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GUSTAVO MALAGI

Flowering phenology and dormancy dynamics of apple tree in contrasting climatic conditions: a case study of phenology modeling in the climate warming context

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Presidente da banca:
Dr. Flávio Gilberto HERTER - (PPGA, UFPel-FAEM, Pelotas, Brasil)

Relatores:
Dr. Luiz Antonio BIASI - (PPGA, UFPR, Curitiba, Brasil)
Dr. André LACOINTE - (INRA [CR], UMR PIAF, Clermont- Ferrand, França)

Examinadora:
Dra. Maria do Carmo Bassols RASEIRA - (EMBRAPA CT, Pelotas, Brasil)

Orientadores:
Dr. Jean Michel LEGAVE - (UMR AGAP, INRA, Montpellier, França)
Dr. Idemir CITADIN - (PPGA, UTFPR, Pato Branco, Brasil)

Co-Orientador / Convidado:
Dr. Marc BONHOMME - (UMR PIAF, INRA, Montpellier, França)

Convidado:
Dr. Jean Luc REGNARD - (Montpellier SupAgro, UMR AGAP, Montpellier, França)

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M. Flávio Gilberto HERTER Prof., UFPel (Brésil)	Président de jury
M. Luiz Antonio BIASI Prof., UFPR (Brésil)	Rapporteur
M. André LACOINTE HDR, CR INRA UMR PIAF (France)	Rapporteur
Mme Maria do Carmo Bassols RASEIRA Dra., Embrapa Clima Temperado (Brésil)	Examinateur
M. Jean Michel LEGAVE HDR Dr., INRA UMR AGAP (France)	Co-Directeur de thèse
M. Idemir CITADIN Prof., UTFPR (Brésil)	Co-Directeur de thèse
M. Marc BONHOMME Dr. IR, INRA UMR PIAF (France)	Co-Encadrant, Invité
M. Jean Luc REGNARD Prof., Montpellier SupAgro, INRA UMR AGAP (France)	Invité

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A persistência é o caminho do êxito.

La persistance est la voie du succès.

Charles Chaplin

À Deus e aos meus pais Ari e Nair,

DEDICO

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Abstract

Weather patterns have changed globally in recent decades causing serious consequences on the development of several plant species. The intensification of phenological advances in the northern hemisphere has raised interest in the understanding of dormancy physiology in temperate species when subjected to warming regimes. The purpose of this study was to compare regional patterns of temperature on the flowering of apple tree cultivars grown under contrasting climates, using an extensive database of temperature and phenology records. Thus, it was possible to understand how temperature controls the flowering process and identify the areas most vulnerable to warming. Historical temperature series collected for Western Europe (cold winter), Morocco and Southern Brazil (warm winter) were used. Historical dates of two flowering stages and the time between them were analyzed. All areas in Western Europe experienced warming in the spring, whereas a slight warming of winter was found in the mild areas. The observed advancement of blooming dates and the short blooming time were linked to spring temperature increase in Western Europe, with the southern region being more vulnerable to future warming. In Morocco and Southern Brazil minimal warming was found in recent decades but winter was most affected. Weak phenological changes and long flowering periods were associated with winter temperatures. Phenology patterns and intermediate temperatures were observed in Morocco. Moreover, an additional study was conducted in Brazil and France to understand the mechanism of dormancy in apple trees, the intrinsic factors that controls it, and to analyze the regional and varietal variability. The kinetics of dormancy in vegetative buds was determined by a "One-bud cutting test" and in floral buds by "Tabuenca's test." Vegetative buds showed fluctuating dormancy during autumn-winter-spring in France, which was deeper in winter. In Brazil, the vegetative buds showed weak variations and shallow depth in the equivalent period. The significant increase in fresh and dry weight of flower primordia characterized the transitional phase between endo- and ecodormancy. However, ecodormancy establishment was related to the significant increase in dry weight of floral primordia and their ability to rehydrate. The transition between these phases was fast in Brazil and slower in France. Sequential models, able to estimate annual blooming dates in both hemispheres were selected from historical temperature and phenology data. Two models were validated to estimate the stages BBCH 61 (NHGoldenF1) and BBCH 65 (NHGoldenF2) of Golden Delicious in Western Europe. These models showed high accuracy and greater efficiency in relation to thermal models. Considering a CPR scenario of intense increase in temperature by the end of this century, there was estimated a trend to extend the period of chill accumulation, especially in Nîmes, and advance dates for flowering in Angers, by the end of this century, according the two NH models. Under both scenarios and locations, there was a significant reduction in the ecodormancy phase, with the lowest values recorded at Nîmes. The projected changes in temperature in the mediterranean region of Europe can extend the flowering period, resulting in physiologic disturbances as today in mild climate. Other concepts of models and/or the inclusion of multiple factors in the model should be considered in the near future, considering that warming in Europe may compromise the applicability of the sequential models, as observed currently in southern Brazil.

