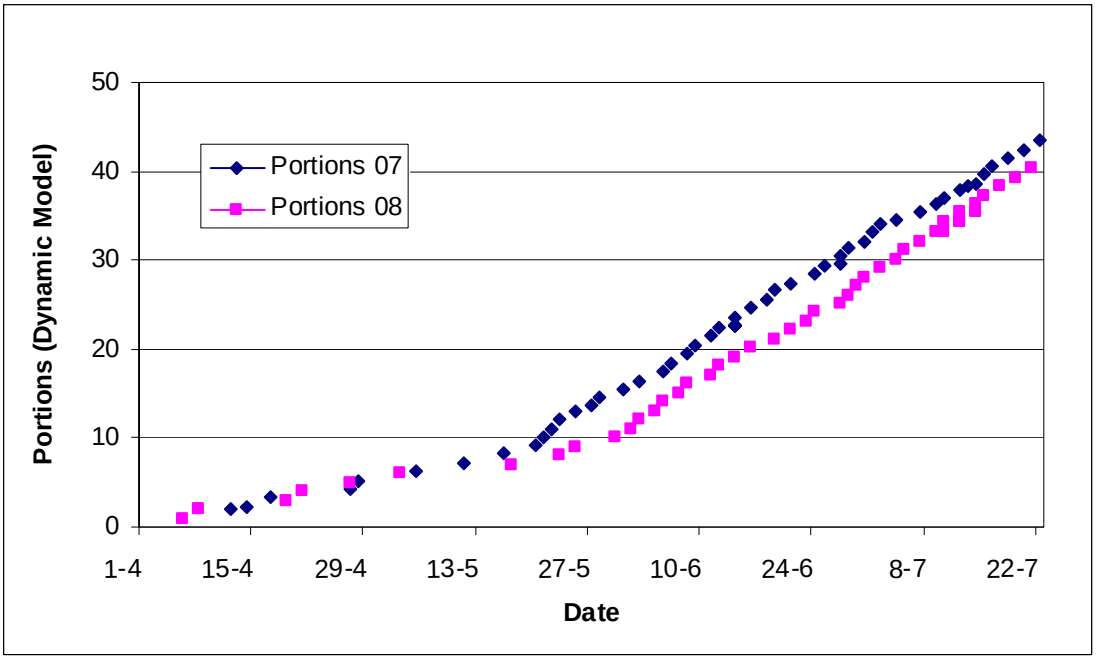


Figure 2. Portion accumulation (Dynamic Model) in Villiersdorp in 2007 and 2008.

The chill accumulated in field conditions by the dates of shoot sampling is shown in Table 2. These data can be used to estimate the quantity of chilling requirement satisfied in field conditions on the sampling dates: 64% of 'Palsteyn´s' CR in June 2007, 0% in April 2008, and 50% in June 2008.

Table 1. Dormancy release dates and chilling requirements of 'Palsteyn' in 2007 and 2008.

Year	Dormancy release Date	CR (Utah Model)	CR (Dynamic Model)	H<7 ºC
2007	1-7-07	335	33.2	211
2008	5-7-08	212.2	30.05	84

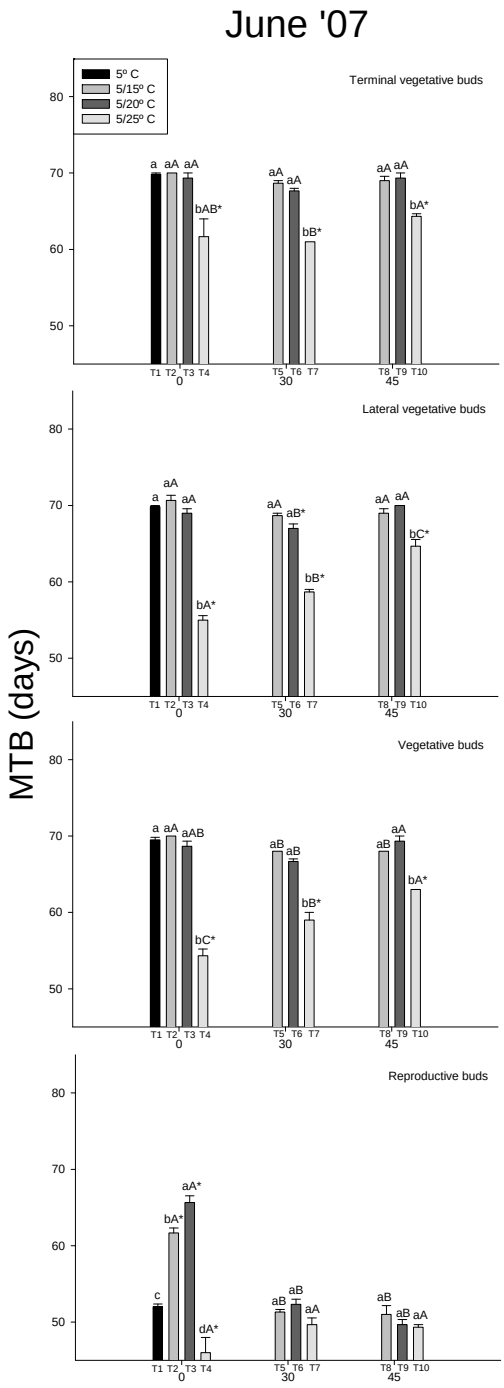
Table 2. Shoot sampling dates and chill accumulation at these times as determined by Utah Model (CU), Dynamic Model (Portions) and Hours below 7 ºC.

Year	Sampling Date	CU (Utah Model)	Portions (Dynamic Model)	H<7 ºC
2007	14/06/2007	215	23.6	115
2008 a	22/04/2008	0	4.0	5
2008 b	18/06/2008	107	20.1	43

1.3.2. Effect of different temperature cycles applied after 64% of chilling requirements had been satisfied (June 2007)

The first sampling was carried out when 64% of the 'Palsteyn' CR had been satisfied (214 CU accumulated) in 2007. The MTB values of the different types of bud, after application of different temperature cycles, are shown in Figure 3. For terminal vegetative buds, cycles of 5/15 and 5/20 ºC - T2 and T3, respectively - for 60 days (no pre-treatment of 5 ºC) showed no significant differences compared with continuous 5 ºC (T1). Application of 5/25 ºC (T4) resulted significantly more effective than T1, reaching budburst with lower MTB, even before forcing took place, whereas T1, T2, and T3 needed more than 9 days of forcing conditions to reach budburst. When a one-month pre-treatment of 5 ºC was given (T5, T6, and T7), similar results were obtained and only 5/25 ºC (T7) resulted significantly more effective than T1, T5, and T6. The MTB values obtained for each temperature cycle did not show significant differences compared to the same cycle without a one–month of 5 ºC pre-treatment. Finally, when a 45–day, 5 ºC pre-treatment was applied, similar results were obtained. The 5/25 ºC cycle (T10) was a significantly more effective cycle than T1, T8, or T9. However, for the 5/25 ºC treatment, the pre-treatment of 45 days at 5 ºC significantly increased the MTB compared with a pre-treatment of 30 days at 5 ºC.

Regarding lateral vegetative buds, only a few differences compared to terminal vegetative buds were found. Thus, 5/25 ºC was, significantly, the most effective cycle with regard to obtaining a low MTB, and both in the case of no pre-treatment (T4) and 30 days pre-treatment at 5 ºC (T7) budburst occurred even before forcing. Within the 5/25 ºC temperature cycle, significant differences were found among no pre-treatment and pre-treatment at 5 ºC for 30 or 45 days. The lower was the duration of the pre-treatment at 5 ºC, the higher the efficiency. On the other hand, 5/20 ºC with a pre-treatment of 30 days at 5 ºC (T6) was significantly more efficient than continuous 5 ºC (T1) or 5/20 ºC without pre-treatment (T3) or with 60 days pre-treatment at 5 ºC (T9) (Figure 3).



Treatment and days of 5º C pre-treatment (out of 60 days of total treatment)

Within each 5 ºC pre-treatment, different lower case letters for different temperature cycles show significant differences (P< 0.05) according to Tukey´s test.
For each temperature cycle, different capital letters in 5 ºC pre-treatments show significant differences (P< 0.05) according to Tukey´s test.
Means followed by * differ significantly from Treatment 1 (T1)

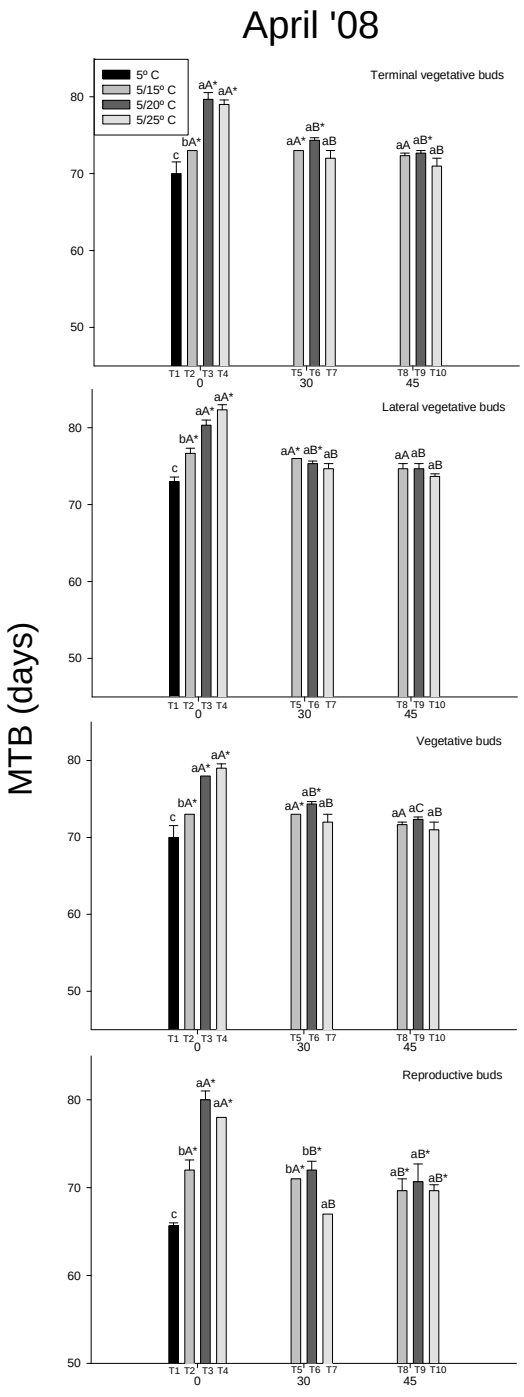
Figure 3. Mean time to budbreak in June 2007 (215 CU accumulated, 64% CR satisfied) of the different types of buds studied, using different temperature cycles.

When all vegetative buds were considered, that is to say both laterals and terminals, similar results were obtained. The most effective cycle was 5/25 ºC , reaching budburst even before forcing when no pre-treatment of 5 ºC or a one-month pre-treatment was applied. Within temperature cycles, the effect of the 5 ºC pre-treatment was different depending on its duration. For the 5/25 ºC cycle, the shorter the pre-treatment the lower the MTB. In the 5/20 ºC cycle, 30 days of pre-treatment was significantly more effective than no pre-treatment or 45 days of pre-treatment, whereas for the 5/15 ºC cycle, when no pre-treatment was applied the MTB was significantly higher than with 30 or 45 days of pre-treatment.

Reproductive buds were also evaluated and different trends were observed in comparison with vegetative buds (Figure 3). More accentuated differences among treatments were obtained when no pre-treatment was applied. The 5/25 ºC cycle (T4) was significantly the most effective treatment, and continuous 5 ºC (T1) was significantly more effective than 5/15 ºC (T2). The highest MTB of all the treatments occurred with 5/20 ºC (T3). When pre-treatment was applied, no significant differences were obtained among temperature cycles. In most of the treatments, budburst occurred even before the forcing, showing the shallower endodormant state of reproductive buds compared to vegetative, either lateral or terminal.

1.3.3. Effect of different temperature cycles applied when no chill had been accumulated (April 2008)

The results of the second sampling date are shown in Figure 4. By the time the shoots were sampled, no chill had been accumulated in the field. In terminal vegetative buds, the most efficient treatment was continuous 5 ºC, which contrasts with the results obtained when 64% chill had been accumulated (Figure 3) and the 5/25 ºC cycle was the most effective treatment. When no pre-treatment at 5 ºC was applied, 5/15 ºC was significantly more efficient than 5/20 ºC or 5/25 ºC, whereas after 30 or 45 days of pre-treatment, no significant differences were found among these temperature cycles. Besides, the 5/20 ºC and 5/25 ºC cycles significantly reduced the MTB after pre-treatment. Thus, pre-treatment at 5 ºC for 30 or 45 days significantly increased the temperature efficiency to break dormancy in the 5-20 ºC and 5-25 ºC cycles but not for 5-15 ºC. After the pre-treatment, 5/25 ºC resulted as efficient as continuous 5 ºC.



Within each 5 ºC pre-treatment, different lower case letters for different temperature cycles show significant differences (P< 0.05) according to Tukey´s test.
For each temperature cycle, different capital letters in 5 ºC pre-treatments show significant differences (P< 0.05) according to Tukey´s test.
Means followed by * differ significantly from Treatment 1 (T1)

Figure 4. Mean time to budbreak in April 2008 (0 CU, 0 CR satisfied) of the different types of buds studied, using different temperature cycles.

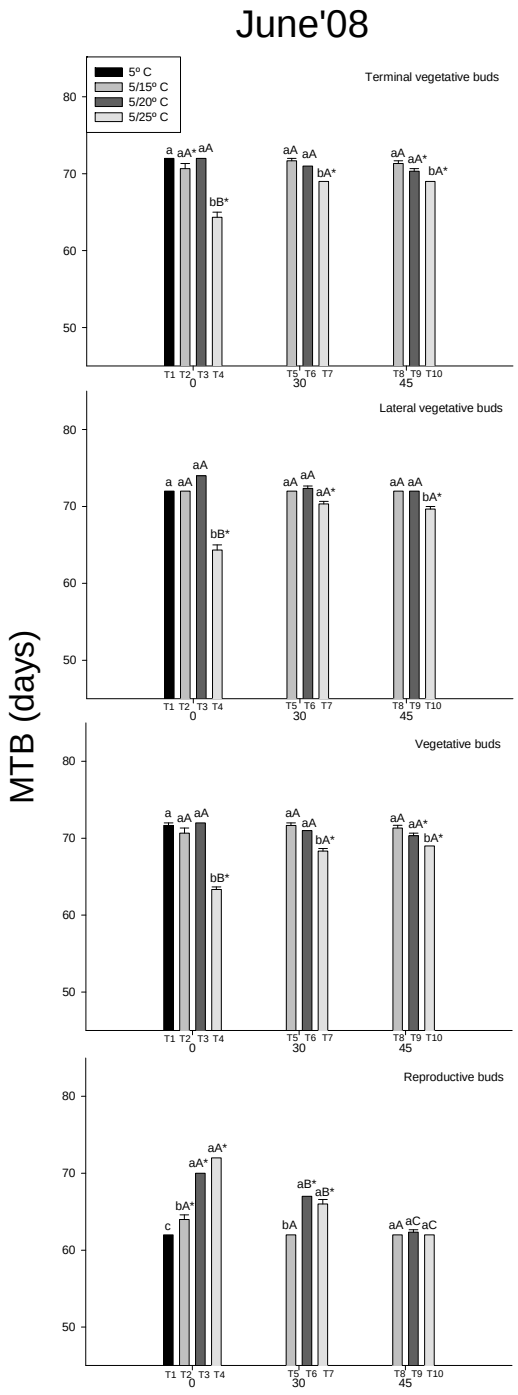
Similar behavior was found in lateral vegetative buds. The only differences were the generalized increase of 2-3 days in MTB and the absence of significant differences between T9 and T1. When vegetative buds were evaluated, similar results were obtained compared with terminal vegetative buds. The effectiveness of the pre-treatment varied slightly. Thus, after 30 days of pre-treatment at 5ºC, all cycles except for 5/15 ºC had significantly increased efficiency, whereas after 45 days only the 5/20 ºC cycle (T9) exhibited a reduction when compared to the pre-treatment of 30 days.

In reproductive buds, MTB values were, in most of the treatments, lower than in vegetative buds (Figure 4). Continuous 5 ºC was the most efficient treatment, showing significant differences from the rest of the treatments, except for T7 (5/25 ºC cycle after 30 days of pre-treatment) which was as efficient as continuous 5 ºC. After 30 days of pre-treatment, 5/25 ºC was significantly more efficient than the 5/15 ºC or 5/20 ºC cycles. The 5/15 ºC cycle improved its efficiency after 45 days of pre-treatment (T8), relative to no pre-treatment (T2) or 30 days of pre-treatment (T5).

1.3.4. Effect of different temperature cycles applied after 50% of chilling requirements had been satisfied (June 2008)

By the time of this sampling date, 107 CU had been accumulated, representing 50% of the 'Palsteyn' CR in that year (Table 2). Shoots were cut by June, four days later than in 2007, but chill accumulation measured as Chill Units was almost half of the 2007 value at harvest. The MTB values of the different buds and treatments are shown in Figure 5. Similar results to June 2007 (when 64% CR had been satisfied) were obtained in June 2008 when 50% of CR had been satisfied, although MTB was slightly higher than in 2007.

In terminal vegetative buds, 5/25 ºC was significantly the most effective cycle, especially when no 5 ºC pre-treatment was applied (Figure 5). The 5 ºC pre-treatment did not increase significantly the effectiveness of any treatment. What is more, 5 ºC pre-treatment significantly reduced the effectiveness of the 5/25 ºC cycle. No significant differences were found between continuous 5 ºC (T1) and the 5/15 ºC cycle, with or without pre-treatment (T5 and T8), or the 5/20 ºC cycle without treatment (T3) or with 30 days pre-treatment (T6). As for lateral vegetative buds, the 5/25 ºC cycle was also the most efficient cycle, especially when no pre-treatment was applied. Similar values were obtained in lateral and terminal vegetative buds, which contrasts with the differences obtained in April 2008.



Within each 5 ºC pre-treatment, different lower case letters for different temperature cycles show significant differences (P< 0.05) according to Tukey´s test.
For each temperature cycle, different capital letters in 5 ºC pre-treatments show significant differences (P< 0.05) according to Tukey´s test.
Means followed by * differ significantly from Treatment 1 (T1)

Figure 5. Mean time to budbreak in June 2008 (107 CU accumulated, 50% CR satisfied) of the different types of buds studied, using different temperature cycles.

However, unlike terminal vegetative buds no significant differences were found in lateral buds between continuous 5 ºC and 5/15 ºC and 5/20 ºC cycles, with or without pre-treatment. When all vegetative buds were considered, the results were practically the same as those of terminal vegetative buds (Figure 5).

Considering the reproductive buds in general, MTB values were intermediate between those of shoots collected in June 2007 (64% CR satisfied) and April 2008 (0 CR satisfied). When no pre-treatment was applied, 5 ºC was, significantly, the most effective treatment followed by the 5/15 ºC cycle. After 30 days pre-treatment at 5 ºC, the 5/15 ºC cycle was as efficient as continuous 5 ºC and significantly more so than the 5/20 ºC and 5/25 ºC cycles. However, after 45 days of pre-treatment at 5 ºC, no significant differences were found either among cycles or between cycles and continuous 5 ºC. Pre-treatments at 5 ºC significantly increased the efficiency of the cycles, except for 5/15 ºC - which already was the most effective combination. As for the other sampling dates, the MTB of reproductive buds was inferior to that of vegetative buds, showing a shallower endodormancy.

1.3.5. Comparison of MTB among sampling dates

Pre-treatment at continuous 5 ºC easily satisfied the whole CR of 'Palsteyn' apricot cultivar. Thus, 30, 45, and 60 days at 5 ºC represented 720, 1080, and 1440 CU (Utah Model) or 21.57, 32.26, and 43.15 Portions (Dynamic Model), respectively. In addition, when shoots were collected in June 2007 and June 2008, more than 50% of the 'Palsteyn' CR had already been already accumulated in field conditions. Therefore, every treatment represented more Chill Units or Portions than the CR calculated for 'Palsteyn' during two consecutive years. In spite of this fact, important differences were found for the same treatments among the different sampling dates (Tables 3-6). In terminal and vegetative buds (Tables 3 and 5, respectively), 60 days at continuous 5 ºC (T1) gave no significant differences in MTB among sampling dates, whereas differences were found in lateral vegetative buds (Table 4) and, especially, in reproductive buds (Table 6).

When temperature cycles were applied (T2-T10), with or without a 5 ºC pre-treatment, significant differences were found among sampling dates for almost all treatments, in both vegetative and reproductive buds (Tables 3-6). The MTB values of the April 2008 sampling, when 0 CU had been accumulated, were significantly higher than on the other sampling dates (June 2007 and June 2008), which had a certain amount of chill accumulated in the field, for most treatments and types of bud. For this sampling date (April 2008), no budburst occurred before the forcing

conditions were imposed. Only when the 5 ºC pre-treatment was applied for 45 days was a similar behavior observed for both April 2008 and June 2008, for the 5/15 ºC and 5/25 ºC cycles in the case of terminal vegetative buds (Table 3) and vegetative buds (Table 5).