Keywords: global warming, temperature, phenology prediction.

Resumo

Os padrões climáticos têm sofrido alterações em escala global nas últimas décadas e consequências graves sobre o desenvolvimento de diversas espécies vegetais têm sido observadas. A intensificação dos avanços fenológicos no hemisfério norte tem despertado o interesse sobre a compreensão da fisiologia da dormência de espécies temperadas quando submetidas a regimes de aquecimento. A proposta desse estudo foi comparar os padrões regionais de temperatura e floração de cultivares de macieiras cultivadas em climas contrastantes, a partir de uma extensa base de dados de temperatura e fenologia. Assim, foi possível compreender como a temperatura controla o processo de floração e diagnosticar as zonas de produção mais vulneráveis ao aquecimento. Séries históricas de temperatura foram coletadas na Europa Ocidental (inverno frio), Marrocos e no sul do Brasil (inverno quente). Datas de ocorrência de dois estágios de floração e o tempo transcorrido entre eles foram analisados. Verificou-se o aquecimento de todas as áreas da Europa Ocidental na primavera, e um leve aquecimento do período invernal na zona sul dessa região. Nesses locais os avanços das datas de floração e o curto tempo de floração ocorreram pela elevação das temperaturas primaveris. A região sul mostrou-se mais vulnerável a futuros aquecimentos. No Marrocos e sul do Brasil o aquecimento foi mínimo nas últimas décadas sendo o inverno mais afetado. Leves mudanças fenológicas e longos períodos de floração estiveram associados às temperaturas invernais. Padrões de fenologia e temperatura intermediárias foram observados no Marrocos. Por outro lado, estudo complementar foi conduzido no Brasil e na França visando compreender o mecanismo de dormência da macieira, os fatores intrínsecos que a controlam, além de analisar as variabilidades regionais e varietais. A cinética da dormência foi determinada em gemas vegetativas por “One-bud cutting test” e em gemas florais por “Tabuenca’s test”. Gemas vegetativas apresentaram dormência oscilante durante outono-inverno-primavera na França, sendo profunda no inverno. No Brasil, as gemas vegetativas apresentaram dormência com variações amenas no período equivalente e com profundidade superficial. O aumento significativo do peso fresco e seco dos primórdios florais caracterizou a fase transitória entre endo- e ecodormência. No entanto, o estabelecimento da ecodormência esteve relacionado com o aumento significativo do peso seco e da capacidade de reidratação dos primórdios florais. A transição entre essas fases foi acelerada no Brasil e mais lenta na França. Ainda, a partir de dados históricos de temperatura e fenologia, foram selecionados modelos fenológicos sequenciais capazes de estimar regionalmente as datas anuais de floração em ambos os hemisférios. Dois modelos foram validados para a estimação do estágio de floração BBCH 61 (NHGoldenF1) e BBCH 65 (NHGoldenF2) de Golden Delicious, na Europa Ocidental. Esses modelos apresentaram elevada acuracidade e apresentaram eficiência superior aos modelos termais. Considerando um cenário RCP de incremento intenso da temperatura até o final do século, observou-se tendência de alongamento do período de acúmulo de frio, principalmente em Nîmes, e antecipação das datas de floração em Angers, até o final desse século, segundo os dois modelos NH. Em ambos os cenários e locais, observou-se redução significativa da fase de ecodormência até o final desse século, com os menores valores registrados em Nîmes. Essa perspectiva de mudanças na temperatura na região Mediterrânea da Europa predispõe futuramente o alongamento do período de floração, resultando distúrbios fisiológicos característicos de regiões de clima ameno hoje. Outros conceitos de modelos ou a inclusão de múltiplos fatores durante a parametrização devem ser considerados em um futuro breve, haja vista ainda, que o agravamento climático na Europa, poderá comprometer a aplicabilidade dos modelos sequências, como se observou na atual situação climática do sul do Brasil.