Table 3. Comparison of MTB in terminal vegetative buds of the different sampling dates.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
April'08 (0 CR)	70.0 a	73.0 a	79.7 a	79.0 a	73.0 a	74.3 a	72.0 a	72.3 a	72.7 a	71.0 a
June'07 (60% CR)	69.8 a	70.0 b	69.3 b	61.7 b	68.7 c	67.7 c	61.0 c	69.0 b	69.3 b	64.3 b
June'08 (54% CR)	72.0 a	70.7 b	72.0 b	64.3 b	71.7 b	71.0 b	69.0 b	71.3 a	70.3 b	69.0 a

Different letters in each column show significant differences (P< 0.05) among sampling dates according to Tukey´s test.

Table 4. Comparison of MTB in lateral vegetative buds of the different sampling dates.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
April '08 (0 CR)	73.0 a	76.7 a	80.3 a	82.3 a	76.0 a	75.3 a	74.7 a	74.7 a	74.7 a	73.7 a
June'07 (60% CR)	69.8 b	70.7 b	69.0 c	55.0 c	68.7 c	67.0 c	58.7 c	69.0 c	70.0 c	64.7 c
June'08 (54% CR)	72.0 a	72.0 b	74.0 b	64.3 b	72.0 b	72.3 b	70.3 b	72.0 b	72.0 b	69.7 b

Different letters in each column show significant differences (P< 0.05) among sampling dates according to Tukey´s test.

Table 5. Comparison of MTB in vegetative buds of the different sampling dates.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
April'08 (0 CR)	70.0 a	73.0 a	78.0 a	79.0 a	73.0 a	74.3 a	72.0 a	71.7 a	72.3 a	71.0 a
June'07 (60% CR)	69.5 a	70.0 b	68.7 c	54.3 c	68.0 c	66.7 c	59.0 c	68.0 b	69.3 b	63.0 b
June'08 (54% CR)	71.7 a	70.7 b	72.0 b	63.3 b	71.7 b	71.0 b	68.3 b	71.3 a	70.3 b	69.0 a

Different letters in each column show significant differences (P< 0.05) among sampling dates according to Tukey´s test.

Table 6. Comparison of MTB in reproductive buds of the different sampling dates.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
April'08 (0 CR)	65.7 a	72.0 a	80.0 a	78.0 a	71.0 a	72.0 a	67.0 a	69.7 a	70.7 a	69.7 a
June'07 (60% CR)	52.0 c	61.7 b	65.7 c	46.0 c	51.3 c	52.3 c	49.7 b	51.0 c	49.7 c	49.3 c
June'08 (54% CR)	62.0 b	64.0 b	70.0 b	72.0 b	62.0 b	67.0 b	66.0 a	62.0 b	62.3 b	62.0 b

Different letters in each column show significant differences (P< 0.05) among sampling dates according to Tukey´s test

Besides, the effect of the temperature cycles differed significantly between June 2007 (214 CU accumulated, 64% of the 'Palsteyn' CR) and June 2008 (107 CU accumulated, 50% of the

'Palsteyn' CR). For terminal vegetative buds, the MTB values of June 2007 were significantly lower than in June 2008 and April 2008, for all treatments (Table 3). In the case of lateral vegetative buds and vegetative buds (considering terminal and lateral), significant differences between June 2007 and June 2008 were found for all treatments except the 5/15 ºC cycle without pre-treatment (T2) and the 5/20 ºC cycle with 45 days of pre-treatment, in vegetative buds (Tables 4 and 5). Similar differences were found for reproductive buds although the MTB values were lower than in vegetative buds. The MTB values of June 2007 were significantly lower than in June 2008, for all treatments.

1.4. Discussion

Many of the experiments developed in previous works, aimed at elucidating the effect of different temperatures or temperature cycles on dormancy release, were carried out with plant material collected at the end of October-beginning of November. This period is commonly associated with the deepest endodormant state in the northern hemisphere (Fuchigami *et al*., 1977) and it would correspond to the end of April-beginning of May in the southern hemisphere. Thus, the plant material collected in April 2008 would correspond to the state of maximum endodormancy. By that time, as usual for that area, no chill accumulation had been registered. On the other hand, according to Weinberger (1950), the effective winter-chilling period extends from November 1 to February 15 (May 1 to August 15 in the southern hemisphere).

The MTB results obtained after the application of different temperature cycles to plant material collected in April were similar to those obtained in previous works (Couvillon and Erez, 1985b; Naor *et al*., 2003), even though those results were obtained using daily cycles of 16-8h instead of the 19-5h cycle used in our experiment.

When no chill accumulation had been achieved, neither in field nor in laboratory conditions (no pre-treatment at 5 ºC), the high-temperature (5/20 and 5/25 ºC) cycles applied gave the highest MTB values. The most effective temperature was 5 ºC. The 5/15 ºC cycle showed an efficiency with respect to release of dormancy intermediate between those of 5 ºC and the cycles with higher temperatures. This synergic effect of moderate and low temperatures has been described previously (Guerriero *et al*., 1985b; Erez and Couvillon, 1987).

Chilling pre-treatment at continuous 5 ºC exerted an important influence over the effect of the daily cycle, for the plant material collected in April 2008. After the chilling pre-treatment, MTB

values were significantly lower for all treatments. However, when no chill was accumulated in field conditions the high-temperature cycles did not have MTB percentages lower than those of continuous 5 ºC, even after 30 or 45 days of 5 ºC pre-treatment. On the other hand, MTB values for samples collected in April 2008 when no chill had been accumulated in field conditions, were significantly higher for all treatments (even with 5 ºC pre-treatment) than MTB values for plan material collected in June 2007 or June 2008 when more than 50% CR had been satisfied. Thus, it seems that the efficiency of chilling in field conditions of shoots on the tree is higher than that for shoots under laboratory conditions presumably due to progressive physiological changes. The identification of the stage of the buds is essential for a correct interpretation of results (Fuchigami and Nee, 1987; Falussi and Calamasi, 2003).

Once a certain amount of cold had been accumulated in field conditions, cycles with moderate or high temperatures increased its efficiency in dormancy breaking (Tables 3-6). For the sampling dates of June 2007 and June 2008, the 5/25 ºC treatment was more efficient than 5 ºC, even though it represented a chill accumulation 21% inferior to that of continuous 5 ºC. The 5/15 ºC and 5/20 ºC treatments also increased its efficiency when applied after partial chill accumulation in field conditions. These results agree with previous work (Erez and Lavee, 1971; Young, 1992) that showed that moderate and high temperatures can be more efficient at breaking dormancy when applied in advanced stages of dormancy release. Couvillon and Erez (1985b) showed a higher efficiency, with regard to increasing budbreak, of daily cycles of 20/ 4 ºC with thermoperiods of 2/22 h or 4/20 h compared with the continuous application of 4 ºC. Both thermoperiods and continuous 4 ºC were organized to contribute the same quantity of chill accumulation. These results agree with ours, even though in our case less chilling was applied in the daily cycle with high temperatures.

The sharp increase in dormancy-breaking efficiency arising from the application of 5/20 ºC to 5/25 ºC, after partial chilling had been accumulated, could indicate the existence of a temperature threshold between 20 and 25 ºC. From this threshold on, it seems that dormancy-breaking efficiency abruptly increases after partial chilling has been accumulated. This could be related to the capacity of high temperatures to break dormancy (Chandler, 1960; Tamura *et al*., 1993) and to the lack of chilling-negation effect when the tree is in an advanced stage of endodormancy release (Couvillon and Erez, 1985b). Erez and Lavee (1971) showed that moderately high temperatures acquire an efficiency to break dormancy only after a partial chill accumulation. However, our results contrast

with those of Erez *et al*. (1979a) and Shaltout and Unrath (1983), who stated that an hour at temperatures from 21 ºC to 24 ºC, and presumably higher, in a diurnal cycle should have a value of -2 CU.

In contrast, the data in Tables 3-6 indicate that the chill receptivity of shoots could be conditioned by the state of development when they are separated from the tree. This is especially clear in the data obtained after continuous pre-treatment at 5 ºC for 45 days. Theoretically, all treatments satisfied the 'Palsteyn' CR according to both the Utah and Dynamic Models. However, the MTB values of many treatments and sampling dates differed significantly. Tehnranifar *et al*. (1998) showed that strawberry plants lifted in December had a more intense response to cold than others lifted in November. Thus, it seems that the physiological state of the plant material on the cutting date can condition the effect of cold in dormancy overcoming (Crabbé and Barnola, 1996). However, to explain the differences, it is necessary to take into account the possibility of a residual effect (Spiegel-Roy and Alston, 1979), even when the models indicate the fulfilment of the CR (Couvillon and Erez, 1985a; Guerriero *et al*., 2002; Ruiz *et al*., 2007). Besides, the possibility of a reduction of the heat requirement after a higher chilling accumulation should be considered (Couvillon and Erez, 1985a; Felker and Robitaille, 1985).

All types of apricot bud studied showed a similar behavior under the different temperature cycles. The competitive effect of vegetative and reproductive buds and the correlative inhibition complicate the comparison of MTB among type of buds (Guerriero *et al*., 1987). For all sampling dates, the reproductive buds showed the lowest endodormant state, which agrees with the lower chilling requirements of reproductive buds in comparison with vegetative buds (Scalabrelli and Couvillon, 1986; Erez, 2000). Naor *et al*. (2003) found in apple that lateral vegetative buds had higher chilling requirements than terminal vegetative and reproductive buds. Besides, the influence of the stage of dormancy on the differing intensities of dormancy in the different types of bud has been shown (Barnola and Crabbé, 1991; Cook *et al*., 1998). In 'Palsteyn', the differences in chilling requirements between terminal and lateral vegetative buds were not very acute. Lateral vegetative buds tended to have a slightly higher value.

1.5. Conclusions

The efficiency of the different treatments was highly influenced by the state of bud dormancy when shoots were cut. When no chill had been accumulated prior to the cutting date,

continuous 5 ºC was the most efficient treatment, followed by 5/15 ºC. However, when shoots had already received a certain chill accumulation in field conditions, 5/25 ºC was always the most efficient treatment, whereas 5/15 ºC and 5/20 ºC became as efficient as 5 ºC. After a pre-treatment of 5 ºC and when no chill had been accumulated in field conditions, the efficiency of the treatments tended to equalize, especially after 45 days of pre-treatment. A similar trend in all treatments was observed within the different types of bud; even tough reproductive buds had lower CR. Almost no differences in CR were observed between terminal and lateral vegetative buds. The notable efficiency showed by the combination 5/25 ºC compared with 5/20 ºC and 5/15 ºC, after partial chilling in field conditions, could indicate a qualitative more than a quantitative change. Besides, the results showed that high temperatures, such as 25 ºC, can be very efficient for dormancy release when applied in a daily cycle with low temperatures after partial chilling has been accumulated. Chilling was substantially more efficient when applied in field conditions than in the growth chamber.

2. EFFECTS OF CHILLING TEMPERATURES ON APRICOT DORMANCY PROGRESSION WHEN APPLIED AFTER DIFFERENT AMOUNTS OF FIELD CHILL ACCUMULATION.

2.1. Introduction

Dormancy in temperate-zone deciduous trees is a phase of development that allows the trees to survive unfavourable conditions during the winter (Faust *et al*., 1987). It is a well stated fact that buds of many deciduous trees and shrubs require a period of chilling to overcome dormancy (Coville, 1920; Chandler *et al*., 1937; Lang *et al*., 1987). Even when there are indications that other factors such as light, mist, heat, etc., affect endodormancy, chilling period has been considered the main factor controlling growth cessation and dormancy progression in temperate woody species (Jacobs *et al*., 2002, Heide and Prestrud, 2005). In addition, low temperatures are the most significant factor affecting dormancy completion (Chandler, 1960; Erez and Lavee, 1971; Freeman and Martin, 1981). The relative contribution of temperatures to dormancy completion has been frequently tested. Initially only temperatures below 45 ºF (7.2 ºC) were considered useful and equally effective in breaking dormancy (Weinberger, 1950). Later it was demonstrated that temperatures higher than 7.2 ºC were also effective, and the relative contribution of different temperatures was found to vary (Richardson *et al*., 1974). Moderately high temperatures were found to negate chilling (Erez *et al*., 1979a).

Based on the results of these studies, different models aimed at estimating chilling accumulation were developed, mainly for peaches, but also for other species (Erez and Lavee, 1971; Richardson *et al*., 1974; Gilreath and Buchanan, 1981b; Shaltout and Unrath, 1983; Erez and Couvillon, 1987). Moreover, moderate temperatures, initially considered neutral, were found to have a synergistic effect when applied alternated with low temperatures in a daily cycle (Erez *et al*., 1979a; Felker and Robitaille, 1985; Guerriero *et al*., 1985b; Erez and Couvillon, 1987). This finding, together with others related to the effect of cycle length on chilling negation (Erez *et al*., 1979b), allowed the development of a new model to estimate chill accumulation, the Dynamic Model (Fishman *et al*., 1987a; 1987b).

All the referenced models assigned a constant contribution to breaking dormancy to each temperature, independent of depth of dormancy (Fuchigami and Nee, 1987). However, there is

strong evidence that the effect of temperatures on dormancy release depends on the stage of rest. Thompson *et al*. (1975) reported that chilling early in the dormant period was less effective than later chilling. No chilling negation was found when exposure of 'Redhaven' peach to 23ºC was applied following the accumulation of three-fourths of CR (Couvillon and Erez, 1985b). Erez and Couvillon (1987) found that the effect of moderate temperatures on rest completion seemed to increase when applied during the latter stages of dormancy. Trees of 'MM111' apple exposed to 15 ºC during the first or second 500 h of chilling at 5 ºC had significantly less bud break than those exposed to 15 ºC during the last 500 h of the chilling period (Young, 1992). The vegetative growth of strawberry after cold storage was greater on plants lifted on December 6 than on plants lifted on November 6 (Tehranifar *et al*., 1998). In a recent work, we have found that the efficiency of different daily temperature cycles in regards to dormancy release was completely dependent on the stage of dormancy in the apricot cultivar 'Palsteyn' (unpublished results). If all this evidence truly indicates a general trend, the new models for estimating chill accumulation should incorporate this variable effect of temperatures. The aim of this work was to elucidate the effect of four different chilling temperatures on the progression of dormancy in vegetative and reproductive buds in apricot cultivars when applied after different amounts of chill accumulated in field conditions.

2.2. Materials and methods

2.2.1. Plant material

The plant material used in this experiment was collected from three apricot genotypes: one old traditional cultivar called 'Pepito del Rubio'; and two selections of intraspecific crosses, 'Z505-2' and 'Z308-9', both released from the CEBAS-CSIC apricot breeding program. All genotypes were grown in an experimental orchard situated in the southeast of Spain (Aljunzarejo-Murcia, altitude 350 m, lat. 38º21'N, long. 1º18'O). Seven year-old trees were cultivated according to habitual apricot orchard management, with drip irrigation and a 7m x 7m distance of plantation. Fifty trees per genotype were selected because of the need for many materials for the experiment. Approximately every 15 days from October 14 to January 10, 120 50 cm-long shoots per genotype were cut from the trees and defoliated when necessary.

2.2.2. Chilling accumulation

Hourly temperatures during autumn and winter were collected in the orchard with an automatic data-logger (Escort ® Datalogging Systems, Buchanan, Virginia, USA, 2002). This data was used to calculate total chill accumulation as well as chill accumulated by the time of sampling shoots in the field. The starting date for chilling accumulation was considered to be when both a consistent chilling accumulation had occurred and the temperatures producing a negative effect (chilling negation) were scarce (Richardson *et al*., 1974; Erez *et al*., 1979b; Guerriero *et al*., 2002). Chilling accumulation was assessed by Chill Units of the Utah Model (Richardson *et al*., 1974); chill hours (Hours below 7 ºC) (Weinberger, 1950); and Portions of the Dynamic Model (Fishman *et al*., 1987a; 1987b).

2.2.3. Chilling requirements for breaking dormancy

In order to establish the percentage of CR satisfied on each sampling date, the chilling requirements of each variety were calculated according to Ruiz *et al*. (2007). Three branches per genotype were collected periodically from the beginning of January to mid-February and forced for ten days at 25 ºC and 70% humidity. Bud weight evolution and the bud development stage were evaluated. Chilling requirement satisfaction was considered when 30% of flower buds had attained the Baggiolini B-C stage (Baggiolini, 1952) and their weight had increased 30% compared to the relatively constant previous value.

2.2.4. Temperature treatments

The shoots were cut to 40 cm long and stored at 1, 4, 7 and 10 ºC for 60 days at 75% RH Thirty shoots per variety, temperature treatment and sampling date were used. The shoots were placed horizontally on watered vermiculite and covered with polyethylene film to avoid desiccation. After the cold treatments, shoots were cut to 30cm long and bundled in three replicated bundles of ten shoots each.

2.2.5. Forcing conditions and bud burst scoring

The bundles of ten shoots each were placed at random in 5 L buckets and forced with their bases in ca. 1 L of tap water, containing 5 mL/L of household bleach (5% sodium hypochlorite) to avoid microorganism proliferation. All shoots were forced at 25ºC with a light source of ca. 200

μmoles m s of photosintetically active radiation (93% "cool white" fluorescent and 7% incandescent). During forcing, the bottom 1 cm of the shoots was removed weekly, and shoots were checked three times a week in order to establish the mean time to bud break (MTB) in each kind of bud until 50% bud burst. The time in days for the occurrence of bud burst was established when budburst was observed in at least one bud per shoot on five shoots per bundle. The types of bud considered were: terminal vegetative, lateral vegetative and reproductive.

2.2.6. Statistical analysis

Statistical analytical procedures were performed using SPSS® 15.0 software for Windows (Chicago, IL). Differences in mean time to budburst among treatments and sampling dates were analyzed by ANOVA for each type of bud in order to determine the efficiency of the different temperature treatments applied at various stages of endodormancy in the different kinds of buds evaluated.

2.3. Results

Values of maximum and minimum temperatures from October 15 to January 10 are shown in Figure 1. In our field conditions, autumn and early winter are characterised by relatively warm temperatures. Chill accumulated at each sampling date is shown in Table 1. Chill accumulation began at the end of October and by the last sampling date, January 10, more than 700 CU had accumulated. Results are shown in Hours below 7 ºC (Weinberger, 1950); CU (Richardson *et al*., 1974); and Portions (Fishman *et al*., 1987a; 1987b). Artificial chilling accumulated in each 60 day treatment is also shown in Table 1. Chilling treatments of 1, 4, and 7 ºC accumulated 1440 Hours below 7.2 ºC, whereas the 10 ºC treatment logically did not accumulate any hours. With regard to CU accumulation, 1 ºC treatment did not accumulate CU, whereas 4 and 7 ºC treatments accumulated 1440 CU, and 10 ºC treatment accumulated 720 CU. Finally, portion accumulation gradually increased from the 1 ºC treatment (17.8 Portions) to 7 and 10 ºC treatments (46.6 Portions in both cases).

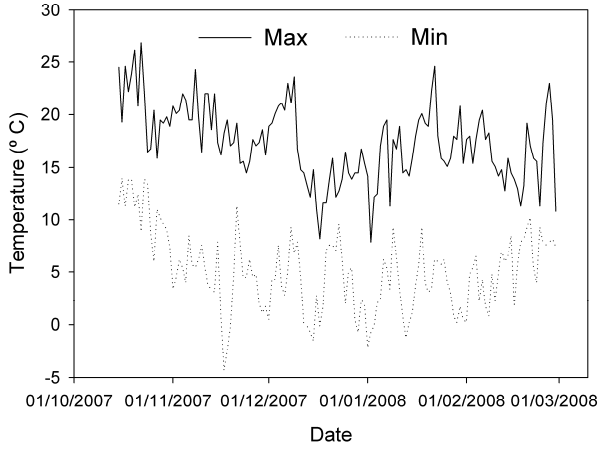


Figure 1. Maximum and minimum temperatures in the orchard throughout the duration of the experiment.

Table 1. Chill accumulation registered for the different variables and categories studied. (A) Chilling requirement of the genotypes in field conditions. (B) Artificial chill accumulated in each 60 day treatment of cold storage. (C) Chill accumulated in field conditions on the different sampling dates.

	Variable	Category	Chill Units	Portions	H < 7 ºC
A	Genotype	308-9	1001	60.51	787
		505-2	849	51.72	670
		Pepito del Rubio	911	54.72	701
B	Treatment (ºC)	1 ºC	0	17.83	1440
		4 ºC	1440	38.04	1440
		7 ºC	1440	46.64	1440
		10 ºC	720	46.14	0
C	Sampling Date	15.10.2007	-	-	-
		29.10.2007	8	2.01	3
		13.11.2007	91.5	9.75	79
		27.11.2007	221.5	18.09	181
		12.12.2007	340	26.53	296
		27.12.2007	558.5	37.21	415
		10.01.2008	731	43.63	566

Figure 2 shows the weight evolution of the flower buds according to chilling accumulation. An arrow indicates the time of dormancy breaking for each genotype in relation to the weight

evolution of the flower buds and the phenological stage (data not shown in the figures). This point indicates the chilling requirements for each genotype, measured by Chill Units, and it coincides with the values shown in Table 1. 'Z505-2' and 'Pepito del Rubio' showed medium chilling requirements, 849 CU and 911 CU respectively, whereas 'Z308-9' showed medium-high chilling requirements (1001 CU). The reference cultivars 'Búlida' and 'Orange Red' had 865 and 1130 CU, respectively. Chilling requirements calculated by CU, Hours below 7 ºC and Portions for each genotype are shown in Table 1.