Palavras-chave: aquecimento global, temperatura, predição da fenologia.

Résumé

Les conditions météorologiques ont changé globalement au cours des dernières décennies et d'importantes conséquences sur le développement des plantes ont été observées. L'approfondissement des études sur la phénologie dans l'hémisphère nord a conduit à davantage prendre en compte la compréhension de la physiologie de la dormance des espèces fruitières tempérées depuis qu'elles sont soumises à une augmentation du réchauffement climatique. Pour cela l'objectif de notre étude est de comparer les évolutions régionales de température et de floraison de cultivars de pommiers cultivés dans des climats contrastés, à partir d'une vaste base de données de température et phénologie. Ainsi, il a été possible de comprendre comment la température contrôle le processus de floraison et de localiser les zones de production les plus vulnérables face au réchauffement climatique. Des séries historiques de température ont été recueillies en Europe occidentale (climat tempréré), au Maroc et dans le sud du Brésil (climat doux). Les dates observée de deux stades de la floraison et la durée entre ces stades ont été analysées. Nous avons constaté un réchauffement printannier dans toutes les régions d'Europe occidentale, et un léger réchauffement durant la dormance en région méditerranéenne. Dans ces sites, des avancées des dates de floraison et une courte durée de floraison ont eu lieu sous l'effet de l'augmentation des températures printanières. Dans l'avenir la région méditerranéenne apparaît la plus vulnérable face au réchauffement climatique durant la dormance. Au Maroc et dans le sud du Brésil le réchauffement a été relativement faible au cours des dernières décennies. Au Brésil des changements phénologiques limités et de longues durées de floraison ont été associés aux évolutions des températures hivernales et printanières. Des changements intermédiaires pour la phénologies et la température ont été observés au Maroc. Une étude complémentaire a été réalisée au Brésil et en France visant la compréhension du mécanisme de la dormance du pommier, les facteurs intrinsèques qui le contrôlent, ainsi que l'analyse de la variabilité régionale et variétale. La cinétique de dormance des bourgeons végétatifs a été déterminée par le "Test de bouture de noeud-isolé" et pour les bourgeons floraux par le "Test de Tabuenca". Les bourgeons végétatifs ont montré une dormance oscillant cours de l'automne-hiver-printemps en France, étant particulièrement profonde en hiver. Au Brésil, les bourgeons végétatifs ont montré de faibles variations de la profondeur de la dormance durant une période équivalente. L'augmentation significative des poids frais et secs des ébauches florales a caractérisé la phase de transition entre endo et ecodormance. Cependant, la mise en place de l'ecodormance apparait liée à l'augmentation significative du poids sec et à la capacité de réhydratation des ébauches florales. La transition entre ces phases a été courte au Brésil et inversement plus lente en France. Par ailleurs, à partir de l'historique des données de température et de phénologie ont été sélectionnés des modèles phénologiques séquentiels capables d'estimer régionalement les dates annuelles de floraison dans les deux hémisphères. Deux modèles ont été validés pour l'estimation du stade de floraison BBCH 61 (NHGoldenF1) et BBCH 65 (NHGoldenF2) de Golden Delicious en Europe occidentale. Ces modèles ont montré une précision supérieure au modèle thermique. En considérant un scénario RCP de forte augmentation de la température jusqu'au la fin du siècle, nous avons trouvé une forte tendance à l'allongement de la période d'accumulation de froid, surtout à Nîmes, et à l'avancement des dates de floraison à Angers. Dans ces deux sites, une réduction significative dans la phase d'ecodormance se produirait à la fin du siècle, avec les valeurs les plus basses pour le site de Nîmes. L'évolution de la température en région méditerranéenne européenne est susceptible d’induire des conséquences importantes sur la physiologie du pommier, semblables à celles enregistrée aujourd'hui en régions à climat doux. D'autres concepts de modèles doivent être considérés en Europe dans l’avenir, face à un accroissement inéluctable du réchauffement climatique, qui ne peut être pris en compte par des modèles séquentielles, comme cela a été montré dans la situation climatique actuelle dans le sud Brésil.

Mots-clés: réchauffement climatique, température, prédiction phénologique.

Summary

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Study plan

Western Europe has faced the serious consequences of climate change, mainly by increase of temperatures during spring. This has led to advancements in flowering dates of some apple cultivars. Future projections have indicated that an intensification of the warming will occur, and considering that it affects the chill accumulation period, this can cause a delay in flowering. This result may be accompanied by serious physiological disorders such as heterogeneity in budburst and flowering, which could compromise the final yield. Therefore, Western Europe could soon be facing a subtropical scenario due to warming. Thus, the cultivation of temperate fruit species will be conditioned to the development of adaptation tactics currently employed in the subtropical region of Brazil. The study of dormancy and the selection of phenological models may be useful in developing strategies for adaptation to warming.

The objectives of this study were to compare the divergences in long term data series of temperatures and phenology of apple cultivars, in three macro-regions with contrasting climates (Southern Brazil, Western Europe and Morocco) to determine the magnitude of changes. The kinetics of dormancy was also studied for the vegetative and floral buds of many apple cultivars at different locations, during dormancy, to understand the factors involved in flowering control. Attempts to select regional blooming models were also performed using historical temperature and blooming date data, and endodormancy release dates. Thus, the study was divided into four main areas to compare contrasting climates:

1) Spatiotemporal variability of temperatures;
2) Spatiotemporal variability of flowering dates;
3) Dormancy of vegetative and floral buds of apples;
4) Development of regional phenological models;

Therefore, the results of this study could contribute to future challenge solutions of fruit production in temperate regions. These knowledge and tools can be useful in breeding programs focused on the selection of varieties adapted to new climate scenarios.

General introduction

Climate change represents one of the most serious global challenges to affect several agro-meteorological, environmental, economic, political, and social processes. Current research efforts have focused on the possible causes of climate change allowing the estimation of current interference and the future trends (IPCC, 2013). For years, climate change has been predominately attributed to a combination of natural causes and anthropogenic activities (Kalis and Merkt, 2003; van Dijk et al., 2013). The emission of greenhouse gases such as carbon dioxide, methane, and nitrous oxide has been linked to climate change (IPCC, 2013). The quantitative assessment of the impact of climate change as well as future predictions, regarding the matter, have also been discussed in the literature (Nuccitelli et al., 2013). However, regardless of the causes, climate change is now a reality that appears to be irreversible (Solomon et al., 2009). Thus, actions to prevent current and future damages to the environment and human activities are crucial (Reusswig, 2013).

Previous studies have shown that climate change affects the frequency, intensity, and duration of extreme global events such as temperature, precipitation, drought, flooding, and heat/cold waves (IPCC, 2007). The impact of such events is largely because societies and ecosystems are not adapted to counter the consequences (Coumou et al., 2013). Likewise, economic activities such as agriculture are more vulnerable to climate change, especially extreme events (EuropeAid, 2009), and thus, can be severely affected. Because it is hard to predict extreme events, it is not always possible to immediately react in order to reduce their effect. North America has experienced an overall increase in heavy precipitation in the last century (Groisman et al., 2005). In the subtropical region of Brazil, a 58% increase in the rate of heavy precipitation has been reported in the last century, whereas in Northern Europe, the increase was 16% in the last 60 years (Groisman et al., 2005). Moreover, higher incidences of droughts (the Amazon basin in 2005), floods, cyclones (Katrina, Brazil in 2004), storms, and the ocean-atmosphere phenomena as *La Niña* and *El Niño* have been observed in South America (EuropeAid, 2009). In recent years, a higher number of heat waves have also been observed, including those that affected Europe in 2003 (Schar et al., 2004), Australia in 2004 (Tong et al., 2010), Greece in 2007

(Founda and Giannakopoulos, 2009), Russia in 2010 (WMO, 2010), the United States in 2012 (NOAA, 2014), and Brazil in 2014 (Reuters, 2014).

Apart from these events, a constant increase has been noted in the average global temperature in the past few decades, and this has been regarded as the main impact factor for climate change. Thus, global temperature is the most important measurable variable for evaluating the magnitude of climate change (Wang and Dickinson, 2013). The annual increase in the average global temperature was 0,29°C per decade from 1979 to 2012. Optimistic predictions suggest an increase of up to 0,5–1,0°C above average by 2050 and up to 1,0°C by 2100. Less optimistic predictions estimate an increase of up to 2,0°C and 4,0°C by 2050 and 2100, respectively (IPCC, 2013).

Current indicators and future predictions of temperature increase call for a better understanding of its existing relations with temperate tree phenology (Galán et al., 2001). This would provide valuable data for the planning, organization, and execution of diverse management activities. The establishment of these relations would allow the use of changes in temporal phenology as indicators of climate change (Ruml and Vulic, 2005). In the last decade, the United Kingdom has utilized phenology as an indicator of climate change (Menzel, 2002), particularly that involving changes in temperature.