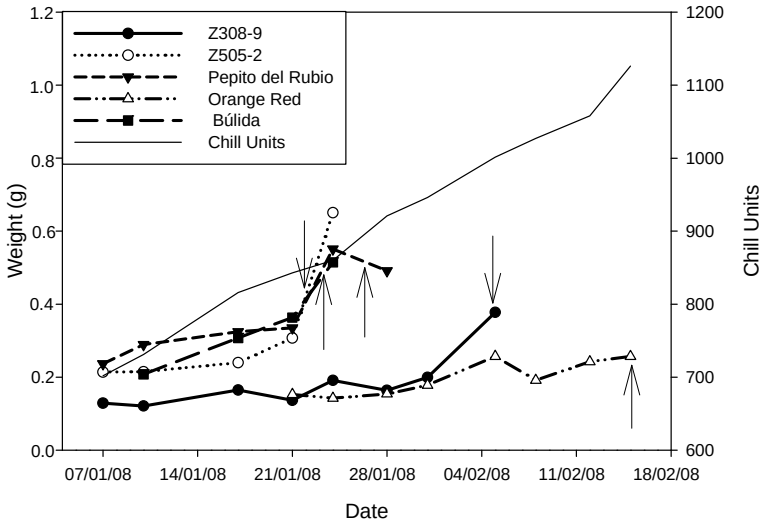


Figure 2. Breaking of dormancy in apricot varieties according to the weight progression of the flower buds and chill accumulation during this period. Values of 'Orange Red' and 'Búlida' are also shown as reference cultivars. Arrows indicate the moment of dormancy release for each genotype.

According to ANOVA results (Table 2), significant differences were found in MTB for the variables Chill Accumulation (sampling date); Genotype; and the interactions between Temperature*Chill Accumulation and Genotype*Chill Accumulation in lateral and terminal vegetative buds. Differences in MTB among temperature treatments were significant in lateral vegetative buds and marginally significant in reproductive buds. As for reproductive buds, the results were similar to those in vegetative buds, but no significant differences were found for Genotype.

Table 2. *F*-values obtained in the ANOVA for the studied variables and interactions for the MTB for the different types of buds.

Type of bud	Variable	*DF*	*MS*	*F-value*	*P*
Terminal Vegetative	Temperature	3	11.797	2.430	0.068
	Chill Accumulation (sampling date)	6	55.293	11.388	0.000
	Genotype	2	31.810	6.551	0.002
	Temperature * Chill Accumulation	18	24.378	5.021	0.000
	Temperature * Genotype	6	1.922	0.396	0.881
	Genotype * Chill Accumulation	12	10.433	2.149	0.018
	Error	134	4.855		
Lateral Vegetative	Temperature	3	52.083	15.120	0.000
	Chill Accumulation (sampling date)	6	93.963	27.278	0.000
	Genotype	2	111.028	32.232	0.000
	Temperature * Chill Accumulation	18	53.555	15.547	0.000
	Temperature * Genotype	6	5.808	1.686	0.126
	Genotype * Chill Accumulation	12	13.270	3.852	0.000
	Error	187	3.445		
Reproductive	Temperature	3	3.997	2.737	0.048
	Chill Accumulation (sampling date)	6	49.176	33.672	0.000
	Genotype	2	1.612	1.104	0.336
	Temperature * Chill Accumulation	15	3.798	2.600	0.003
	Temperature * Genotype	3	1.128	0.772	0.512
	Genotype * Chill Accumulation	8	8.175	5.598	0.000
	Error	90	1.460		

Regarding MTB progression, terminal vegetative buds and lateral vegetative buds showed a similar pattern (Figure 3A and Figure 3B respectively). However, some differences should be remarked, principally in the first stages of the experiment. On the first sampling date, all treatments showed a low MTB value (ca. 8 days), except for 10 ºC, which had a value greater than 15 days. Subsequently, the 7 ºC treatment slightly decreased the MTB of the lateral buds, while the other treatments increased the MTB value to greater than 12 days. On the third sampling date, all MTB values converged to a value of ca. 14 days, as had occurred in terminal vegetative buds. Thereafter, the progression was similar as in lateral vegetative buds. A progressive decrease in MTB occurred in all treatments, except for the last sampling date, when a slight increase was found in the 1 ºC and 7 ºC treatments.

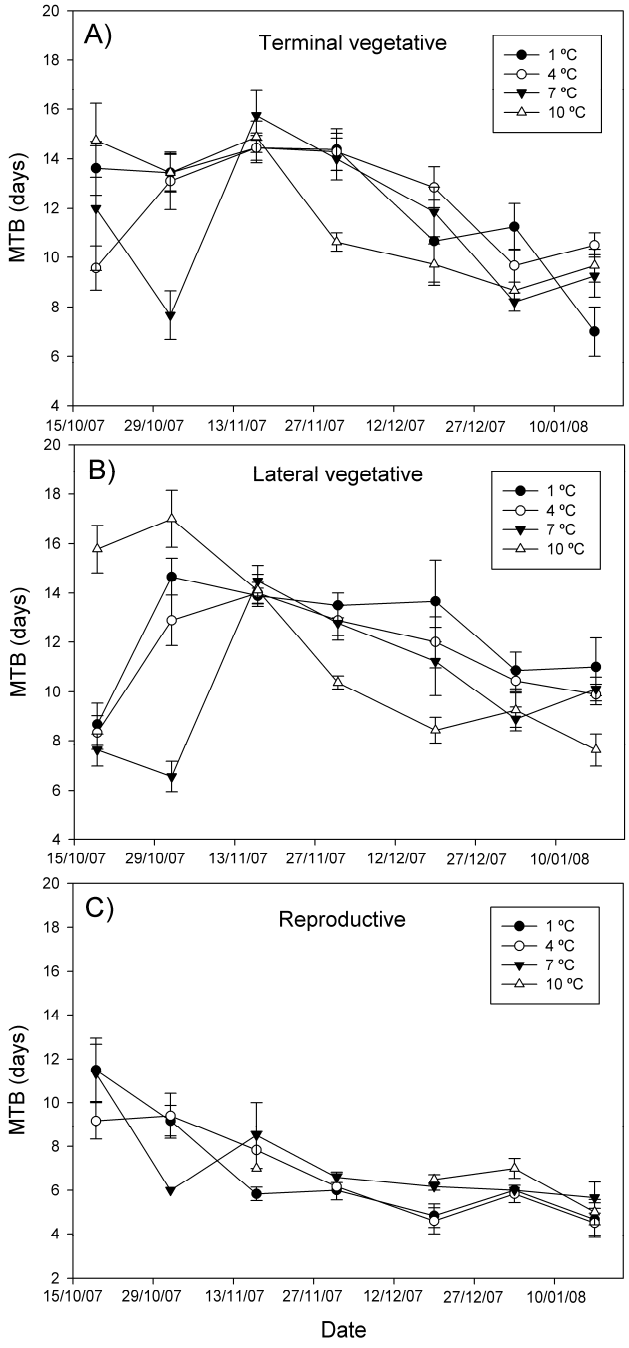


Figure 3. MTB progression of terminal vegetative buds (A), lateral vegetative buds (B) and reproductive buds (C), after treatment at different temperatures. Data show mean values of the three cultivars.

With regard to reproductive buds (Figure 3C), the MTB progression was completely different from that of the lateral and terminal vegetative buds. The MTB values were generally lower in reproductive buds except for on the first sampling date, when the values were similar to or higher than in lateral vegetative buds, i.e. 12 days for all treatments, except for the 4 ºC treatment,

which had a value of ca. 9 days. After the first sampling date, a gradual decrease in MTB occurred in all treatments, which contrasts with the progression of MTB shown by both lateral and terminal vegetative buds. It is important to note the effect of the 10 ºC treatments on reproductive buds. During these treatments, a high percentage of bud drop was observed (data not shown) until ca. 350 Chill Units had accumulated (November 27), which involved missing values in MTB.. From November on, the 10 ºC treatment performed similarly to the rest of treatments.

2.4. Discussion

In terminal and lateral vegetative buds, the 10 ºC treatment showed the highest MTB values of all treatments when applied before the advent of chilling temperatures and before the onset of the maximum depth of dormancy. This could be also interpreted as a higher capacity of 10 ºC to induce a deeper dormancy, especially in lateral vegetative buds, where the increase was sharper. Lavarenne *et al*. (1975) found that moderately cold temperatures are more efficient than extreme cold temperatures to increase the depth of dormancy. Besides, only in the 10 ºC treatment MTB did not increase when chill accumulation began. In late October-early November, coinciding with the onset of chill accumulation, a generalized increase in MTB was observed in all treatments in both terminal and lateral vegetative buds, which could be associated with dormancy depth intensification. By the beginning of November, the MTB of all treatments were very similar. The relatively abrupt intensification of dormancy in this stage has already been reported by several authors (Arias and Crabbé, 1975; Walser *et al*., 1981; Amling and Amling, 1985). The same temperatures that later release dormancy through chilling accumulation also deepen the dormant state when applied before the tree reaches the endodormant state (Crabbé, 1994; Faust *et al*., 1997). However, Gariglio *et al*. (2006) stated that chilling caused a continuous decline in the intensity of rest. The later date of shoot sampling, when the maximum rest had likely been achieved, could explain this difference. Cook and Jacobs (2000) observed that “Granny Smith” and “Golden Delicious” apple shoots from a cold area reached maximum dormancy before any considerable chilling accumulated (<100 CU) whereas those from a warmer area reached maximum dormancy after 600 CU.

Lavarenne *et al*. (1975) found that the maximum depth of dormancy in *Fraxinus excelsior* L. took place around mid-November in a colder area than the southeast of Spain. This could indicate that other factors can influence the entrance into dormancy. Heide (2008) showed the important

interaction of photoperiod and temperature in the control of growth and dormancy in *Prunus* species. Besides, it seems that the advent of chilling temperatures (Figure 1) in field conditions had a higher effect over dormancy induction than did the application of low temperatures in laboratory conditions over cut shoots.

The acute drop of MTB obtained during the 7 ºC treatment at the end of October in all types of buds (Figure 3) is difficult to explain, considering the phase in dormancy intensification when it took place. The higher efficiency of this temperature to break endodormancy (Richardson *et al*., 1974; Shaltout and Unrath, 1983; Erez and Couvillon 1987), and the higher efficiency of moderate and low temperatures as the season advances, could explain this result (Tehranifar *et al*., 1998).

In lateral and terminal vegetative buds all temperature treatments showed a similar MTB value when the maximum depth of dormancy had been achieved (beginning of November). This stage seems to be an equilibrium point between the dormancy induction and dormancy release effect of the chilling temperatures applied. At this point, a qualitative change in the effect of chill temperatures over growth inhibition was produced. Afterwards, as chill accumulated in field conditions, a continuous reduction in MTB in all temperature treatments was observed. This is in accordance with previous work carried out by Gariglio *et al*. (2006). Similar results were obtained by Cook and Jacobs (2000) in a cold area. However, apple shoots from a warmer area reached maximum dormancy after 600 CU, and therefore, temperatures that normally promote chill requirement satisfaction enhanced dormancy (Cook and Jacobs, 2000).

From the beginning of chill accumulation, the most efficient treatment to induce budbreak in both terminal and lateral vegetative buds was 10 ºC. Also, the 7 ºC treatment showed a higher efficiency than the 1 and 4 ºC treatments, especially in the lateral vegetative buds. The least efficient treatment was that of 1 ºC. The models developed in peach that quantify the chill accumulated to overcome dormancy usually assign a higher efficiency at breaking dormancy to temperatures between 4-7 ºC than to 10 ºC (Richardson *et al*., 1974; Erez y Couvillon, 1987). Other authors working on apple have assigned more efficiency to 1 ºC than to 10 ºC. (Del Real-Laborde, 1990; Naor *et al*., 2003). Our results indicate that in apricot, after the satisfaction of ca. 20% of the CR of the genotypes in the field, the most efficient temperature was 10 ºC. Therefore, it could be argued that, to a certain extent, the efficiency of temperatures changes as the physiological state of the plant evolves in the season (Laverenne *et al*., 1975). The date of collection of the plant material in trials attempting to elucidate aspects of dormancy induction and release seems to have an

important effect on the posterior result of the applied temperatures. The variation between a few days near the maximum dormancy stage can be crucial for the posterior results. This could explain the uneven results that are frequently obtained, which are related to the uncertainty as to how to determine the maximum dormancy state.

In reproductive buds no trend of increase in MTB was observed. The continuous decrease of MTB along the season could indicate that the maximum depth of dormancy was already established in the reproductive buds when our trial began, before any considerable chilling accumulation. In comparison to vegetative buds, the different response of reproductive buds to chilling temperatures along the season and the shallower dormancy shown, could indicate the different chilling requirements of this type of buds, as previously reported (Guerriero *et al*., 1985a; Erez, 2000). It also could suggest differences in the mechanism of dormancy release that allow reproductive buds to have a shallower dormancy intensity and a dormancy release earlier in the season compared to vegetative buds.

2.5. Conclusions

Similar results were obtained in both lateral and terminal vegetative buds regarding the effect of chilling temperatures on dormancy progression. Maximum depth of dormancy in vegetative buds was achieved by mid-November - when 100 CU had accumulated in field conditions - in all treatments except for 10 ºC in lateral buds. What is more, treatment at 10 ºC seemed to induce maximum dormancy in lateral vegetative buds but also to release bud dormancy earlier thereafter in both terminal and lateral vegetative buds. On the other hand, the other temperature treatments resulted in similar behaviour. Reproductive buds showed a shallower endodormancy and an earlier dormancy release than vegetative buds. Maximum depth of reproductive bud dormancy was achieved by mid- October, when no chill had accumulated in field conditions. Thereafter, a gradual decrease of MTB was observed for all temperature treatments. When low amount of chill had accumulated in field conditions, a high flower bud drop was observed in forcing conditions after the 10 ºC treatment. Results show the stage of dormancy has a strong influence on the effect of the different temperatures. A non-linear effect of different temperatures along the dormancy cycle was obtained, especially in the superior range of temperatures traditionally considered to release dormancy. Thus, the introduction of this differential effect could help to improve the models to estimate dormancy release.

CHAPTER 4. EFFECTS OF SHADING AND THIDIAZURON-OIL TREATMENT ON DORMANCY BREAKING, FLOWERING AND FRUIT SET.

1. Introduction

Apricot culture is greatly restricted by climatic conditions, especially related to low chill accumulation in several growing areas, with a significant influence on adaptation and hence in productivity (Quamme *et al*., 1982; Guerriero and Bartolini, 1991). Incomplete dormancy release affects tree behaviour in three main ways: a late bud break, a low level of bud break and a lack of uniformity of leafing and bloom, resulting in a higher flower bud drop (Legave *et al*., 1982; Viti and Monteleone, 1991, 1995; Erez, 2000).

The breaking of dormancy depends on the accumulation of winter chilling, which according to some authors is also a factor determining the installation of dormancy in an initial phase (Crabbé, 1994; Crabbé and Barnola, 1996). The most efficient temperatures to break dormancy are around 7 ºC (Weinberger, 1950; Richardson *et al*., 1974; Gilreath and Buchanan, 1981b, Shaltout and Unrath, 1983), while temperatures below 0 ºC are inefficient for dormancy breaking (Erez *et al*., 1971; Richardson *et al*., 1974). Temperatures above 16-18 ºC exert a negative effect on the accumulation (Richardson *et al*., 1974), which is level-, duration- and cycle-dependent (Erez and Fishman, 1998). Moderate temperatures (13-16 ºC) combined in a daily cycle with low temperatures enhance the chilling effect (Erez and Fishman, 1998). Temperatures above 16-18 ºC, due to sunshine, are frequent in warm-winter areas and affect negatively the breaking of dormancy (Richardson *et al*., 1974; Erez and Lavee, 1971). Couvillon and Erez (1985b) found that the main cause of poor bud break is the increase in daytime temperatures and not the lack of low night-time temperatures. Researchers have tried to improve dormancy breakage by reducing maximum daily temperatures, through sprinkling (Gilreath and Buchanan, 1981a; Erez and Couvillon, 1983; Nir *et al*., 1986; Erez, 1995), shading (Buchanan *et al*., 1977) and reflective films and evaporative cooling devices (Honjo *et al*., 2005). Shading could reduce a few degrees the maximum temperatures during the insolation hours. Therefore, shading can eliminate the negative effect of high temperatures, and enhance positive effect of moderate temperatures in combination with low temperatures.

On the other hand, the role of light as a factor regulating dormancy was studied by Erez *et al*. (1968), and they reported that a reduction in the amount of light supplied during the mid-rest period caused a better vegetative bud opening in peach as compared with natural winter daylight. Westergaard and Eriksen (1997) suggested a close relationship between autumn temperature and the strength of the induced dormancy in *Acer platanoides* L.

Although the physiological processes related to the dormancy still remain unclear, a group of chemical products have been identified, either by chance or try-and-error, to have a positive effect on dormancy overcoming when applied correctly. Concerning the growth regulators, gibberellins and cytokinins have also been frecuently used (Wang *et al*., 1986; Lloyd and Firth, 1993). A special mention deserves the cytokinin Thidiazuron ([TDZ] N-phenil-N-1,2,3-thiodiazol-5-il-urea), which in combination with mineral oil also shows an important action over dormancy release.

Nevertheless, there are still some gaps regarding not only the physiology of its action but also other related to its application. It is generally indicated that the applications of dormancy breaking chemicals should be accomplished when a considerable part of the chilling requirements have been satisfied (Erez, 1987a). However, it seems a more complex matter, and a further exploration should be done for the different crops and locations. This derives from the fact that, in general, the plant follows a different dynamics to the usual when the treatment is not performed. Another question that should be clarified, for its economic and practical relevance, is where the limit are to apply these treatments with a beneficial effect, in terms of date of the year, level of CR of the cultivar and the chilling accumulation of the area. Besides, it has been shown that regular application of some treatments, not only increases the percentage of budburst but also has a depressive effect over the vegetation (Costa *et al*., 2004).

The apricot cultivar ‘Poppy’ (U.S. Plant Pat. No. 9,593) has exhibited some production problems in some warm apricot production areas in Murcia (south-eastern Spain). Despite being a rather low-chilling cultivar, some production problems have been reported (personal communication), which may be related to a lack of adaptation to the warm climatic conditions of the area where it is cultivated. In apricot species, abnormally-underdeveloped pistils are caused generally by abortion of floral primordia, due to cellular necrosis (Legave, 1978), which is related to cultivar adaptation to different cropping areas. An important influence of weather conditions has been observed, as well as a significant variability among cultivars (Guerriero and Monteleone, 1988; Legave *et al*., 2006). A detrimental effect of warm winters on flower bud development has been observed (Erez, 1999). Brown (1958) and Monet and Bastard (1971) indicated an increase of flower bud abscission in peach under high temperature conditions in winter.

The purpose of this work was to determine the effect of shading, during different stages of dormancy, on flowering, pistil abortion, fructification and ripening in apricot (cv. ‘Poppy’) in a

warm area. In addition, these results were compared with the effect of a combined treatment of mineral oil and thidiazuron (N-phenyl-N-1,2,3-thiodiazol-5-il-urea), a cytokinin used to break bud dormancy (Wang *et al*., 1986; Steffens and Stutte, 1989).

2. Materials and methods

2.1. Plant material

One block per treatment, with ten seven-year-old 'Poppy' apricot trees each, was selected in 2005. All trees were cultivated in the same experimental orchard (south-eastern Spain: 38° N latitude, 1° W longitude and 150 m altitude) according to habitual apricot orchard management. Trees were drip-irrigated with a planting distance of 6 x 8 m. The fruitlets were thinned by hand. The CR of 'Poppy' in this area were calculated previously following the protocol used by Ruiz *et al*. (2007). After determination in three years, an average value of 720 Chill Units (CU) was determined as the CR of 'Poppy'. Despite the low chilling requirement of 'Poppy', productivity problems have been reported associated with the low chill accumulation registered in the area of the experiment.

2.2. Treatments

The experiments were conducted from autumn to spring during three consecutive years (2005-2006; 2006-2007; 2007-2008). Blocks of 10 trees each were selected for the treatments which were carried out as follows:

2005-2006: Treatment 1: shading during endodormancy (from November 1 to January 9, when 720 CU were accumulated); Treatment 2: thidiazuron (TDZ) + winter oil treatment; Treatment 3: shading in autumn from October 1 to November 16.

2006-2007: Treatment 1: shading during endodormancy (November 1 - February 1, when 720 CU were accumulated); Treatment 2: TDZ + winter oil treatment; Treatment 4: shading during late endodormancy (December 15 – February 1).

2007-2008: Treatment 1: shading during endodormancy (November 1 - February 1, when 720 CU were accumulated); Treatment 2: TDZ + winter oil treatment; Treatment 4: shading during late endodormancy (December 15 – February 1).

In each year, a control treatment was carried out.

The shade cloth was removed when the chilling requirements of 'Poppy', calculated previously for our area (~ 720 CU), had been satisfied. Shading was performed with a high tunnel structure, built with steel arcs and covered with a high-density, polyethylene shade cloth. The commercial shade cloth intercepted 80% of the incident radiation. Treatment 2 consisted of an application of 400 mg/L of Dropp ® (48.7% TDZ) and 25 g/L of 'Oil Oro Invierno'® winter oil (83%). The TDZ treatment date was chosen taking into account the point at which 2/3 to 3/4 of the chilling requirement had accumulated (Erez, 1987a) that is when 480-540 CU had been accumulated in the field.

2.3. Temperature measurement and chilling accumulation

Temperatures were measured with TMC50-IT-A sensors and recorded with the HOBO U12 data-logger (Onset Computer Corporation, Massachusetts, USA). Two sensors were used for shaded and two for non-shaded treatments. To avoid tree shadow, sensors were sited in the top parts of trees. Temperature was registered hourly, to calculate chilling accumulation.

The initial date for chilling accumulation was considered to be when a consistent chilling accumulation occurred and temperatures producing a negative effect (chilling negation) (Richardson *et al*., 1974; Guerriero *et al*., 2002) were scarce. Chilling accumulation was assessed as Chill Units of the Utah Model (Richardson *et al*., 1974). Previous studies (Ruiz *et al*., 2007) have reported a high correlation between Chill Units and Portions under our climatic conditions, that is to say, between the Utah and Dynamic (Fishman *et al*., 1987a, 1987b) Models. Thus, only chill accumulation by Utah Model will be shown.

2.4. Determination of flowering percentage, flowering date, pistil abortion, fruit set, and ripening cycle.

Three two-year-old shoots per tree were chosen in each treatment. The total number of flower buds was measured. Later, the number of flowers developed was recorded and expressed as a percentage of the total number of flower buds in those shoots (flowering percentage). Flowering date was determined as F_{50}, when 50% of flowers were open. Three sets of 100 flower buds at stage E-F of Baggiolini (1952) were collected per treatment and examined visually to determine pistil abortion percentage. One month after bloom, fruit set percentage (number of fruits per total remaining flowers) was calculated, as an expression of flower quality. Also, to determine the productivity in the different treatments, fruit set was calculated as the number of fruits per total

number of flower buds (fruits/buds). Due to the high uniformity in each treatment, flowering and ripening dates were determined per treatment instead of per tree. The ripening cycle was determined as the number of days from F_{50} to harvest.

2.5. Statistical analysis

Statistical analytical procedures were performed using the SPSS® 15.0 software for Windows (Chicago, IL). Differences among treatments concerning flowering, pistil abortion, fruit set and fructification percentage were analyzed by ANOVA. Pearson correlation coefficients were determined.

3. Results

3.1. Effect of shading on temperatures and chilling accumulation

The shade cloth reduced direct solar exposure and temperature in treatments 1, 3 and 4, especially during the maximum irradiation period, around the solar midday (Figure 1). Differences of up to 5 ºC were registered between shaded and non-shaded trees.

Maximum mean temperatures in non-shaded trees of treatment 3 were above 26 ºC, showing the warmth of the autumn in this area, while in treatments 1 and 4 they were around 18 ºC. In spite of these differences in maximum temperatures, the difference between the maximum mean temperatures of shaded and non-shaded trees was 2.5-3 ºC in all treatments. Therefore, the decrease of direct irradiation reduced, to a similar extent, the temperature in all treatments. The low standard errors for treatments 3 and 4 (Figure 1 - middle and lower) reflect the temperature constancy obtained by shading during the autumn and winter, respectively, compared with the high variability of temperatures from the end of autumn to winter in control (Treatment 1, Figure 1 - upper).

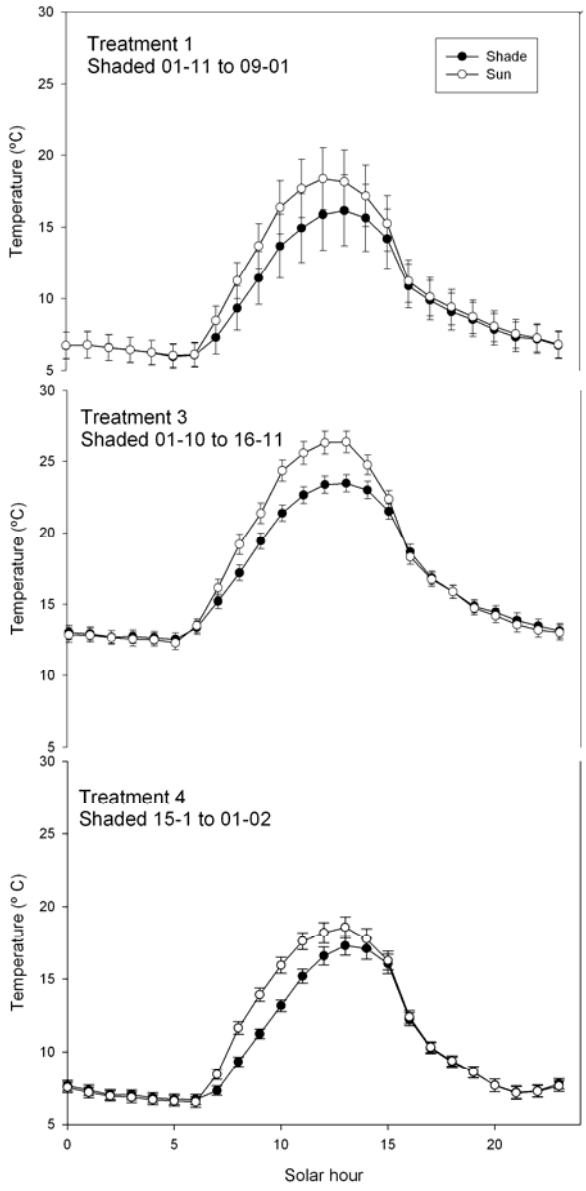


Figure 1. Mean hourly temperature during shading for treatments 1 (upper), 3 (middle) and 4 (lower), in comparison with non-shaded trees. Bars represent standard errors.

The effective chilling accumulation began in the first week of November in 2005 and 2007, in all treatments (Figure 2). In 2006, the warm autumn delayed the start of the chilling accumulation until the first week of December. In spite of this delay, a similar total chilling accumulation, but with a completely different distribution, was achieved in 2006-2007 in comparison with the 2007-2008 period (Figure 2). The total chill accumulation in 2005-2006 was higher than in the other two periods studied and unusually high for this area (Figures 2 and 3).

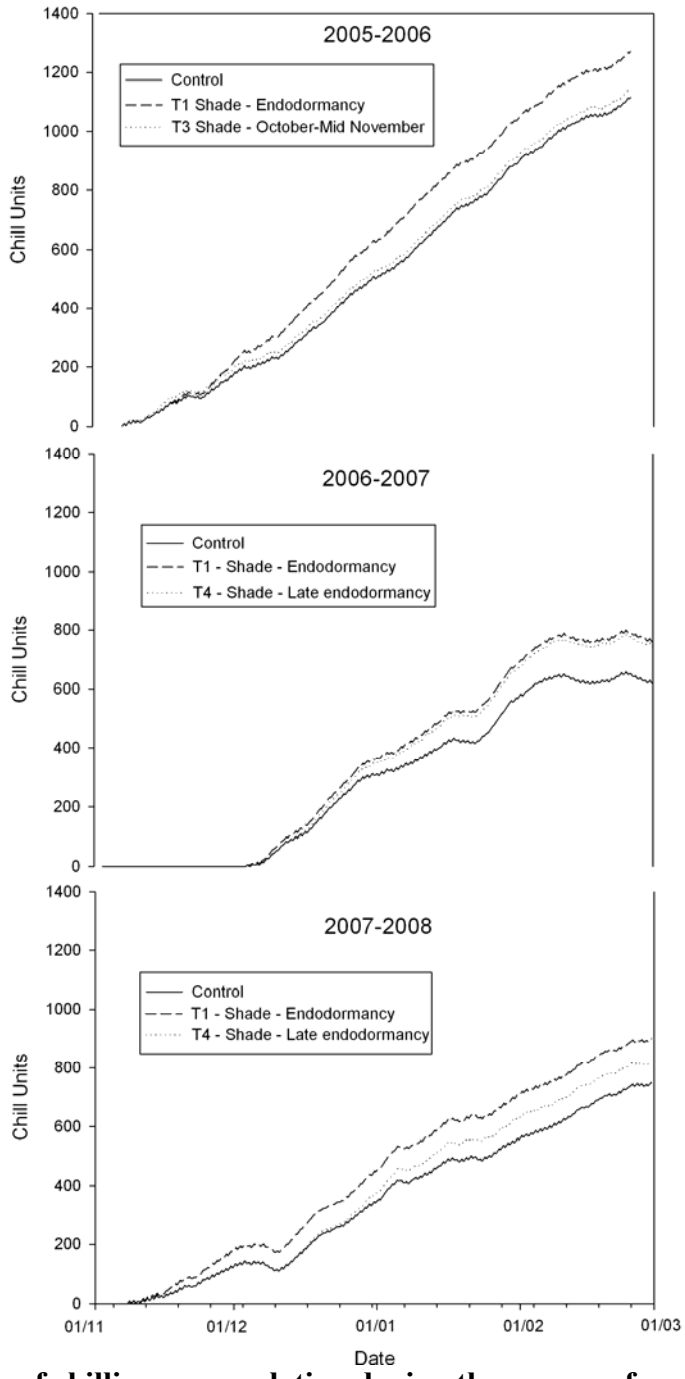


Figure 2. Progression of chilling accumulation during three years, for each treatment.

The difference in chill unit accumulation between the control and treatment 1 was almost constant among the periods studied (Figure 3). It ranged from 131.5 CU in 2007-2008 to 153 CU in 2005-2006. The difference arose mainly in the first 1.5 months of the chill accumulation period

(Figure 2). This portion represented up to 22.5 % in the 2006-2007 period of the total chill accumulation registered in non-shaded trees (control and treatment 2).

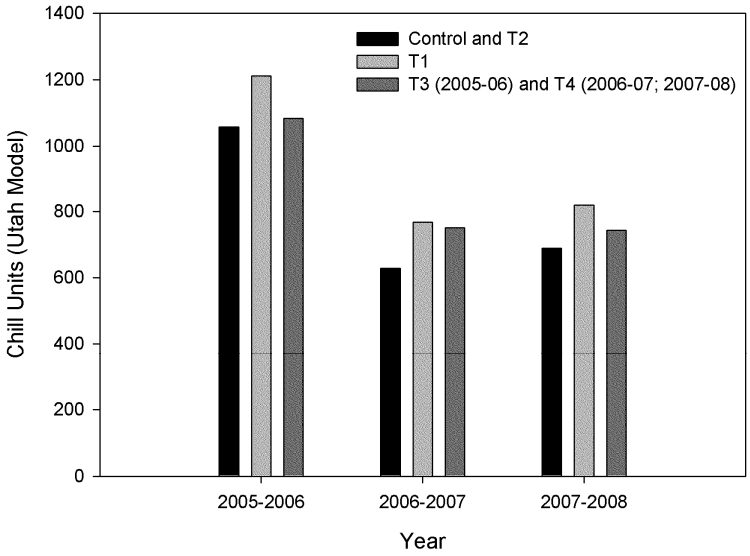


Figure 3. Total chill accumulation until February 15, by year and treatment

Treatment 3 in 2005-2006 had almost the same chilling accumulation than control (Figure 3), because chill accumulation began in November, just a few days before the shade was removed. For treatment 4, the accumulation was almost the same as in Treatment 1 in 2006-2007, because chilling accumulation began just before the trees were covered. In 2007-2008, treatment 4 achieved ca. 75 CU more than the control, representing 10% of the total chill accumulation.

3.2 Effects of shading and thidiazuron + oil treatment on flowering date and flowering percentage.

Treatment 1 brought forward flowering time 4 days in 2006, 3 days in 2007 and 0 days in 2008, compared with the control (Table 1). The application of TDZ and winter oil (Treatment 2) brought forward the flowering date, compared to the control, by 14 (2006), 7 (2007) and 10 days (2008).

The reduction of maximum daily temperatures through shading during the onset of dormancy (Treatment 3) did not cause differences between Treatment 3 and the control regarding the flowering date in 2006 (Table 1). Shading during late endodormancy (Treatment 4) had an uneven effect on the flowering date. In 2007, flowering was 5 days earlier than for the control, while in 2008 an unexpected delay of 9 days was recorded (Table 1).

Differences among years regarding flowering date were observed. Similar flowering date was obtained in 2006 and 2007 both in control and treatment 1, while flowering date was 5 days later in 2007 with treatment 2 (Table 1). However, flowering date was significantly earlier in 2008 than in 2006 and 2007 for control trees, treatment 1 and treatment 2 (Table 1). Only for treatment 4, flowering date was later in 2008.

The flowering percentages (Table 1) were highly variable among years and treatments. A clear relationship between the proper satisfaction of chilling requirements and high flowering percentage was observed. In 2006, all treatments gave a flowering percentage significantly higher than in 2007 or 2008, except for treatment 2 in 2007. Excluding treatment 2, 2007 had the lowest flowering percentages of the three periods studied. Flowering percentage in control trees was three times higher in 2006 than in 2007. Treatment 2 (TDZ + oil) had a significantly-higher flowering percentage over the three years, compared with the rest of the treatments (Table 1). Shading treatments did not increase significantly flowering percentages in comparison with control, with the exception of treatment 4 in 2008 which showed significantly higher flowering percentage (Table 1).

Table 1. Flowering date (F_{50}), flowering percentage, pistil abortion, fruit set percentage, fruits/buds percentage, harvest date and ripening cycle per treatment and year.

Treatment	Description	F_{50}	Flowering[1] (%)	Pistil abortion[1] (%)	Fruit set[1] (%)	Fruits/Buds[1] (%)	Harvest date	Ripening cycle (days)
					2005-2006			
Control	Control	06/03	65.8 Ab	12.9 Ba	30.5 Aa	20.1 Aab	14/05	69
1	Shade Endodormancy	02/03	57.0 Ac	9.6 Aa	29.7 Aa	16.9 Aa	12/05	71
2	TDZ+oil	20/02	90.4 Aa	16.0 Ba	24.7 Aa	22.3 Ab	11/05	80
3	Shade October	06/03	64.2 Abc	10.8 Ba	25.8 Aa	16.6 Aa	14/05	69
					2006-2007			
Control	Control	04/03	22.7 Cb	29.2 Ab	13.6 Bbc	3.1 Cb	14/05	71
1	Shade Endodormancy	01/03	26.2 Bb	16.7 Ac	16.6 Bbc	4.4 Bab	12/05	72
2	TDZ+oil	25/02	84.0 ABa	74.7 Aa	9.9 Bc	8.4 Ba	09/05	73
4	Shade Late Endodormancy	27/02	32.1 Bb	39.9 Ab	26.0 Aa	8.3 Ba	09/05	71
					2007-2008			
Control	Control	22/02	33.3 Bc	17.1 Bbc	33.0 Aa	11.0 Ba	07/05	75
1	Shade Endodormancy	22/02	33.4 Bc	14.5 Ac	25.0 Ab	8.3 Bb	04/05	72
2	TDZ+oil	12/02	76.9 Ba	71.9 Aa	8.9 Bc	6.8 Bb	28/04	76
4	Shade Late Endodormancy	03/03	52.7 Ab	32.9 Ab	22.0 Ab	11.6 Aa	08/05	66

3.3 Pistil abortion, fruit set, harvest date and ripening cycle

No significant differences among treatments regarding pistil abortion were found in 2006. However, in 2007 and 2008 treatment with TDZ + oil showed by far the highest percentages of pistil abortion, whereas treatment 1 showed significantly the lowest pistil abortion. Intermediate values were obtained with control trees and treatment 4 (Table 1). Pistil abortion was significantly higher in the control trees in 2007 (Table 1), which was the year with lower chill accumulation, than in 2006 or 2008. In treatment 4, no significant differences were found among years. In treatment 2, pistil abortion was increased significantly in the years with low chill accumulation (2006-2007 and 2007-2008).

As for pistil abortion, low chill accumulation had a negative influence on the fruit set percentage. The lowest fruit set percentages for all treatments, except treatment 4, occurred in 2007. Although similar low amounts of chill were accumulated in 2007 and 2008 (Figure 2), fruit set was significantly higher in control trees and treatment 2 in 2008 compared to 2007 (Table 1). In 2006, no significant differences were found among treatments, whereas significant differences were observed in 2007 and 2008, especially with treatment 2 which showed the lowest fruit set values (Table 1). The high flowering percentage achieved in treatment 2 did not translate into a high fruit set value because of the high pistil abortion rate.

Productivity was influenced highly by the uneven chilling accumulation registered during the three years of study (Table 1). For all treatments, the highest values of fruit/bud occurred in 2006. In 2007 and 2008, a considerable drop in the fruit/bud percentage was experienced, particularly in 2007 - the year with the lowest chilling accumulation. For example, in control trees, decreases of 85% and 50% in fruits/buds percentage were registered in 2007 and 2008, respectively, in comparison with 2006. Similar behavior was found in treatment 1. Treatment 2 showed a significantly-higher fruits/buds percentage in 2006 compared to 2007 and 2008, but no significant differences were found between 2007 and 2008 (Table 1). A certain influence of alternate bearing in 2007 cannot be excluded; however, no significant differences were found among the different years for the buds/cm^2 values (data not shown).

The harvest date was similar in 2006 and 2007 despite the difference in chilling accumulation (Table 1). In 2006, Treatment 3 and the control were harvested on the same date. Two and three days of precocity were obtained in treatments 1 and 2, respectively. In 2007, treatment 1

was harvested two days before the control, while the precocity obtained in treatments 2 and 4 was five days relative to the control.

In 2006, the ripening cycle was two and eleven days longer in treatments 1 and 2, respectively, compared with the control (Table 1). In 2007, the ripening cycle was almost constant among treatments, only one and two days of delay, respectively, occurred in treatments 1 and 2. In 2008, the ripening cycle of treatment 1 was three days shorter than that of the control, whereas in treatment 2 it was one day longer. Treatment 4 had an unexpectedly-short cycle, nine days less than that of the control (Table 1). In 2008, harvest date was one week earlier than in 2006 and 2007 for control and treatment 1, while harvest date for treatment 2 was 14 and 12 days earlier than in 2006 and 2007 respectively. Harvest date for treatment 4 was similar in 2007 and 2008.

3.4 Correlation among variables

A significant negative correlation was found between chill accumulation and pistil abortion (R = -0.642) (Table 2). Positive significant correlations were also found between chill accumulation and fruit set (R = 0.628) (Table 2) and between chill accumulation and fruits/buds percentage (R = 0.825). In addition, positive significant correlations were found between flowering percentage and fruits/buds (R = 0.606) and between fruit set and flowers/buds (R = 0.628).

Table 2. Correlation matrix among the variables studied.

	F_{50}	F (%)	P (%)	FS (%)	FB (%)	H	CU
F_{50}	1.000	-0.298	-0.489	0.319	0.164	0.860*	0.284
F (%)	-	1.000	0.354	-0.173	0.606*	-0.150	0.336
P (%)	-	-	1.000	-0.776*	-0.462	-0.596*	-0.642*
FS (%)	-	-	-	1.000	0.628*	0.359	0.628*
FB (%)	-	-	-	-	1.000	0.346	0.825*
H	-	-	-	-	-	1.000	0.325
CU	-	-	-	-	-	-	1.000

* Correlations significant at $p < 0.05$. Abbreviations: F_{50}: flowering date; F: flowering percentage; P: pistil abortion; FS: fruit set percentage; FB: fruits/buds percentage; H: harvest date; CU:Chill Units accumulation.

A significant negative correlation was found between pistil abortion and fruit set (R = -0.776), showing the direct connection between both parameters. An interesting negative correlation

was found between harvest date and pistil abortion (R = -0.596). An expected, positive correlation was also found between the flowering and harvest dates (R = 0.860).

4. Discussion

4.1. Effect of shading on temperatures and chilling accumulation

Shading treatments were an effective method to reduce direct solar exposure and temperature, especially during the maximum irradiation period (Figure 1). Differences of up to 5 ºC were registered between shaded and non-shaded trees. Similar results were obtained by Honjo *et al*. (2005), using reflective film and evaporative cooling systems. Buchanan *et al*. (1977) reduced the temperature by 5 to 10 ºC, through shading and sprinkling.

The oscillation in the chilling accumulation in the three studied years can be considered as evidence for the irregular behaviour of 'Poppy' in this area. According to Figure 2, the chilling requirements of 'Poppy' were satisfied in all three years. However, an important residual effect of dormancy (Spiegel-Roy and Alston, 1979) was found in 2007 and 2008, since flowering, fruit set and fruits/buds were significantly lower, and pistil abortion significantly higher, than in 2006. These results suggest that in warm-winter areas, a high chilling accumulation, and therefore, an adequate chilling requirement satisfaction have a strong influence on improving flowering and fruit set percentages. In addition, ours results suggest that the established concept of chilling requirements (Guerriero *et al*., 2002; Ruiz *et al*., 2007) may not be an accurate way to asses the complete dormancy release of a cultivar. In this way, used models for assessing chilling requirements (Weinberger, 1950; Richardson *et al*., 1974; Fishman *et al*., 1987a, 1987b) may not be completely accurate for establishing the chilling requirements for dormancy release, and temperature should be analysed together with other climatic factors, such as time of temperature application and combination of cold and warmth, in order to improve the chilling requirements assessment.