Temperate perennial species are more vulnerable to changes in temperature, because their development is largely dependent on this abiotic factor (Walthall et al., 2012). Reductions in the photoperiod and temperature, particularly during the fall, often lead to a decrease in physiological processes and the beginning of the endodormancy phase. To overcome this phase, accumulated winter chill is required, which varies among species and cultivars (Hauagge and Cummins, 1991). Once the chill requirement is fulfilled, plants enter the ecodormancy phase, which ends with heat accumulation during the start of spring and bloom if environmental conditions are favorable.

When growing under mild climate areas such as Southern Brazil, temperate trees rarely fulfill their chill requirement during the winter (Hawerroth et al., 2010). As a consequence, abnormalities develop, which include smaller leaf area, low spur density, apical dominance, uneven budburst, lower fruit quality, longer flowering period, deformed stigma, lower production of pollen grains, decrease in stigma receptivity, and lower fruit set (Petri and Leite, 2004). Certain products such as hydrogen cyanamide are used to compensate for the lack of chill (Jackson and Bepete, 1995; Mohamed, 2008), inducing budbreak when applied during onset of budding in early spring. A wide variety of chemicals can be used to control vegetative growth. All this management not only leads to an increase in production costs, but also contributes to environmental decline. On the other hand, the selection of low chill cultivars has equally contributed to overcoming these barriers.

Recently, the phenology of temperate perennial species that grow in cold climates have been strongly impacted by the constant increase in temperature. This scenario has conditioned these species to a situation that is growing even closer to those of subtropical climates. For example, an increase in temperature during the late autumn-winter has led to a reduction in accumulated chill in several production zones (Baldocchi and Wong, 2008; Luedeling et al., 2011). Whereas the increase in temperature during the early spring has resulted in the advancement of flowering dates (Guédon and Legave, 2008), an increase in risk of damage due to late frost (Gleizer et al., 2007), and desynchronization of pollination in species that depend on cross-pollination (Miller-Rushing et al., 2007; Petri and Leite, 2004) were also reported. During the summer, the elevation in temperature and hydric stress has increased the amount of damage caused to fruits (Legave, 2009).

The intensity of the effect of temperature on phenology of these species, however, depends on regional temperature changes (IPCC, 2007). Regional studies have examined historical trends in temperature, which in turn facilitates temperature predictions, their impacts on phenology and the development of adaptation strategies compatible for each area. In this way, studies have been performed on strategic agricultural production areas in Africa (Kruger and Sekele, 2013), Asia (Revadekar et al., 2013), South America (Skansi et al., 2013), Europe (Viola et al., 2013), and Oceania (Wang and Dickinson, 2013). Thus, according to the temperature scenario and future phenology events, the fruit production will be conditioned to adapt to changes in temperature using responses that are: (i) independent of the temporal dimension of warming, (ii) short-term, and (iii) long-term (Kurukulasuriya and Rosenthal, 2003).

In the first case, it is important that fruit growers discuss current experiences and use these experiences to develop simple adaptation strategies (NZCCP, 2001). The study of the potential impact of current and future warming on production, as performed by Warrick et al. (2001), may also help to make decisions. At the same time, government agencies should provide financial support to fruit-producing activities through fiscal incentives, financing, subsidies, and other measures, to maintain fruit growing activities and encouraging new investments. Agronomic support is essential for achieving an increase in productivity without incurring on extremely high costs. In the second case, an immediate response to warming may be achieved by adjusting crop management and diversifying the cultivated varieties or species (Kurukulasuriya and Rosenthal, 2003).

In the third case, the development of technologies capable of reducing the damage caused by the increase in temperature, such as the use of highly efficient irrigation systems, would constitute a plausible alternative (Kurukulasuriya and Rosenthal, 2003). On the other hand, future warming of high latitude regions, that are today suitable for producing temperate fruits, can be moved poleward or to higher altitude regions. However, the warming of regions bordering the poles would be decisive for the definitive replacement of temperate species by those of subtropical origin (Mendelsohn, 2000).