4.2. Effects of shading and thidiazuron + oil treatment on flowering date and flowering percentage

Although a high correlation has been reported between chilling requirements of apricot cultivars and flowering date (Ruiz *et al*., 2007), the significant increase in chilling accumulation

obtained by shading treatments, and consequently earlier chilling requirements satisfaction, did not result in the expected flowering date precocity.

On the other hand, the more chilling that was accumulated, the higher was the precocity achieved by the application of TDZ and winter oil. Izadyar and Wang (1999) showed that the ability of TDZ to stimulate breaking of dormancy begins at the end of endodormancy. Costa *et al*. (2004), working on apple, suggested the application of TDZ in an oil base (Lift®) 5 to 6 weeks before expected full bloom. However, Steffens and Stutte (1989) found that TDZ application was more effective prior to chill accumulation in apple, whereas Boozer and Pitts (2002) found no differences in flower bud development between treatments with Dropp ® plus dormant oil at 74% of chill hours (Weinberger, 1950) accumulation and at the early bud-swell stage. Experiments oriented to determine the optimum stage for TDZ application could be interesting.

The reduction of maximum daily temperatures through shading during the onset of dormancy (Treatment 3) did not cause differences between Treatment 3 and the control regarding the flowering date. On the contrary, Westergaard and Eriksen (1997) reported the influence of high autumn temperatures on the depth of dormancy in *Acer platanoides*. The different range of the applied temperatures, the lower magnitude of the differences and the limited effect on the hours of solar radiation exposure could have been the causes of these different results.

Differences between years concerning flowering time are common in apricot cultivars, being more marked in early cultivars (Egea *et al*., 1999). In 2005-2006, a general delay in flowering was experienced due to the low temperatures during January. The trees overcame endodormancy but entered in ecodormancy until temperatures were higher and favourable for flowering. In 2006-2007, flowering dates were similar to 2005-2006, but in this case the delay was caused not by low temperatures but by the low chilling accumulation. The scarce and late chill accumulation was the basis of a delay in the flowering dates. These results agree with those of Browning and Miller (1992), who showed that high temperatures in November delayed budbreak and flowering time in pear. The most precocious period, by far, was 2007-2008. The considerable chilling accumulation until mid-December, followed by a short period of high temperatures and the resumption of chilling accumulation, could have triggered the early blossoming. Weinberger (1950) stated that intensive early-winter chilling hastens the rest-breaking processes and shortens the rest period.

On the other hand, in 2006, the year with the highest chilling accumulation, flowering percentages were significantly higher than in 2007 and 2008 (Table 1). Other authors have indicated

that chill requirements not being completely satisfied could be the reason for higher bud abscission (Legave, 1978; Legave *et al.*, 1982), and therefore lower flowering percentage.

Treatment with TDZ + oil showed a significantly-higher flowering percentage over the three years, compared with the rest of the treatments. Wang *et al*. (1986) and Steffens and Stutte (1989), in apple, and Izadyar and Wang (1999), in blackberry, also confirmed the capacity of TDZ to increase bloom percentage. Treatment 2 also made more uniform flowering, which can be very important with regard to avoiding competition between the late bloom and already-established sinks (Erez, 2000). In 2007-2008, shading during late endodormancy (Treatment 4) caused a significant higher flowering percentage as compared with control (Table 1). It could be related to the higher chilling accumulation obtained by shading, but also due to the reduction in the amount of light supplied during the late-rest period, as it was reported in previous work (Erez *et al*., 1968).

4.3. Effects of shading and thidiazuron + oil treatment on pistil abortion, fruit set, harvest date and ripening cycle

Pistil abortion is a physiological problem very frequent in apricot and it can reduce fruit set and affect productivity (Legave *et al*., 2006). When a high amount of chill was accumulated and CR were satisfied completely (2005-2006), low pistil abortion percentage was observed, and no significant differences were found among treatments (Table 1). On the contrary, pistil abortion was significantly higher in 2007 and 2008, when low chill was accumulated (Table 1). This results are in accordance with the following hypothesis: warm winters may have an important effect on the abnormal development of flower bud in dormancy (Erez, 1999). Given the high flower density of 'Poppy' (data not shown), pistil abortion could produce an early, beneficial flower thinning. However, the high percentage of aborted pistils limited the fruiting excessively and reduced the yield in 2007 and 2008, findings which are in agreement with those of Legave *et al*. (2006). The insufficient chill accumulation may have been responsible for the sharp increase of anomalies in flower bud formation after the application of TDZ and winter oil in 2007 and 2008.

In 2007 and 2008, when low chill was accumulated, treatment with TDZ + oil showed by far the highest percentages of pistil abortion (Table 1). Taking into account the negative effect of insufficient chill accumulation on flower bud development (Erez, 1999, 2000), it can be suggested that TDZ and winter oil trigger a general blossoming of flowers. When the winter fully satisfies the chilling requirement, fewer defective flowers appear whereas when not enough chill is accumulated

this rate significantly increases. Therefore, the insufficient chill accumulation may have been responsible for the sharp increase of anomalies in flower bud formation after the application of TDZ and winter oil in 2007 and 2008.

Warm pre-blossom temperatures also have been described as the cause of underdevelopment of the pistil at the time of flower opening in apricot (Rodrigo and Herrero, 2002). However, no significant differences among treatments were found in the maximum temperatures of the seven days before anthesis (data not shown). Thus, the differences in pistil abortion were not related to different thermal regimes just prior to anthesis.

As for pistil abortion, low chill accumulation had a negative influence on the fruit set percentage (Table 1). This agrees with the findings of Weinberger (1956), where warm temperatures at the beginning of the winter were related to flower bud drop in peach. Egea *et al*. (2004b), studying the apricot cultivar 'Orange Red', also found a significant flower bud drop when chilling requirements were not satisfied adequately. Erez (2000) associated warm conditions during winter with abnormal flower development, especially abnormal ovary development, and severe drop of floral buds. The lowest fruit set percentages for all treatments, except treatment 4, occurred in 2007 which was the year with lowest chill accumulation, whereas in 2006, the coldest, no significant differences were found among treatments.

Fruit set was significantly higher in control trees and treatment 2 in 2008 compared to 2007 (Table 1). Although similar low amounts of chill were accumulated in 2007 and 2008, the distributions were completely different (Figure 2). Therefore, it should be stated that the distribution of the chill accumulation exerted an important influence on fruit set. The low chill accumulation in autumn 2006 could have led to a lower fruit set in 2007, compared with 2008. During autumn 2007, more than 200 chills units were accumulated in treatment T1. In 2006 and 2007, the control treatment gave the highest value of fruit set. The high flowering percentage achieved in treatment 2 did not translate into a high fruit set value because of the high pistil abortion rate.

Erez and Lavee (1971), working with peach, reported that long, warm periods after partial chilling requirements fulfillment not only did not negate the chilling effect, but actually improved the level of bud break. Similar results were obtained by Young (1992) in apple and by Erez and Couvillon (1985a) in peach. On the other hand, Thompson *et al*. (1975) stated that interruption of chilling by periods of high temperatures reduced subsequent growth in apple, while Weinberger (1950) affirmed that intensive, early-winter chill hastens the rest-breaking process and shortens the

rest period. In our study, a period of 8 days of negative chill accumulation after the accumulation of ca. 22% of the total accumulation in 2007-2008, may has been related to the significantly-higher flowering, fruit set and fruits/buds ratio, as well as the significantly-lower pistil abortion in 2008 compared to 2007 in the control treatment. Currently, the chill distribution throughout the autumn and winter months is not taken into account by climatic models for chill accumulation assessment.

The high chill accumulation in the 2005-2006 period may have minimized the variation in harvest date among the treatments. As for flowering date, these similar dates in the different years have very different causes. In 2006, the late harvest date was probably due partly to the low temperatures prior to flowering, which caused a prolonged ecodormancy period, whereas in 2007 the extremely-low chill accumulation caused a delay in both flowering and harvesting. This delay not only reduced the precocity of this relatively low-chill cultivar, but also reduced the flowering, fruit set and fruits/buds percentages and increased pistil abortion. In 2006, the precocity gained in flowering time by application of TDZ and oil was substantially reduced by the elongation of the ripening cycle. The high energetic demand due to the higher number of flowers and fruits could have been the cause of delayed harvest. In 2007, the high rate of pistil abortion in treatment 2 significantly reduced fruit set and carbohydrate consumption. This lower competition for nutrients may have contributed to the shorter ripening cycle in treatment 2 compared to the previous year.

4.4. Correlation among variables

A significant negative correlation was found between chill accumulation and pistil abortion (R = -0.64). Legave (1978) stated that pistil abortion is related to the cultivar´s adaptation to different cropping areas. The low values of chilling accumulation increased the pistil abortion, showing the lack of adaptation of this cultivar to the studied area.

Positive significant correlations were also found between chill accumulation and fruit set (R = 0.628) and between chill accumulation and fruits/buds percentage (R = 0.825), showing the need of satisfying the chilling requirement in order to have proper fruit set and production (Erez, 2000). In addition, positive, significant correlations were found between flowering and fruits/buds ratio (R = 0.606) and between fruit set and flowers/buds (R = 0.606), as in previous work on apricot (Ruiz and Egea, 2008).

The direct connection between pistil abortion and fruit set (R = -0.776) differs from that of Ruiz and Egea (2008). The synergic effect of the low chill accumulation and the TDZ+oil treatment

on pistil abortion may explain part of this significant correlation. An interesting negative correlation was found between harvest date and pistil abortion (R = -0.596); this may mean that the lower the pistil abortion, the higher the number of fruits and therefore the competition among fruits, which can lead to a lengthening of the ripening cycle.

5. Conclusions

Shading during the autumn-winter period reduced maximum daily temperatures by up to 5 ºC in the climatic conditions of the experiment, so that the chill unit accumulation could be more than 20% greater than for non-shaded trees. However, the important increase in chilling accumulation (calculated with the Utah Model) obtained by shading treatments, and consequently earlier CR satisfaction, did not result in the same proportion with the expected precocity on flowering and ripening dates.

No effect was found with regard to reducing high daytime temperatures, through the reduction of incident radiation with shade cloth, during the stage of entering into dormancy. The flowering and harvesting dates remained the same as for control trees. However, shading during endodormancy can hasten dormancy release, flowering time and harvest date. Hence, shading at this stage could be an interesting cultural practice to hasten dormancy and to bring forward flowering date, especially in winters with insufficient chill to satisfy the CR of the cultivars. Shading during late endodormancy had variable results among the years, regarding flowering and ripening precocity. The application of treatment 3 (shading from October 1 to November 16) does not seem to be adequate to achieving harvest date precocity in low-chill apricot cultivars in the climatic area studied.

The treatment with TDZ and winter oil made flowering significantly earlier and more uniform. In addition, flowering percentages were by far higher than in control trees and shading treatments. Pistil abortion percentage was also strongly increased by using TDZ and winter oil when there was low chilling accumulation, which led to a reduced fruit set percentage. However this was the result of the high number of new flowers that blossomed after the TDZ+oil treatment. Nonetheless, an increase in productivity greater than 250% was obtained compared to the control when insufficient chill was accumulated. It can be suggested that TDZ and winter oil trigger a general blossoming of flowers and that fewer defective flowers appear when the winter fully

satisfies the chilling requirement, whereas the rate of deceptive flowers increases when not enough chill is accumulated. On the other hand, the precocity achieved by TDZ and winter oil was up to nine days for harvest date. Thus, this treatment is an interesting option for early-season apricots in the studied area.

Significant year-to-year variation occurred for flowering percentage, pistil abortion, fruit set and fruits/buds percentage, which indicates a high influence of the chill accumulation of each year and the effect of other environmental conditions. The progression of the chill accumulation exerted an important effect on the overcoming of dormancy when CR were hardly achieved. An early chill accumulation followed by a short period of high temperatures significantly increased the flowering, fruit set and fruits/buds rates and significantly reduced pistil abortion. A significant correlation was found between fruit set and chill accumulation, which was also highly correlated with fruits/buds percentage. Besides this, chill accumulation was correlated significantly with pistil abortion.

CHAPTER 5. OPTIMIZATION OF THE USE OF SSR MARKERS IN THE MOLECULAR CHARACTERIZATION OF APRICOT DESCENDANTS USING MEGAPLEX PCR AND IDENTIFICATION OF QTLs CONTROLLING FLOWERING TIME.

1. Introduction

Flowering time is one of the most important traits studied in apricot breeding programs in Spain due to its direct relation to chilling requirements (Ruiz *et al*., 2007) being also indicative of cold hardiness risk. Flowering time is determined basically by chilling requirements, which breaks bud endodormancy, and by heat requirements, which promotes an active resumption of floral primordia growth (Bailey *et al.*, 1978; Andrés and Duran, 1999; Ruiz *et al.*, 2007).

Conventional genetic strategies such as specific inter-variety crosses and subsequent selection of interesting genotypes have been used in breeding programmes for obtaining apricot cultivars characterized by early or late flowering time, low or high chilling requirements, respectively. While the ability of breeders to create descendants is almost unlimited, the evaluation of flowering time of a large number of seedlings is the limiting factor in these programs. Evaluation of flowering time is a long and tedious process due to the long juvenile period of the apricot trees, the influence of this juvenility in the expression of this trait and the influence of environmental factors, especially winter temperatures, over different years. Therefore, in order to plan an efficient breeding program to obtain early or late flowering cultivars, it would be very important to have a reliable method for the flowering time evaluation in young seedlings from the controlled-cross breeding programs. The development of strategies which make possible an early selection of those descendants showing the demanded traits has a great importance in order to improve breeding programmes efficiency.

Flowering time is transmitted as a quantitative (polygenic) trait in *Prunus* species with a high heritability being early flowering (low chilling requirements) described as dominant (Arora *et al.*, 2003). However, preliminary results obtained at CEBAS-CSIC of Murcia could indicate the exception of 'Orange Red'® cultivar (Hough and Bailey, 1982) where late flowering seem to be dominant. In addition, a great influence of the year has also been described in the flower development in apricot (Egea and Burgos, 1998; Ruiz and Egea, 2007).

On the other hand, recent advances in DNA markers offer plant breeder a rapid and precise alternative approach to conventional selection schemes to improve quantitative traits (Tanksley and Hewitt, 1988). Using detailed molecular linkage maps, quantitative trait loci (QTLs) affecting important traits could be mapped, genetically evaluated and selected through linked markers. The

development of co-dominant molecular markers for marker assistant selection (MAS) in breeding strategies is a powerful strategy in order to improve breeding programmes efficiency.

DNA marker technology has become an essential tool for the molecular characterization of plant species. In addition, from the end of the 1980's, the utilization of PCR-based markers has increased the opportunities for molecular characterization and mapping of populations in a wide range of plant species including fruit crops (Wünsch and Hormaza 2002). One of the most used PCR-based markers are Simple Sequence Repeat markers (SSR, i.e. microsatellites). This type of markers has been described as the best DNA markers for the assessment of genetic diversity within plant species because of their high polymorphism, abundance, and codominant inheritance (Gupta *et al*., 1996; Wünsch and Hormaza, 2002). In the case of *Prunus* species, hundreds of primer pairs flanking SSRs have been cloned and sequenced in different species including peach, apricot, cherry, and almond (Aranzana *et al*., 2003; Martínez-Gómez *et al*., 2003a; Dondini *et al*., 2007). Molecular studies using SSR markers are performed routinely in fruit breeding programs, including apricot, allowing the characterization of progenitors, the identification of accidental pollinations and the design of new crosses (Hormaza, 2002; Zhebentyayeva *et al*., 2003; Sánchez-Pérez *et al*., 2005). In addition, further studies using SSR markers in progenies segregating for agronomic traits are being performed for the development of genetic maps and markers associated with genes or QTLs involved in the inheritance of the agronomic traits in many *Prunus* species including apricot (Martínez-Gómez *et al*., 2003a; Dirlewanger *et al*., 2004; Dondini *et al*., 2007).

To reduce cost and improve the efficiency and throughput of the molecular characterization assays using PCR-based markers, multiplex PCR, a variant of the PCR in which more than one target sequence is amplified using more than one pair of primers (and usually less than four), are being assayed in molecular studies (Sánchez-Pérez *et al*., 2004; Hayden *et al*., 2008; Patocchi *et al*., 2009). On the other hand, megaplex PCR is a very recent and robust technology for highly multiplexed amplification of specific DNA sequences. It uses target-specific pairs of PCR primers (more than four) that are physically separated (Meuzelaar *et al*., 2007). To date, this methodology has not been applied in *Prunus* species. The most important advantage of these techniques (multiplex and megaplex PCR) is the empirical choice of many oligonucleotide primers to improve specificity in the molecular characterization assays and to facilitate the automation of this process.

Additionally, the identification of genomic regions or quantitative traits loci (QTLs) involved in traits of interest is the first step in order to identify candidate genes related to these

traits. The development of specific molecular markers related to these candidate genes will be performed at a subsequent stage. Up to now, scarce works have been carried out regarding identification of QTLs related to traits of interest on apricot species. First results on identification of QTLs linked to fruit quality traits on apricot have been recently presented (Ruiz *et al*., 2008), and QTL analysis related to Sharka resistance have been carried out by different authors on this species (Hurtado *et al*., 2002; Vilanova *et al*., 2003; Lambert *et al*., 2007; Soriano *et al*., 2008; Lalli *et al*., 2008). However, no works regarding identification of QTLs controlling chilling requirements have been reported on apricot species, excepting preliminary results which have been presented recently (Olukolu *et al*., 2008). In this work, QTL analysis using two linkage maps with phenotypic trait data of dormancy budburst resulted in seven QTLs on linkage groups (LG) 1, 2, 3, 5, 6, 7 and 8 (Olukolu *et al*., 2008). A similar recent study conducted in peach for detection of vegetative bud dormancy QTLs also found three QTLs related to chilling requirements located in LGs 1, 4 and 7 of the *Prunus* reference map (Chaparro and Beckman, 2008). On the other hand, one QTL for the blooming date was also found in the linkage group 4 both in a genetic map of almond (Sánchez-Pérez *et al*., 2006) and a genetic map of rose (Hibrand-Saint Oyant *et al*., 2008)

The aims of this chapter were the optimization of the use of SSR markers in the molecular characterization of apricot breeding progenies and the construction of genetic linkage maps using multiplex and megaplex PCR, as well as the identification of QTLs related to flowering time are discussed.

2. Material and methods

2.1. Plant material.

The plant material assayed included the North America late flowering cultivar ‘Orange Red’® (with chilling requirements around 1286 Chill Units, CU), the Spanish early flowering cultivar ‘Currot’ (628 CU) (Ruiz *et al.*, 2007), and a BC1 progeny of 74 seedlings from the cross made in 2001 between the F1 selection ‘Z506-07’ (‘Orange Red’ x ‘Currot’) and the Spanish cultivar ‘Currot’. All genotypes were cultivated in the same experimental orchard belonging to CEBAS-CSIC, located in Cieza, Murcia, Southeast Spain (lat. 37º N, long. 1º W).

2.2. Methods

2.2.1. Phenotypic analysis

Phenotyping was carried out during three consecutive years with different Chill Units (CU) accumulated until February 15: 2005 (1205 CU), 2006 (1413 CU), and 2007 (1029 CU). We evaluated the flowering time [Julian days (natural days from January 1) until 50% of the flowers were opened] in the BC1 progeny assayed, and the progenitors 'Currot', 'Orange Red', and 'Z506-07'.

2.2.2. Molecular characterization based on multiplex and megaplex PCR using SSRs markers

2.2.2.1. DNA isolation

Total genomic DNA was isolated using the procedure described by Doyle and Doyle (1987). Approximately 50 mg of young leaves were ground in a 1.5-ml Eppendorf tube with 750 µl of CTAB extraction buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.2% mercaptoethanol, 0.1% $NaHSO_3$). Samples were incubated at 65 ºC for 20 min, mixed with an equal volume of 24:1 chloroform-isoamyl alcohol, and centrifuged at 6,000 g for 20 min. The upper phase was recovered and mixed with an equal volume of isopropanol at –20 °C. The nucleic acid precipitated was washed in 400 µl of 10 mM NH_4Ac in 76% ethanol, dried, resuspended in 50 µl of TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), and incubated with 0.5µg of RNase A at 37 ºC for 30 min, to digest RNA.

2.2.2.2. SSR analysis

Extracted apricot genomic DNA was PCR-amplified using 120 pairs of primers flanking SSR sequences, previously cloned and sequenced in peach (44 SSRs) (Cipriani *et al*., 1999; Sosinski *et al*., 2000; Dirlewanger *et al*., 2002) and apricot (76 SSRs) (Hagen *et al*., 2004; Messina *et al*., 2004) (Table 1). SSR-PCR amplifications were performed in 5 µL reaction mixture containing 2.5 µL of commercial Taq PCR Master Mix Kit (Qiagen, Hilden, Germany), 20 nM of each forward primer labelled with a fluorescent chemical [6-FAM (blue); VIC (green); NED (yellow); PET (red)] (Applied Biosystems, Foster City, California, USA), 20 nM of each unlabelled reverse primer, 5 ng of genomic DNA and 1 µL of dH_2O. Amplification was performed for 40 cycles at 94 ºC for 30 sec, 58 ºC for 1min 30 sec, and 72 ºC for 1 min, for denaturation, annealing, and primer extension, respectively.

2.2.2.3. Multiplex and megaplex PCR development

Initially, thirty multiplex PCR using four SSR markers were designed (Table 1). Each SSR marker was labelled with one of the four dyes available. These SSR markers were applied in the progenitors and in several individuals of the progeny to check the size of the amplified bands in our apricot genotypes. These results were applied in the development of megaplex PCR using more than four SSR markers attending the size of the resulted PCR products and the dye of the SSR.

2.2.2.4. SSR Fragment analysis

Amplified PCR products were separated and analyzed by an automated sequencer capillary electrophoresis 3130x Genetic Analyzer (Applied Biosystems, Carlsbad, USA). The size standard used in the sequencer was Gene Scan™ 500 Rox™ (-250) and allele sizes were scored using in the analysis software GeneMapper v4.0 (Applied Biosystems).

In addition for the analysis of one SSR, amplified PCR products were separated using low melting Metaphor® agarose (Cambrex, East Rutherford, NJ, USA) 3% gels stained with GelRed™ Nucleic Acid Gel Sating® (Biotium, Hatwad, CA, USA) and visualized under UV light.

Table 1. SSR markers assayed ordered attending the size of the amplified sequence in the original species assayed and multiplex PCR (indicated with different labelling) performed in the molecular characterization of the apricot breeding progeny.

SSRs	Reference	Annealing Temperature	Original size (bp)	Labelling
BPPCT-024	Dirlewanger *et al*., 2002	57-60	96	6-FAM
AMPA095	Hagen *et al*., 2004	56	100	NED
UDAp-474	Messina *et al*., 2004	56	100	PET
UDP98-406b	Cipriani *et al*., 1999	57-63	101	VIC
AMPA118	Hagen *et al*., 2004	55	104	6-FAM
UDP98-405b	Cipriani *et al*., 1999	57-63	104	NED
UDP98-024	Cipriani *et al*., 1999	57-63	105	PET
AMPA124	Hagen *et al*., 2004	55	110	VIC
BPPCT-035C	Dirlewanger *et al*., 2002	57-60	113	6-FAM
UDAp-493	Messina *et al*., 2004	50	113	NED
BPPCT-006C	Dirlewanger *et al*., 2002	57-60	117	PET
UDAp-439	Messina *et al*., 2004	56	118	VIC
UDAp-470	Messina *et al*., 2004	56	118	6-FAM
AMPA116	Hagen *et al*., 2004	55	119	NED
AMPA115	Hagen *et al*., 2004	55	125	PET
UDP98-409b	Cipriani *et al*., 1999	57-63	129	VIC
UDP98-412	Cipriani *et al*., 1999	57-63	129	6-FAM
UDP97-401b	Cipriani *et al*., 1999	57-63	130	NED
UDP96-010b	Cipriani *et al*., 1999	57-63	131	PET
AMPA122	Hagen *et al*., 2004	55	132	VIC
UDAp-432	Messina *et al*., 2004	56	132	6-FAM
BPPCT-026	Dirlewanger *et al*., 2002	57-60	134	NED
BPPCT-038	Dirlewanger *et al*., 2002	57-60	135	PET
BPPCT-040	Dirlewanger *et al*., 2002	57-60	135	VIC
UDAp-435 c	Messina *et al*., 2004	50	135	6-FAM
AMPA113	Hagen *et al*., 2004	55	136	NED
UDP97-402b	Cipriani *et al*., 1999	57-63	136	PET
UDAp-491	Messina *et al*., 2004	56	140	VIC
UDAp-412 a b	Messina *et al*., 2004	56	141	6-FAM
UDAp-463	Messina *et al*., 2004	56	141	NED
UDP96-003b	Cipriani *et al*., 1999	57-63	143	PET
UDP98-021	Cipriani *et al*., 1999	57-63	145	VIC
AMPA110	Hagen *et al*., 2004	55	146	6-FAM
UDAp-454	Messina *et al*., 2004	56	147	NED
BPPCT-008b	Dirlewanger *et al*., 2002	57-60	148	PET
BPPCT-007	Dirlewanger *et al*., 2002	57-60	149	VIC
UDAp-405	Messina *et al*., 2004	56	149	6-FAM
UDAp-446	Messina *et al*., 2004	56	149	NED
UDAp-462	Messina *et al*., 2004	50	149	PET
UDP97-403b	Cipriani *et al*., 1999	57-63	150	VIC
UDP98-411	Cipriani *et al*., 1999	57-63	150	6-FAM
UDAp-486	Messina *et al*., 2004	56	153	NED
BPPCT-039ab	Dirlewanger *et al*., 2002	57-60	154	PET
UDAp-473	Messina *et al*., 2004	56	154	VIC

Table 1. (Continuation). SSR markers assayed ordered attending the size of the amplified sequence in the original species assayed and multiplex PCR (indicated with different labelling) performed in the molecular characterization of the apricot breeding progeny.

SSRs	Reference	Annealing Temperature	Original size (bp)	Labelling
AMPA094	Hagen *et al*., 2004	55	155	6-FAM
BPPCT-037	Dirlewanger *et al*., 2002	57-60	155	NED
UDP96-005b	Cipriani *et al*., 1999	57-63	155	PET
UDAp-415	Messina *et al*., 2004	56	156	VIC
UDAp-437	Messina *et al*., 2004	56	156	6-FAM
UDAp-489	Messina *et al*., 2004	56	156	NED
UDAp-468	Messina *et al*., 2004	56	157	PET
UDAp-471	Messina *et al*., 2004	56	157	VIC
UDAp-483	Messina *et al*., 2004	50	157	6-FAM
UDAp-457	Messina *et al*., 2004	50	158	NED
UDAp-410	Messina *et al*., 2004	56	159	PET
UDAp-421 a b	Messina *et al*., 2004	56	159	VIC
UDAp-438	Messina *et al*., 2004	56	159	6-FAM
pchgms5	Sosinski *et al*., 2000	58	160	NED
UDAp-452	Messina *et al*., 2004	50	160	PET
UDAp-458	Messina *et al*., 2004	56	160	VIC
UDAp-466	Messina *et al*., 2004	56	160	6-FAM
UDAp-406	Messina *et al*., 2004	56	161	NED
UDAp-460	Messina *et al*., 2004	56	161	PET
UDAp-496	Messina *et al*., 2004	56	162	VIC
pchgms2	Sosinski *et al*., 2000	58	163	6-FAM
UDAp-444	Messina *et al*., 2004	56	163	NED
BPPCT-012	Dirlewanger *et al*., 2002	57-60	164	PET
BPPCT-028	Dirlewanger *et al*., 2002	57-60	164	VIC
UDAp-497	Messina *et al*., 2004	56	166	6-FAM
UDAp-409	Messina *et al*., 2004	56	167	NED
UDAp-449	Messina *et al*., 2004	56	167	PET
UDAp-456	Messina *et al*., 2004	56	167	VIC
UDAp-451	Messina *et al*., 2004	50	168	6-FAM
UDAp-418	Messina *et al*., 2004	56	169	NED
BPPCT-009	Dirlewanger *et al*., 2002	57-60	171	PET
UDAp-479	Messina *et al*., 2004	56	171	VIC
BPPCT-011	Dirlewanger *et al*., 2002	57-60	172	6-FAM
UDAp-461	Messina *et al*., 2004	56	173	NED
AMPA096	Hagen *et al*., 2004	55	174	PET
BPPCT-017	Dirlewanger *et al*., 2002	57-60	174	VIC
pchgms4	Sosinski *et al*., 2000	58	174	6-FAM
BPPCT-030	Dirlewanger *et al*., 2002	57-60	175	NED
UDAp-420	Messina *et al*., 2004	56	175	PET
UDAp-485	Messina *et al*., 2004	56	177	VIC
UDAp-413	Messina *et al*., 2004	56	179	6-FAM
UDAp-424	Messina *et al*., 2004	50	179	NED
AMPA107	Hagen *et al*., 2004	51	180	PET
BPPCT-033B	Dirlewanger *et al*., 2002	57-60	180	VIC

Table 1. (Continuation). SSR markers assayed ordered attending the size of the amplified sequence in the original species assayed and multiplex PCR (indicated with different labelling) performed in the molecular characterization of the apricot breeding progeny.

SSRs	Reference	Annealing Temperature	Original size (bp)	Labelling
BPPCT-013	Dirlewanger *et al*., 2002	57-60	183	6-FAM
AMPA101	Hagen *et al*., 2004	55	188	NED
UDAp-407	Messina *et al*., 2004	56	188	PET
UDAp-465	Messina *et al*., 2004	56	188	VIC
UDAp-469	Messina *et al*., 2004	50	188	6-FAM
UDAp-430	Messina *et al*., 2004	56	189	NED
UDAp-450	Messina *et al*., 2004	50	189	PET
AMPA105	Hagen *et al*., 2004	55	191	VIC
UDAp-422	Messina *et al*., 2004	50	191	6-FAM
UDAp-423	Messina *et al*., 2004	56	192	NED
UDAp-472	Messina *et al*., 2004	56	192	PET
BPPCT-019C	Dirlewanger *et al*., 2002	57-60	194	VIC
BPPCT-025	Dirlewanger *et al*., 2002	57-60	197	6-FAM
BPPCT-004	Dirlewanger *et al*., 2002	57-60	200	NED
BPPCT-020B	Dirlewanger *et al*., 2002	57-60	200	PET
UDAp-401	Messina *et al*., 2004	56	201	VIC
UDAp-436 a b	Messina *et al*., 2004	56	202	6-FAM
AMPA109	Hagen *et al*., 2004	55	204	NED
UDAp-503	Messina *et al*., 2004	56	204	PET
UDAp-467	Messina *et al*., 2004	56	211	VIC
AMPA100	Hagen *et al*., 2004	55	215	6-FAM
BPPCT-014	Dirlewanger *et al*., 2002	57-60	215	NED
BPPCT-041	Dirlewanger *et al*., 2002	57-60	220	PET
pchcms3	Sosinski *et al*., 2000		220	VIC
BPPCT-018B	Dirlewanger *et al*., 2002	57-60	222	6-FAM
AMPA112	Hagen *et al*., 2004	55	223	NED
pchcms4	Sosinski *et al*., 2000	58	225	PET
UDAp-431ab	Messina *et al*., 2004	56	227	VIC
BPPCT-002	Dirlewanger *et al*., 2002	57-60	229	6-FAM
UDAp-441	Messina *et al*., 2004	56	237	NED
UDAp-499	Messina *et al*., 2004	56	248	PET
BPPCT-027ab	Dirlewanger *et al*., 2002	57-60	249	VIC

2.2.3. Map construction and QTLs analysis

A framework genetic map was constructed using Join Map v. 3.0 software (Van Ooijen and Voorrips, 2001) by using the CP population type. Linkage groups were established with a minimal LOD of 4.0. Quantitative trait loci (QTL) identification related to fruit quality traits was carried out with composite interval mapping by MapQTL 5.0 software. A threshold LOD score value of 3 was chosen for declaring the existence of a QTL.

3. Results

3.1. Phenotypic data

Results of the flowering time evaluation in Julian days in the BC1 progeny from the cross between the F1 selection 'Z506-07' x 'Currot' during three consecutive years are shown in Figure 1. Values of the parents ('Z 506-07' and 'Currot') as well as 'Orange Red' are also shown. Results indicate an effect of delay of flowering time in the descendants, since a considerable number of hybrids showed later flowering time than 'Z 506-07'. In this way, it was evident the genetic influence of 'Orange Red', the latest flowering time cultivar, which was the parental used for releasing 'Z 506-07'. A segregating distribution of hybrids between 'Currot' and 'Orange Red' was observed (Figure 1).

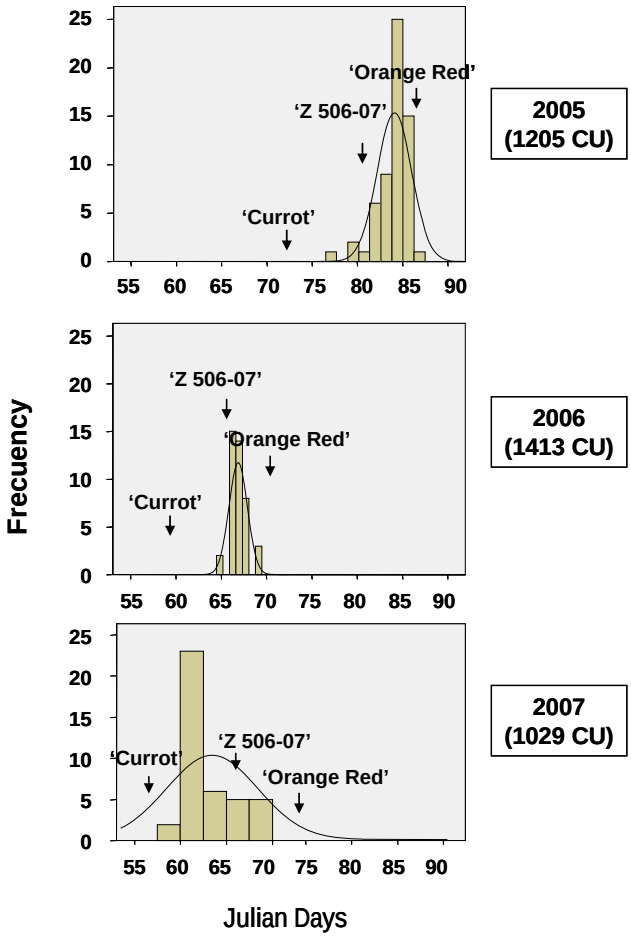


Figure 1. Flowering time phenotyping in Julian days in the BC1 progeny of 73 seedlings from the cross between the F1 selection 'Z506-07' ('Orange Red' x 'Currot') and 'Currot' during three years. Chill Units of the studied years (CU) have also been indicated.

On the other hand, an effect of the year was observed. All genotypes showed significantly later flowering time in 2005 than in 2006 and 2007 (Figure 1). The mean value of flowering time in the progeny differed almost 20 days between 2005 and 2007. Besides, in 2006, which was the year with the highest chill accumulation, flowering times in the studied population were much more grouped, showing minimum differences among descendants.

3.2. Molecular characterization by using SSR markers and megaplex PCR development.

Amplifications were successful in apricot progenitors and in the progeny with 114 of the 120 (95%) SSR markers with a 69% (79 out of 114 markers) polymorphism detected (number of polymorphic bands ranged from 1 to 4 depending on the codominant nature of the maker) in the apricot BC1 descendants studied. In the case of the SSR markers developed in apricot, 96% of these markers were successfully amplified in our two progenitors ('Z506-07' and 'Currot') and in the 74 seedlings of the BC1 progeny with the exception of the UDAp-435c, UDAp-431ab (Messina *et al*., 2004) and AMPA096 (Hagen *et al*., 2004) (Table 2). SSR markers showed a size ranging between 83 and 242 bp. For the majority of the markers, the allele size range matched the one initially described by the authors who developed these markers in both peach (Cipriani *et al*., 1999; Sosinski *et al*., 2000; Dirlewanger *et al*., 2002) and apricot (Hagen *et al*., 2004; Messina *et al*., 2004).

A series of seven megaplex PCRs containing between 6 to 20 SSR markers were assembled for the molecular characterization of the apricot breeding progeny (Table 2). These megaplex PCRs were designed according to the size obtained in our apricot materials (between 83 and 242 bp) by combining the different dyes labelling the SSR markers assayed. In the combination of these SSR markers through megaplex PCR a minimum interval of 5 bp was kept between the SSR markers with the same labelling to avoid band mis-identification (Table 2). 91 of the 120 SSR markers assayed were used in only seven PCR reactions corresponding with seven megaplexes. In megaplex PCR 3, for example, a total of 20 markers were amplified in the same PCR reaction (Figure 2). 60 of these SSR markers amplified in the studied progeny and were used for the screening of the whole progeny evaluating the polymorphic markers to use in the construction of the genetic linkage map. No problems were found in the PCR amplification due to the location of several markers in the same reaction. The percentage of markers amplified in the progeny in each megaplexe ranged from 100% in the case of megaplexe 6 to 28.57% in the megaplexe 7 with a mean value of 66% (Table 3).

Table 2. Megaplex, multiplex and single PCR (indicated with different shaded) designed for the molecular characterization of the apricot breeding progeny using different SSR markers and range of the size of the resulted PCR products.

SSRs	Labelling	Range (bp) Min	Max
Megaplex 1			
AMPA095	NED	85	142
UDP98406	VIC	89	128
UDAp470	6-FAM	84	118
BPPCTO38	PET	80	142
AMPA110	6-FAM	126	146
BPPCT007	VIC	135	158
UDAp405	6-FAM	164	164
UDAp460	PET	148	165
BPPCT028	VIC	169	170
UDAP418	NED	160	168
BPPCT011	6-FAM	173	185
Pchgms4	6-FAM	153	153
UDAp485	VIC	181	211
UDAp430	NED	176	198
UDAp450	PET	185	217
AMPA112	NED	204	222
UDAp499	PET	226	249
PCT027	VIC	238	242
Megaplex 2			
BPPCT-024	6-FAM	94	137
BPPCT-006C	PET	80	143
UDAp-439	VIC	98	135
BPPCT-026	NED	96	141
UDAp-415	VIC	149	161
UDAp-437	6-FAM	146	171
pchgms5	NED	162	173
UDAp-452	PET	154	164
UDAp-449	PET	172	173
UDAp-413	6-FAM	178	288
UDAp-424	NED	185	341
UDAp-401	VIC	171	214
UDAp-503	PET	185	220
pchcms4	PET	232	236
Megaplex 4			
UDAp-493	NED	97	135
AMPA115	PET	83	127
UDAp-491	VIC	139	159
BPPCT-039	PET	133	150
UDAp-421	VIC	112	133
pchgms2	6-FAM	160	169
UDAp-497	6-FAM	144	144
UDAp-420	PET	161	175
BPPCT-013	6-FAM	140	140
UDAp-422	6-FAM	181	198
UDAp-423	NED	171	302
BPPCT-019C	VIC	214	240
BPPCT-025	NED	148	150
UDAp-467	VIC	186	202

SSRs	Labelling	Range (bp) Min	Max
Megaplex 3			
UDP96-010b	PET	83	131
UDAp-432	6-FAM	96	128
BPPCT-040	VIC	108	135
UDP97-402b	PET	141	146
UDAp-463	NED	110	122
UDAp-446	NED	131	153
UDP98-411	6-FAM	167	179
UDAp-438	6-FAM	139	139
UDAp-466	6-FAM	147	162
UDAp-456	VIC	151	169
UDAp-461	NED	162	175
BPPCT-017	VIC	174	186
UDAp-407	PET	193	193
AMPA105	VIC	198	217
UDAp-472	PET	156	184
BPPCT-004	NED	197	203
BPPCT-020B	PET	198	198
AMPA100	6-FAM	186	217
BPPCT-014	NED	186	186
BPPCT-041	PET	215	215
Megaplex 5			
AMPA118	6-FAM	97	108
UDAp-454	NED	97	161
BPPCT-008b	PET	92	128
UDAp-462	PET	147	153
UDP97-403b	VIC	118	118
UDAp-451	6-FAM	131	167
AMPA107	PET	172	242
BPPCT-033b	VIC	126	165
AMPA101	NED	188	188
AMPA109	NED	199	205
BPPCT-002	6-FAM	188	195
Megaplex 6			
UDP98-405b	NED	104	148
UDP98-412	6-FAM	104	116
UDP96-003b	PET	94	106
UDAp-473	VIC	115	145
AMPA094	6-FAM	132	154
UDP96-005b	PET	119	146
UDAp-479	VIC	151	196
Megaplex 7			
UDAp-474	PET	99	100
BPPCT-035C	6-FAM	96	148
UDAp-471	VIC	121	147
UDAp-406	NED	105	179
UDAp-496	VIC	156	192
BPPCT-012	PET	132	169

SSRs	Labelling	Range (bp) Min	Max
Multiplex 8			
UDP98-024	PET	91	146
AMPA124	VIC	108	156
UDAp-444	NED	108	170
UDAp-436ab	6-FAM	103	262
Multiplex 9			
UDP97-401b	NED	109	142
UDAp-412ab	6-FAM	132	152
UDAp-410	PET	122	149
UDAp-465	VIC	119	173
Multiplex 10			
UDAp-468	PET	146	168
UDAp-483	6-FAM	133	160
UDAp-458	VIC	127	170
UDAp-409	NED	109	160
Multiplex 11			
UDP97-401b	NED	112	136
UDP98-409b	VIC	132	161
UDAp-469	6-FAM	141	217
Multiplex 12			
AMPA122	VIC	132	161
UDAp-486	NED	115	154
Multiplex 13			
UDP98-021	VIC	153	153
BPPCT-037	NED	115	161
Single 14			
UDAp-441	NED	120	322
Single 15			
UDAp-489	NED	132	166
Single 16			
UDAp-457	NED	133	166
Single 17			
AMPA113	NED	134	161
Single 18			
BPPCT-030	NED	136	146
No amplification			
UDAp-435c	6-FAM	0	0
BPPCT-018b	6-FAM	0	0
pchcms3	VIC	0	0
UDAp-431ab	VIC	0	0
BPPCT-009	PET	0	0
AMPA096	PET	0	0

Table 3. Amplification of PCR in each megaplex PCR.