In contrast, the selection of varieties adapted to each region is an important tool for the adaptation of temperate fruit species to warming (Walthall et al., 2012). This alternative would place the migration of orchards as a secondary option, consequently minimizing any resulting socioeconomic impact. However, breeding programs in temperate regions today mainly considering fruit quality will have the warming as new challenge. Thus, the selection of new varieties would need to be supported by similar strategies adopted by breeding programs of temperate fruit trees adapted to subtropical climates. To accomplish this, several features, including the control of vigor, flowering, and fruit maturation, should be incorporated into the selection scheme (Topp and Sherman, 2000). However, the selection of varieties with a low chilling requirement for overcoming endodormancy (Topp and Sherman, 2000) would be the priority, as well as varieties with a high heat requirement for overcoming ecodormancy (Citadin et al., 2001). Furthermore, the new varieties should be resistant or tolerant to diseases and opportunistic pests, drought, and stress caused by increased temperature during the flowering and fruit development phases (Kurukulasuriya and Rosenthal, 2003).

However, the breeding of alternative varieties in itself would not be sustainable because, in perennial fruit species, the selection of a new cultivar takes at least 10 years (Walthall et al., 2012). Since warming is viewed as a moving target (Ceccarelli et al., 2013), the late selection of a new cultivar may no longer be useful. Therefore, breeding programs require additional tools other than those used in conventional selection practices for the selection of a new cultivar. For example, a molecular approach would be required to reduce the selection time among generations (Srinivasan et al., 2012). Solutions that are efficient, rapid, and reliable are also needed to quantify the chill requirement of new cultivars.

The increase in temperature due to climate change will lead to the selection of new varieties that show an adequate phenological response to the new temperature level (Hodge, 1991). Considering that phenological dynamics are determined by a complex interaction between the cultivars genetic traits and environmental factors (Ruml and Vulic, 2005), the development of phenological models that mainly relate to phenology and temperature is required. Understanding the chilling requirement of each selected cultivar and knowledge of the historical temperature and phenological events may facilitate in the development of phenological models based on a statistical-biological inference. These models can predict phenological events under various climatic conditions (Hodge, 1991). Annual phenological predictions may assist in agricultural management activities, whereas future predictions can determine the suitability of specific cultivars to potential growing areas. Thus, it is possible to determine whether a cultivar can be cultivated in a predicted temperature condition (Hodge, 1991).

While tropical species will respond spatially to climate change, particularly by temperature changes, temperate species will respond temporally, advancing or delaying phenology (Pau et al., 2011). Understanding the magnitude of temporal variations in temperate species will only be possible through the use of phenological models, which can also be employed as tools for breeding programs that are focused on the selection of varieties adapted to warming. To achieve a satisfactory prediction of phenology, the parameterization must be based on large variability of data found in long series of temperature and phenology (Zhao et al., 2013).

Review

Recently, several international climate studies has reported climate changes patterns for several fruit production regions around the world. The temperature change is often referred on these studies. They show regional differences in temperature rising according to contrasting latitudes in the continents of both hemispheres. According to IPCC (2007) the increase of global average air temperature in the last decades is accompanied by the highest rate of increase recorded in the last 11th century. Results of climate researches assessing the 1979-2005 period have clearly pointed a rise of the mean annual temperature of 0,27°C decade^{-1} for global surface, 0,33°C decade^{-1} in the Northern Hemisphere and 0,13°C decade^{-1} in the Southern Hemisphere (IPCC, 2007). In a large-scale climate evaluation it was found a significant rise of 0,42°C decade^{-1} in Europe, 0,28°C decade^{-1} in North America, 0,28°C decade^{-1} in Africa, 0,28°C decade^{-1} in Asia, 0,12°C decade^{-1} in South America and a not significant rise of 0,05°C decade^{-1} in Australia, considering the 1977-2001 period (Jones and Moberg, 2003).

The marked increase in worldwide air temperature has been particularly the recorded at the end of the 80's, but this may be variable depending on the region and season assessed (IPCC, 2007). Northwestern North America, interior of Asia and Southeastern Brazil are the main areas where the annual mean temperature was warmest (IPCC, 2007). For instance, the Western North America, Northern Europe and China showed a clear warming in the winter, Europe and Northern/Eastern Asia in the spring, the Europe and Northern Africa in the summer and Northern North America and Eastern Asia during autumn (IPCC, 2007).

A greater part of climate research, internationally published, focus on the Northern hemisphere regions where the numbers of long-series temperatures is greater. In the North America some studies have identified changes in winter and spring temperature both time and space (Jones and Moberg, 2003). These changes include the reduction of winter period and the increase of maximum and minimum temperature during spring at some southern areas of the United States (Zhang et al., 2007). Furthermore, the temperatures rise indicates a strong warming in the winter (Schwartz et al., 2006) and spring season after the 70th decade, principally in the Western North America (Cordero et al., 2011).