Megaplex	Number of Primers per megaplex	Number of markers amplified in the progeny	%
1	18	16	88.89
2	14	8	57.14
3	20	11	55.00
4	14	10	71.43
5	11	6	54.55
6	7	7	100.00
7	7	2	28.57
Mean	13	8.57	65.93
Total	91	60	-

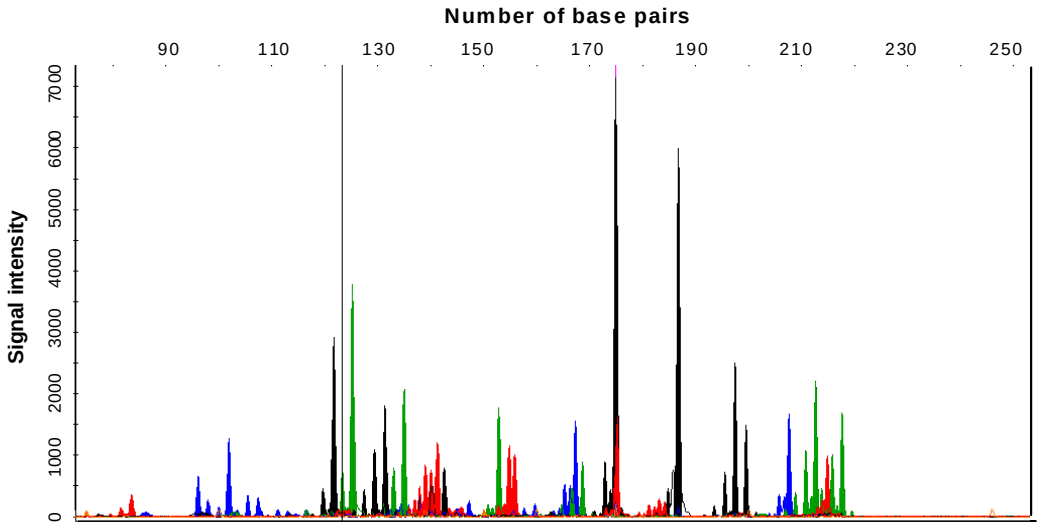


Figure 2. Distribution of amplified products in the megaplex PCR 3 using automated sequencer capillary electrophoresis 3130x Genetic 22 Analyzer (Applied Biosystems). The observed peaks [blue (6-FAM dye), black (NED), red (PET) and green (VIC)] correspond to the 20 different SSR markers analyzed in the megaplex.

On the other hand, obtained results also suggest the suitability of these markers in the identification of accidental pollinations (Figure 3). Thirteen seedlings issued from accidental pollinations were detected, with two seedlings amplifying alleles not present in the parental genotype (contamination of pollen) and 11 with not expected segregation (probably self-pollination).

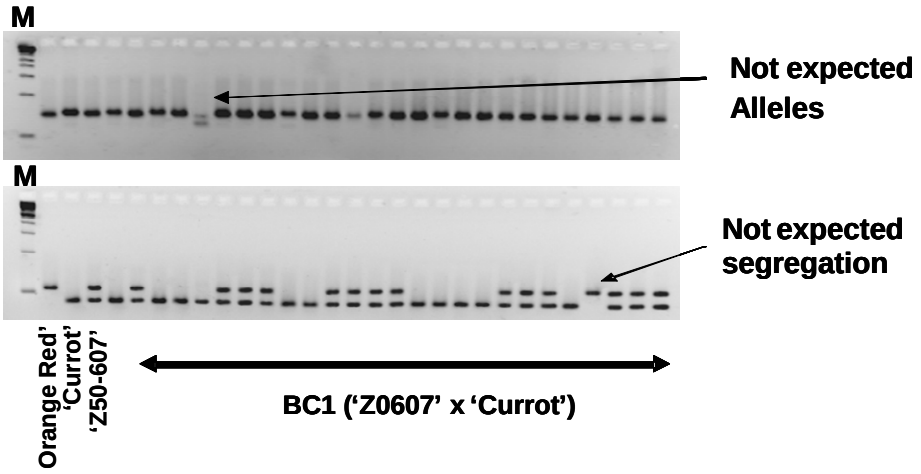


Figure 3. Metaphor© agarose gel (3%) showing the allelic segregation of the UDP98412 and BPPCCT007 SSR markers in the BC1 progeny of 74 seedlings from the cross between the F1 selection 'Z506-07'('Orange Red' x 'Currot') and 'Currot' using SSR markers. Arrows showed the identification of accidentals pollination in this progeny. M, 1Kb DNA Ladder (Invitrogen, Madrid, Spain).

3.3. Map construction and identification of QTLs.

Using the marker data available, two preliminary molecular linkage maps of 'Z506-07' and 'Currot' respectively were constructed (Figure 4). These genetic maps include 37 and 29 SSRs, for 'Z506-07' and 'Currot', respectively (Table 4). The maps cover 241 cM for 'Z506-07' and 226 cM for 'Currot', spanning about 45% and 37% respectively of the apricot genome, compared with the *Prunus* reference map (522 cM) (Aranzana *et al*., 2003; Dirlewanger *et al*., 2004). Considering both maps together, the SSR distribution was relatively uniform with the exception of linkage group 6.

The number of markers per LG varied from none (LGs 6, 7, 8 in 'Currot') to nine (LG1 in 'Z 506-07') (Table 4). The biggest LG obtained were 90.0 cM in LG 1 for 'Z506-07' and 52.9 cM in LG 5 for 'Currot' (Figure 4).

Table 4. Number of mapped SSR markers, amplified but not mapped (between parenthesis), size of the LGs, and marker density (mean distance in cM) in the two linkage maps of 'Z506-07' and 'Currot'.

Linkage group	Number of SSR markers ('Z506-07')	Size of LG (cM)	Marker density (cM)	Number of SSR markers ('Currot')	Size of LG (cM)	Marker density (cM)
1	9	90.0	10.00	5	25.7	5.14
2	5+(1)	38.4	7.68	4	42.4	10.60
3	4	33.7	8.43	5	46.7	9.34
4	4	17.2	4.30	5	47.2	9.44
5	8	18.0	2.25	6	52.9	8.82
6	(1)	0	-	-	-	-
7	3	25.3	8.43	(3)	-	-
8	4	18.2	4.55	-	-	-
Unknown	-	-		4	11.4	2.85
Total	37	237	6.52	29	214	7.70

In addition, one QTL was detected linked to flowering time, that is to say related to chilling requirements. It was located in linkage group 5 of the genetic map of 'Z 506-07' (Figure 4). This QTL was found in the same region in 2005 and 2007, although LOD was significantly higher in 2007 than in 2005 (Figure 5). However, no QTLs for flowering time were found in the genetic linkage map of 'Currot' (Figure 4). .

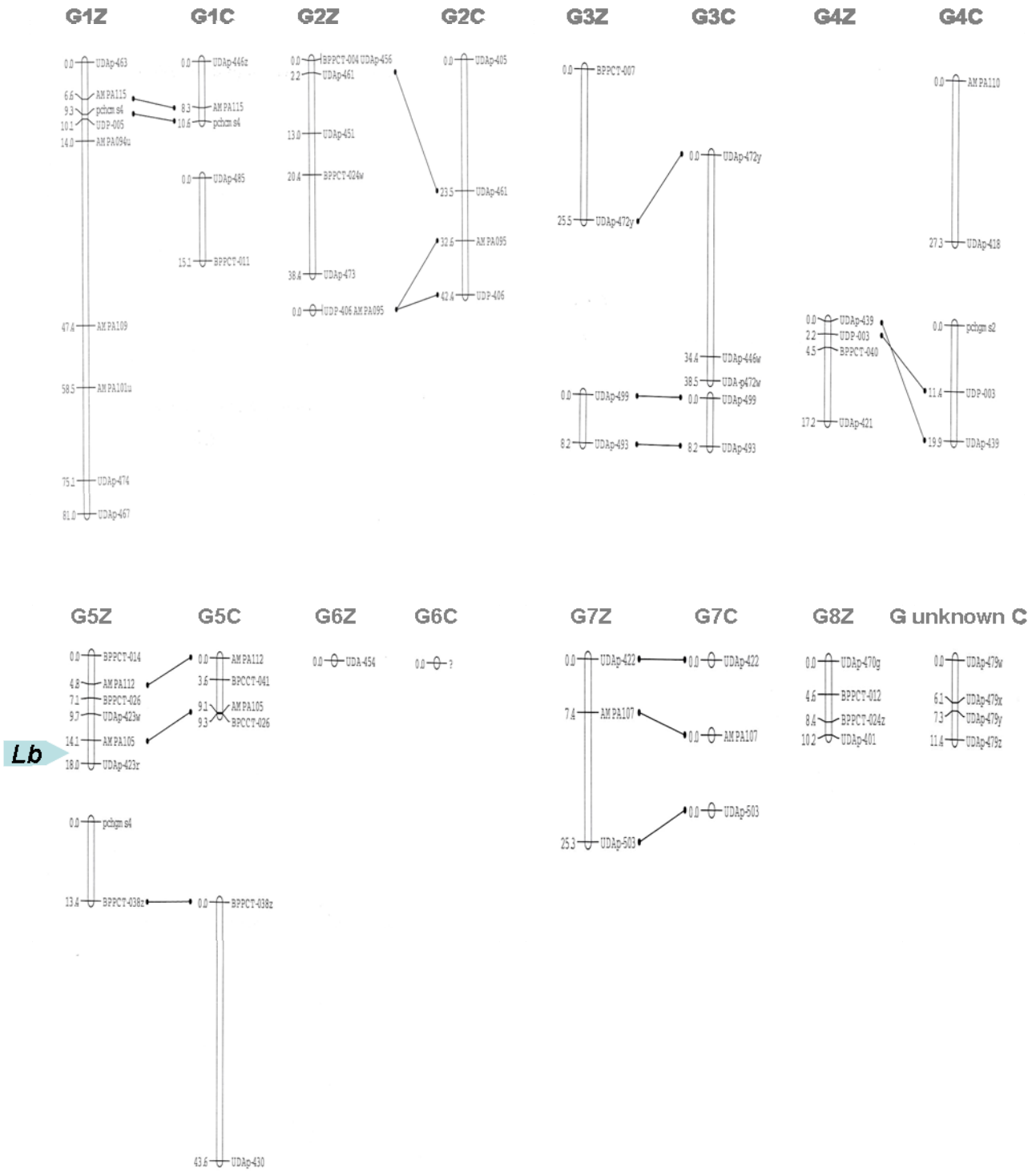


Figure 4. Genetic linkage maps of 'Z506-07' (Z) and 'Currot' (C) obtained with 37 and 29 SSRs, respectively, constructed using JOINMAP, and location of QTL controlling flowering time (*Lb*) is indicated.

Figure 5. QTL analysis for flowering time in the eight identified linkage groups in the genetic linkage maps of 'Z506-07' during the years 2005, 2006 and 2007, and the mean of the three years. Red line indicated the value of the LOD (logarithm of the odds ratio) and blue line the value of the percentage of variance explained for each and QTL.

Finally, QTL analysis showed as a SSR loci (AMPA-105) was linked to this trait (flowering time) in apricot with a LOD value of 7 in year 2005 and 8 in year 2007 (Figure 6).

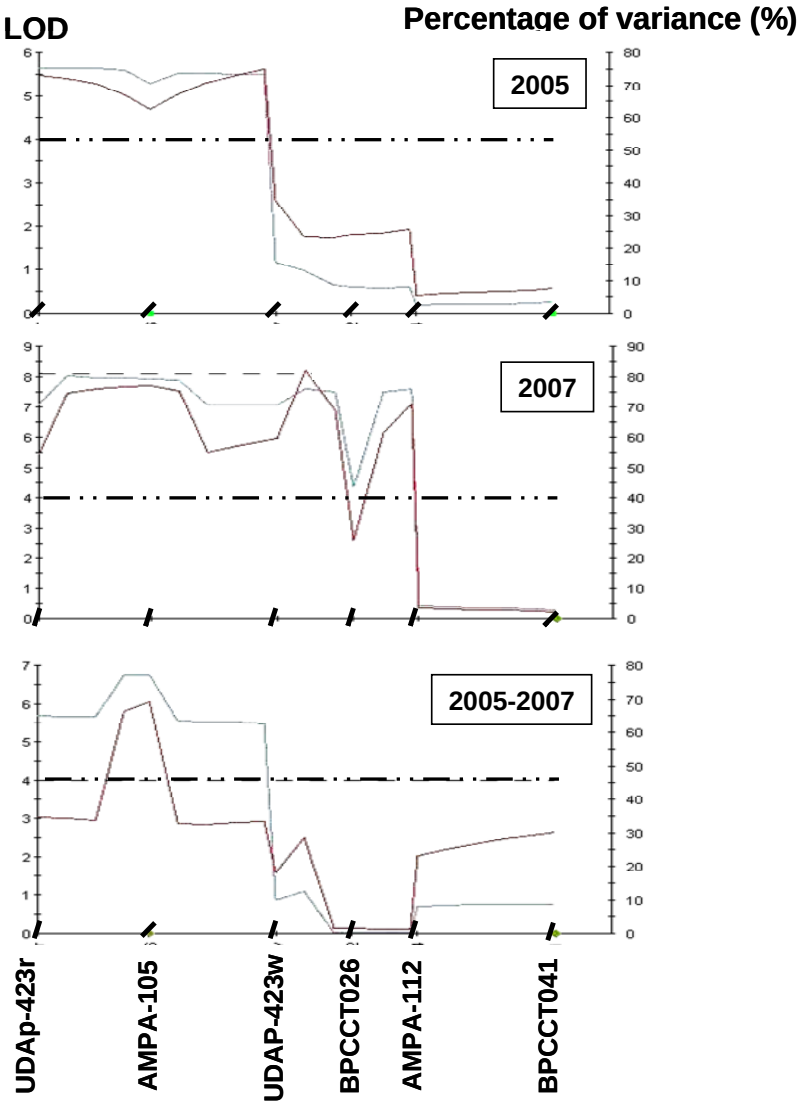


Figure 6. QTLs controlling flowering time identified in the linkage group 5 of 'Z506-07' during the years 2005 and 2007 and the mean of the three years studied (2005, 2006, and 2007). Red line indicated the value of the LOD (logarithm of the odds ratio) and blue line the value of the percentage of variance explained for each and QTL. A threshold LOD score value of 4 (discontinous line) was chosen for declaring the existence of a QTL.

4. Discussion.

Phenotyping results indicate an effect of delay of flowering time in the BC1 descendants from 'Currot' and 'Z 506-07', in contrast with the expected increase of the precocity, being the precocity (low chilling requirements) dominant over the delay (high chilling requirements) (Arora *et al.*, 2003). The genetic origin of 'Z 506-07', released from 'Orange Red' which is the latest flowering time cultivar, is probably the cause of this distribution. Therefore, the objective to advance the flowering time with respect to 'Currot' by using a BC1 cross is not obtained.

On the other hand, an absence of flowering and irregular growth was observed in 13 (17%) aberrant descendants from the BC1 progeny of 74 seedlings from the cross between the F1 selection 'Z506-07' and 'Currot' with an absence of leafing in these aberrant descendants in comparison with the normal flowering and leafing of the rest of descendants of the progeny. A correlation between growth habit, flowering location and juvenility has been observed. The vigorous long branches and the position of flower in the extreme of the branches in the young seedlings affected the flowering time of these seedlings. In the case of vigorous long branches, the flowering time is later than in the case of less vigorous short spours branches. This situation indicated the great effect of the juvenility (seedlings with vigorous long branches and a late flowering) in the evaluation of flowering time in apricot and the risk of evaluation of flowering time during the juvenility period.

A great influence of Chill Units accumulation in the year on the expression of flowering time has been observed. In years with lower chilling requirements accumulation the variability and the standard deviation of flowering time evaluation is higher. Cold year produced a more concentrated flowering time on apricot seedlings (Figure 1). These results indicated that the evaluation of flowering time in apricot during different years is of critical importance. The influence of the year in the flower development in apricot has been previously described in some aspects as the ovule development and the flowering time (Egea and Burgos, 1998; Ruiz and Egea, 2007).

Regarding molecular characterization, a large set of highly polymorphic SSR markers has been identified that are well distributed over the apricot genome. Overall, the results showed a high level of polymorphism in our apricot BC1 population. DNA fingerprints obtained from the amplification of SSR markers are of great importance for different purposes including the certification program to protect new releases from breeding programs, particularly in the cases of species, such as apricot, that are vegetatively propagated (Sánchez-Pérez *et al.*, 2005), or the

development of genetic linkage maps and markers associated with genes or QTLs (Dirlewanger *et al*., 2004). Our results confirmed the well described transferability of SSR markers between *Prunus* species (Cipriani *et al*., 1999; Sosinski *et al*., 2000; Martínez-Gómez *et al*., 2003b; 2003c; Zhebentyayeva *et al*., 2003; Arús *et al*., 2006). Forty one of the forty four (93%) markers initially developed in peach amplified successfully in the apricot progeny assayed.

On the other hand, the percentage of accidental pollination observed agrees with previous results using SSR markers in the study on interspecific hybrids in several *Prunus* species (Arbeloa *et al*., 2005). In addition, the presence of these fortuitous pollinations is a factor that can affect the characterization of apricot progenies.

The development of the megaplex PCR method greatly increased the efficiency and reduced the cost involved in the implementation of this type of molecular characterization studies. In addition, the organization of SSR markers in sets of megaplex PCR also increased the efficiency and reduced the cost of the development of genetic linkage maps in *Prunus* species. Using megaplex PCR, in only one reaction till 20 SSR markers (megaplex 2) were analyzed within an initial set of 120 markers with a range of 96-249 bp (Table 2). These results improved the previous results exposed by Hayden *et al*. (2008) and Patacchi *et al*. (2009) which in one multiplex PCR reaction incorporated 6 and 4 SSR markers respectively. As the 120 markers set had not been tested in our population before selecting markers, the optimization of the process can be fairly increased if it is known the exact size of the amplified products of the markers in the progeny. Thus, the choice and sorting of the primers of the megaplex could be more accurate. Besides, the higher the range of the amplified sequences, the more markers could be included in a single megaplex. The optimization and use of the megaplex can open new dimensions in the multifunctional use of microsatellites for breeders and genetics, multiplying the efficiency and significantly reducing the cost of the analysis.

One of the most important applications of this new methodology is to ease the development of genetic linkage maps. Two preliminary molecular linkage maps of 'Z506-07' and 'Currot' respectively have been constructed (Figure 3). The maps cover 241 cM and 226 cM for 'Z506-07' and 'Currot', respectively, spanning about 45% and 37% of the apricot genome, compared with the *Prunus* reference map (522 cM) (Aranzana *et al*., 2003; Dirlewanger *et al*., 2004). The location of the SSR marker mapped was similar to those previously reported by Aranzana *et al*. (2003) and Dondini *et al*. (2007). Our results showed a high level of colienarity between *Prunus* species and

confirmed a high level of transferability of the markers, as already described by Dirlewanger *et al*. (2004) and Arús *et al*. (2006). This homology among *Prunus* species partly explains the low level of breeding barriers to interspecific gene introgression and highlights the opportunity for successful gene transfer between closely related species (Martínez-Gómez *et al*., 2003c).

Although only preliminary results concerning identification of QTLs linked to flowering time are shown here, one QTL has been found in linkage group 5 of the genetic map of 'Z506-07' (Figure 3) in two different years (2005 and 2007). However, no QTLs for flowering time were found in the genetic linkage map of 'Currot'. It could be explained by the very different genetic background of these apricot cultivars. Our results are in accordance with previous preliminary results obtained in apricot (Olukolu *et al*., 2008) where one QTL was also located in LG 5, although they also found QTLs for flowering time in LG 1, 2, 3, 6, 7 and 8. On the contrary, previous works in *Prunus* identified QTLs for blooming date in different linkage groups, such as in peach (LG 1, 4 and 7) (Chaparro and Beckman, 2008), almond (LG 4) (Sánchez-Pérez *et al*., 2006) and rose (LG 4) (Hibrand-Saint Oyant *et al*., 2008).

We must point out that the results presented in this chapter concerning QTLs identification are only preliminary results. In this sense, the SSR loci (AMPA-105) linked to flowering time must be assayed in other apricot descendants. In the continuation of the work we are working in this moment on the saturation of the genetic linkage maps by new SSR markers in order to make possible a more reliable identification of QTLs related to flowering time. On the other hand, we have recently generated two new apricot progenies characterized by a high segregation regarding chilling requirements, with the purpose of improving the efficiency of this research line. The identification of QTLs involved in the chilling requirements is the first step in order to identify candidate genes related to this trait. It will mean a huge advance for the knowledge of the genetic basis of plant dormancy mechanisms, but also for making possible the development of specific molecular markers associated to this trait. This development will help to improve the efficiency of breeding programmes aimed to release new apricot cultivars with low or high chilling requirements.

5. Conclusions.

Our results indicated that the optimization and use of the megaplex PCR can open new dimensions in the multifunctional use of microsatellites for breeders and genetics, multiplying the efficiency and significantly reducing the cost of the analysis. Using this technique, two linkage

maps in apricot have been developed with 37 and 29 SSR markers respectively. In addition, it was possible to identify in one of the genetic map one QTL linkaged to flowering time in the linkage group 5. One SSR loci (AMPA-105) was linked to this trait in apricot. However, further studies with appropriate crosses between parents, which segregate for these traits, will be necessary to apply efficient marker assisted selection (MAS) strategies in the breeding programmes.

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION

Seasonal progression of dormancy

1. SNC approach

According to our results, the onset of dormancy occurred prior to the advent of chilling accumulation. This is in agreement with previous results found in apple in an area with similar chill accumulation (Cook and Jacobs, 2000), but in contrast with the common belief that buds reach dormancy with the onset of chilling closer to winter (Crabbé, 1994). In general, the maximum values of the mean time to bud break (MTB) obtained, which coincide with the maximum depth of dormancy, are in agreement with previous results in other species in mild winter climates (Balandier et al., 1993a; Balandier et al., 1993b; Cook and Jacobs, 2000). However, our results are in contrast with Rageau (1987) in a temperate climate, showing a lower depth of dormancy in mild winter areas compared to temperate areas. An oscillating pattern in dormancy progression markedly influenced by autumn and early winter temperatures was observed. The differentiation of flower buds exerted an important role over the dormancy intensity of vegetative and reproductive buds. After the differentiation of floral whorls, dormancy depth was always lower in reproductive buds compared to vegetative buds (Figure 7). Besides, the formation of complete flowers was concomitant to a generalised increase in the MTB in both vegetative and reproductive buds. This is in concordance with a recent work on apricot, where it was found that floral differentiation occurs before dormancy onset (Julian 2008).

The earlier dormancy release of flower buds compared to vegetative buds found in this trial is in agreement with Erez (2000). On the other hand, terminal vegetative buds reached a deeper maximum dormancy status compared to lateral vegetative buds, although afterwards a quicker dormancy release was observed in terminal buds compared to lateral buds. This is in accordance with previous results in other species and climatic conditions (Crabbé and Barnola, 1996; Champagnat 1992, 1983; Mauget and Rageau, 1988; Williams et al., 1978). In this manner, it appears that upon entrance into dormancy, a distal shoot-forming or acrotonic tendency exists. This trend shifts to a proximal shoot-forming or basitonic tendency in early winter and then becomes more acrotonic as spring approaches (Barnola and Crabbé, 1991; Cook et al., 1998). These findings

are in agreement with our results. However, an effect of suboptimal chilling over acrotony was found in 2006 in vegetative buds, which could be consistent with the findings of Cook and Jacobs (1999), who stated that suboptimal winter chilling impedes development of acrotony in apple shoots.

2. Induction and release of endodormancy in apricot - effects of different climatic conditions

The dissimilar temperatures registered in the diverse Mediterranean climates studied involved markedly different chill accumulations, which were in accordance to the altitude. In agreement with the results obtained with single node cuttings, buds entered into dormancy prior to the advent of chilling accumulation in warm and moderately cold areas. The decrease of temperatures in late summer, coinciding with the decreasing photoperiod, could trigger the onset of dormancy (Heide, 2008). However, the variation observed in dormancy induction among locations within the same latitude suggests an important influence of temperature. In warm areas dormancy entry occurred earlier than in colder areas. A clinal variation in dormancy progression with warm temperatures could be hypothesized for apricot cultivars in warm-winter areas, as Olsen (2003, 2004) showed for northern trees ecotypes. The assumption that dormancy induction and progression is related to the chilling accumulation seems erroneous according to the results obtained in most of the warm areas and cultivars studied in both countries.

The different approaches to the study of dormancy showed some differences and analogies in the results obtained in the seasonal progression of bud dormancy in apricot. The influence of paradormant tissues can have an important role in these divergences (Cook et al, 1998), especially in the first stages studied in these trials where paradormancy exerts an important influence (Faust, 1987). The SNC may show a closer picture to the state of the bud itself, whereas the evaluation of complete shoots would predict better the field response (Dennis, 2003). In spite of the incipient results regarding the discovery of new indicators of the state of dormancy (Tamura, 1998; Mathiason et al., 2009), the quantification of the state of dormancy through the forcing of a plant, or a part of it (shoots, cuttings, etc.), is still an economic and suitable method. Even though, the use of potted trees or micropropagated plants (Heide, 2005) could solve some problems associated with the possible interference of the wound (Latimer and Robitaille, 1981).

Chilling requirements for breaking of dormancy

1. Comparing chilling requirements in Tuscany (Italy) and Murcia (Spain)

The assessment of CR by the Utah Model showed a high variability of results among years and locations, and therefore, the Utah Model was not completely accurate with regard to the establishment of CR for dormancy release under a Mediterranean climate. Several studies have reported that the Utah Model did not accurately predict dormancy completion under warm climatic conditions (Buchanan et al., 1977; Gilreath and Buchanan, 1979; Erez et al., 1990; Linsley-Noakes and Allan, 1994). The different chill requirement recorded in most cultivars could be due to interferences of other climatic factors, affecting bud development (Garcia et al., 1999). It is probable that, rather than an absolute action of the temperature in terms of chilling measured by the Utah Model, other climatic parameters, such as temperature fluctuations between day and night and the moment of chilling application (late autumn, early winter or mid-winter), could have a very important role as factors regulating the breaking of dormancy.

Moreover, the flowering and fruit-set percentages also were influenced by the environmental conditions occurring in the different years. The inadequate fruit–set, especially in the warmer winter (2006-07), could have been due to an irregular or insufficient satisfaction of CR, but also could be the result of an altered flower bud development determined by a lack of synchronisation of the dormancy cycle, xylem differentiation and microsporogenesis processes (Bartolini et al., 2006a, 2006c), as well as inadequate mobilisation of reserves (Rodrigo et al., 2000).

2. Comparing chilling requirements in the Western Cape (South Africa) and Murcia (Spain)

The variability of the CR among years (Bailey *et al.*, 1982; Tabuenca, 1964; Ruiz, 2007; Valentini et al., 2004) altitude (Balandier, 1993a; Mauget, 1977); coastal or inner areas (Sorensen, 1983; Campbell, 1974); and latitude (Balandier, 1993a; Champagnant, 1983) has been already described in several species. In our study a comparison between very different locations where commercial apricot is successfully grown revealed considerable differences in the CR of the cultivars studied. The range of CR for of the group of cultivars studied was markedly different between countries, and was especially high for the CR calculated by the Utah and Hours-below-7 ºC Models. Likewise, ‘Canino’ and ‘Orange Red’ showed very different CR in both countries. For

example, the variation in CR of 'Canino' between marginal chill conditions (South Africa) and normal chill conditions (Spain) was higher than the variation in CR obtained among low and medium-high CR cultivars studied in Spain. These results are in accordance to the previously found by Balandier et al, (1993a) between tropical and temperate areas in peach cultivars, and suggest that the variability of the climatic conditions can lead to variability in the CR even higher than between different cultivars of the same species. In the field conditions studied, a high correlation coefficient between CU and Portions was found. Previous studies (Linsley-Noakes and Allan, 1994; Erez et al., 1990; Erez et al., 1998; Ruiz et al, 2007) also have reported this high correlation in field conditions with similar winter temperatures. Therefore, with both models similar values of CR would be obtained. Nonetheless, the considerable inaccuracy of the models in different climatic conditions questions the reliability of modelling chill accumulation to determine the endodormancy release of temperate fruits without any link to the physiological processes of the plant. Moreover, these models consider a linear effect of temperatures regardless of the state of dormancy progression or physiology of the plant (Weinberger, 1950; Thompson et al., 1975; Couvillon and Erez, 1985b; Young, 1992; Tehranifar et al., 1998).

No significant differences in HR were found among cultivars and no significant correlation was found between HR and flowering date. Thus our results indicate that HR for flowering are not an intrinsic characteristic of the cultivar in the apricot species. This would suggest that flowering date is determined basically by CR (Brown, 1957; Swartz and Powell, 1981; Couvillon and Erez, 1985b), and HR seems to be a constant thermal integral required for flowering.

Finally, it is worthwhile mentioning the successful cultivation of apricot under marginal CR accumulation areas in South Africa. This suggests the possibility to expand in Spain the cultivated area to marginal chill areas with appropriate culture practices, especially the use of rest breaking agents. This could have an important effect over the production of apricot cultivars oriented to the early fresh market.

Effect of different temperature cycles and chill temperatures over dormancy

1. Effect of temperatures cycles over dormancy evolution and dormancy release

The efficiency of the different temperature treatments over dormancy evolution and dormancy overcoming was highly influenced by the state of bud dormancy when shoots were cut from the tree, which is in agreement with findings obtained by previous studies (Falussi and Calamasi, 2003; Fuchigami and Nee, 1987). When no chill had been accumulated prior to the cutting date, continuous 5 ºC was the most efficient treatment, followed by 5/15 ºC. However, once a certain amount of cold had been accumulated in field conditions, cycles including moderate or high temperatures (5/25 ºC cycle) increased its efficiency in dormancy breaking. These results agree with previous work (Erez and Lavee, 1971; Young, 1992) that showed that moderate and high temperatures can be more efficient at breaking dormancy when applied in advanced stages of dormancy release. What is more, the sharp increase in dormancy-breaking efficiency arising from the application of 5/20 ºC to 5/25 ºC, after partial chilling had been accumulated, could indicate the existence of a temperature threshold between 20 and 25 ºC. This could be related to the capacity of high temperatures to break dormancy (Chandler, 1960; Tamura et al., 1993) and to the lack of chilling-negation effect when the tree is in an advanced stage of endodormancy release (Couvillon and Erez, 1985b). Erez and Lavee (1971) showed that moderately high temperatures acquire an efficiency to break dormancy only after a partial chill accumulation. However, our results contrast with those of Erez et al. (1979a) and Shaltout and Unrath (1983), who stated that an hour at temperatures from 21 ºC to 24 ºC, and presumably higher, in a diurnal cycle should have a value of -2 CU. Taking into account our results this value could be higher than +1 CU when partial chill has been accumulated.

In contrast, some factors should be taken into account. Firstly the chill receptivity of shoots could be conditioned by the state of development when they are separated from the tree (Tehnranifar et al., 1998) or by the physiological state (Crabbé and Barnola, 1996). Secondly, a residual effect (Spiegel-Roy and Alston, 1979), even when the models indicate the fulfilment of the CR (Guerriero et al., 2002; Couvillon and Erez, 1985a; Ruiz et al., 2007), has been described. This effect entails considerable drawbacks in fruit production in field conditions and may have not been possible to detect in our study. Thirdly, the possibility of a reduction of the heat requirement after a

higher chilling accumulation should be considered (Couvillon and Erez, 1985a; Felker and Robitaille, 1985).

2. Effects of chilling temperatures on apricot dormancy progression

The effect of chilling temperatures on dormancy progression and dormancy release also showed interesting results. In vegetative buds, 10 ºC treatment showed the highest MTB values, that is to say the deepest dormancy intensity, of all treatments, when applied before the advent of chilling temperatures and before the onset of the maximum depth of dormancy. Lavarenne et al. (1975) found that moderately cold temperatures are more efficient than extreme cold temperatures to increase the depth of dormancy. The generalised increase in the depth of dormancy in early November, coinciding with the onset of chilling accumulation, has already been reported by several authors (Amling and Amling, 1985; Arias and Crabbé, 1975; Walser et al., 1981). At this time, an equilibrium point between the dormancy induction and dormancy release effect of the chilling temperatures applied was observed. However, it must be noted that in our case a considerable application of chilling temperature was applied before the forcing conditions. On the other hand, it seems that the same temperatures that later release dormancy through chilling accumulation also intensify the dormant state when applied before the tree reaches the endodormant state (Crabbé, 1994; Faust et al., 1997). Results show the stage of dormancy has a strong influence on the effect of the different temperatures. Heide (2008) showed the important interaction of photoperiod and temperature in the control of growth and dormancy in *Prunus* species. Besides, it seems that the advent of chilling temperatures in field conditions had a higher effect over dormancy induction than did the application of low temperatures in laboratory conditions over cut shoots. A non-linear effect of different temperatures along the dormancy cycle was obtained, especially in the superior range of temperatures traditionally considered to release dormancy. Our results indicate that in apricot, after the satisfaction of ca. 20% of the CR of the genotypes in the field, the most efficient temperature was 10 ºC. Whereas, others authors working on apple have assigned more efficiency to 1 ºC than to 10 ºC. (Del Real-Laborde, 1990; Naor et al., 2003). The different efficiency of temperatures as well as the physiologic state of the plant evolved in the season (Laverenne et al., 1975), should be considered before assigning a value to a determined temperature for its efficiency in dormancy release. This non-linear effect of temperatures along dormancy cycle could partly explain the uneven results that are frequently obtained in the determination of the temperature efficiency for

dormancy release. The introduction of this differential effect, both of chill and cycles of temperatures, could help to improve the models to estimate dormancy release, and, consequently, the CR of apricot cultivars.

Effects of shading and TDZ+oil treatment on dormancy breaking, flowering and fruit set

Shading and TDZ+oil treatment have been evaluated as strategies for bringing forward dormancy release and improving flowering percentage and fructification.

The important increase in chilling accumulation (calculated with the Utah Model) obtained by shading treatments (more than 20% greater than for non-shaded trees) and the resultant earlier satisfaction of CR, did not result in the same proportion with the expected precocity on flowering and ripening dates. Due to the high fluctuation of the climate during the years studied, only in the period 2006-2007 insufficient chilling was accumulated for 'Poppy' in our climatic conditions. No effect was found through the reduction of incident radiation with shade cloth during the stage of entering into dormancy. However, shading during late endodormancy produced 5 days precocity for the harvest date in the year with lower chill accumulation. Besides higher flowering and fruit set percentages were obtained. This could be related to the higher chilling accumulation obtained by shading, but also due to the reduction in the amount of light supplied during the late-rest period, as it was reported in previous work (Erez et al., 1968). Perez et al, (2007) suggested that the erratic budbreak in grape could be related to high winter temperatures, which create disturbances to mitochondrial respiratory capacity and oxidative metabolism. In the year with insufficient chill accumulation, an increase in productivity higher than 250% was found when trees were shaded during late endodormancy or treated with TDZ+oil. In the case of shading during endodormancy the increase was higher than 40%.

The TDZ+oil treatment increased flowering percentages which is in accordance with previous results in apple (Wang et al. 1986; Steffens and Stutte, 1989), and in blackberry (Izadyar and Wang, 1999). Besides flowering was more uniform, and a considerable precocity in flowering (by 7 to 14 days) and ripening (3-9 days) dates was obtained. However, pistil abortion percentage was strongly increased by using TDZ and winter oil when there was low chilling accumulation, which led to reduce fruit set percentage. Taking into account the negative effect of insufficient chill

accumulation on flower bud development (Erez, 1999, 2000), it can be suggested that TDZ and winter oil trigger a general blossoming of flowers. When the winter fully satisfies the chilling requirement, fewer defective flowers appear whereas when not enough chill is accumulated this rate increases.

Some interesting correlations in agreement with previous studies were found. A negative correlation was found between chill accumulation and pistil abortion (R = -0.64). Legave (1978) stated that pistil abortion is related to the cultivar´s adaptation to different cropping areas. The low values of chilling accumulation increased the pistil abortion, showing the lack of adaptation of this cultivar to the studied area. Besides, positive correlations were also found between chill accumulation and fruit set (R = 0.628) and between chill accumulation and fruits/buds percentage (R = 0.825), showing the necessity of satisfying the chilling requirement in order to have proper fruit set and production (Erez, 2000).

Considering the possibilities of growing apricot cultivars in warm conditions as the studied in South Africa, together with the substantial increase of fruit/buds percentages obtained in a year with insufficient chilling accumulation, shading of trees during endodormancy and TDZ+oil treatment could be suitable to both increase precocity and improve the performance of low chill cultivars in warm-winter climates.

Using megaplex PCR for molecular characterization and identification of QTLs controlling flowering time

One hundred and twenty apricot and peach simple sequence repeat (SSR) markers were used in the molecular characterization of a BC1 apricot progeny of 61 seedlings derived from the cross between the F1 selection ‘Z506-07’ (‘Orange Red’ x ‘Currot’) and the Spanish cultivar ‘Currot’. The megaplex PCR method was used for the optimization of the use of SSR markers in the molecular characterization, multiplying the efficiency and significantly reducing the cost of the analysis. A series of seven megaplex PCRs containing between 6 and 20 SSR markers were developed for the molecular characterization of the apricot breeding progeny studied. Amplification was successful in apricot progenitors and in the progeny with 114 of the 120 (95%) SSR markers with a suitable level of polymorphism (1.7 alleles/marker) detected in the BC1 descendants studied. The successful amplification performed in meglaplex 2 indicated the possibility of the analysis in

only one megaplex PCR reaction of 18 SSR markers. These results improved the previous results exposed by Hayden et al. (2008) and Patacchi et al. (2009) which in one multiplex PCR reaction incorporated 6 and 4 SSR markers, respectively. Thus, the implementation of megaplex PCR considerably increased the efficiency and reduced the cost of this type of molecular studies.

Using this technique, two linkage maps in apricot have been developed with 37 and 29 SSR markers respectively. The location of the SSR marker mapped was similar to those previously reported by Aranzana et al. (2003) and Dondini et al. (2007). Our results showed a high level of colienarity between *Prunus* species and confirmed a high level of transferability of the markers, as already described by Dirlewanger et al. (2004) and Arús et al. (2006). In addition, it was possible to identify in the genetic map of 'Z 506-7' one QTL related to flowering time in the linkage group 5. One SSR loci (AMPA-105) was linked to this trait in apricot. No works regarding identification of QTLs controlling CR have been reported on apricot species, excepting preliminary results which have been presented recently (Olukolu et al., 2008). In this work, QTL analysis using two linkage maps with phenotypic trait data of dormancy bud-break resulted in seven QTLs on linkage groups (LG) 1, 2, 3, 5, 6, 7 and 8. However, further studies with appropriate crosses between parents, which segregate for these traits, will be necessary to apply efficient MAS strategies in the breeding progammes.

GENERAL CONCLUSIONS

1. The study of endodormancy progression through a single node cuttings approach revealed an oscillating and shallow pattern in the climatic conditions of southeast Spain. This pattern was markedly influenced by autumn and early winter temperatures. A deeper dormancy but an earlier release of dormancy was found in terminal vegetative buds compared to lateral vegetative buds.

2. Studying dormancy progression using detached shoots, apricot buds entered into dormancy prior to the advent of chilling accumulation in warm and moderately cold areas, a result which coincides with the results obtained with the single node cutting method. Thus, the assumption that dormancy induction is related to chilling accumulation seems erroneous according to the results obtained in most of the warm areas and cultivars studied in Spain and South Africa.

3. The variation observed in dormancy induction among locations within the same latitude suggests an important influence of temperature. In warm areas, dormancy entry occurred earlier than in colder areas. A clinal variation in dormancy progression with warm temperatures could be hypothesized for apricot cultivars in warm-winter areas.

4. The range of chill requirements (CR) for the group of cultivars studied was markedly different between countries (Spain, Italy and South Africa), especially when warm winter and cold winter areas are compared. Marked differences of CR between cultivars with similar CR were observed when a moderately cold area (Murcia, Spain) and a very warm area (Villiersdorp and Ladismith, Western Cape, South Africa) were compared. This variability of results for the determination of CR indicates the inaccuracy of traditional methods, which should be revised to introduce aspects such as the differential effect of temperatures according to the moment of application, as well as the variable depth of dormancy, which depends on the conditions of onset.

5. High temperatures (25 ºC), which usually have a negative effect over dormancy overcoming, can be very efficient when applied in a daily cycle with low temperatures after partial chilling has been accumulated. Besides, the efficiency and effect of different low temperatures was highly dependant on the moment of application. Chilling was substantially more efficient when

applied in field conditions than in the growth chamber, which highlights the importance of physiological processes along dormancy maintenance and release in the tree.

6. Our results indicate that heat requirements for flowering after breaking of dormancy are not a specific characteristic of each cultivar in the apricot species, and that flowering date is mainly determined by the chilling requirement of the cultivars.

7. In conditions of insufficient chill accumulation, the treatment with TDZ+oil and shading during endodormancy or late endodormancy substantially increased flowering uniformity, flowering and fruit set percentages and can induce a considerable precocity in both flowering and ripening times. Therefore, the shading of trees during endodormancy and a TDZ+oil treatment could be suitable for increasing precocity and improving the performance of low chill cultivars in warm winter climates, for example in the Western Cape in South Africa and in some locations in Spain.

8. The megaplex PCR optimized the use of microsatellite markers and significantly reduced the costs and the time of the analysis for molecular characterization. Besides, two preliminary linkage maps have been developed for ‘Z506-07’ and ‘Currot’, and one QTL linked to flowering time has been identified in the linkage group 5. One SSR loci (AMPA-105) was linked to this trait in apricot. However, further studies will be necessary to confirm these results. The development of specific molecular markers will help to improve the efficiency of breeding programs aimed at releasing new apricot cultivars with low or high CR.

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ANEXES

ANEXE 1. TABLE INDEX

ANEXE 2. FIGURE INDEX

ANEXE 3. INDEX (SPANISH)