However, the warming in Europe started later than in North America, being widely pronounced in the last three decades (Rebetez and Reinhard, 2007;Guédon and Legave, 2008; Rutishauser et al., 2009; Viola et al., 2013), with spring and summer being the most affected seasons. In France, many sites presented a sudden change of annual mean temperature trend since the 1988 (Guédon and Legave, 2008). Similarly to Northern America the marked increase of mean temperatures in Western Europe occurred predominantly in the spring season (Menzel et al., 2006; Rebetez and Reinhard, 2007; Guédon and Legave, 2008;Gordo and Sanz, 2010) and in the summer (Rebetez and Reinhard, 2007) whereas the winter seems not to be getting warmer (Schwartz et al., 2006). The equivalent increasing on the maximum and minimum temperature rates were found on a study undertaken at Catalonia region, in Spain. The summer and spring season were both more warmed in this region (Martínez et al., 2010). However, we can find diverse patterns of climate on low latitudes of Europe. Southern Italy showed an expressive increasing trend of medium and maximum temperature in all seasons, notably for winter (Viola et al., 2013). Northern Africa is another important zone where the warming has been reported and it stimulated research mainly towards crop adaptations (CIHEAM, 2010). However, there are a few research results published in international journals, reporting the extension of climate changes.

Nevertheless, when we take into account a large geographic zone distribution we can find different patterns of climate changes. An overview of temperature trends in Western, Eastern and Northern Asia may reveal this. While in Japan and Northern/Eastern Asia experienced a warming in the spring accompanied by an increase of average temperature of whole months (Doi and Katano, 2008; Ma and Zhou, 2012) the annual mean temperature in Western Asia increased on both winter and spring seasons, since 80’s (Yu et al., 2010).

On the other hand, Southern hemisphere shows a limited number of climate and phenological studies. The lack of long-series of data is the main reason which prevents these studies in Southern hemisphere (Rumpff et al., 2010). This region presents naturally a less inter-annual variability of temperature and seasonal contrast than Northern hemisphere, possibly due a greater ocean fraction (Jones and Moberg, 2003). Nevertheless, recent research has demonstrated a significant climate change in the Southern hemisphere principally in Southern Brazil, Southern Africa and

Australia. In Southern Brazil (which includes Paraná, Santa Catarina and Rio Grande do Sul states) the climate is mildest than the majority temperate fruit crop areas showing normally the elevated annual mean temperatures. Recent research has indicated climate change wherein the minimum temperature is pointed as mainly climate variable changed in this zone. In addition, there is a stronger evidence of minimum temperature rise after the 90th decade, substantially during the winter (JJA) (Marengo and Camargo, 2008) and in the autumn season (MAM) (Sansigolo and Kayano, 2010). The intense warming registered in the recent decades in Southern Brazil may be linked to *El Niño* phenomenon. According to Marengo and Camargo (2008) the five years greatest warmed in this region between 1960 and 2002 occurred at the same time of more intense *El Niño* activity. But, despite to be evident the minimum temperature rise in Southern Brazil the rise rate seems to be variable according to the land surface size analyzed and the way how climate data are assessed.

The Southern Africa has also recorded a significant warming in their temperate fruit zone production. Southwestern Cape region suffered a warming of 2ºC between 1973 and 2009 driven substantially by the minimum temperature increase during the early spring (Grab and Craparo, 2011). This warming is greater than those measured at global scale. Similarly, the Southeastern Australian region has been warmed substantially by the increase of annual mean maximum temperature mainly (Murphy and Timbal, 2008). They also verified that warming has been occurred in all seasons except in the autumn which has experienced a cooling driven substantially by *El Niño* phenomenon.

This deep understanding of long-term temperature change, especially in the annual and seasonal context is essential to analyze the behavior of plant phenology. Recently, the stronger evidence of warming in temperate zones has been firstly identified by phenology studies (Schwartz and Hanes, 2010). Therefore, the temperature is the most important factor which influences the phenological phases in fruit trees (Chmielewski et al., 2004). Hence, the air temperature change is often associated with phenology changes in several plant species, as reported in many studies considering perennial plants (Doi and Katano, 2008; Gordo and Sanz, 2010). The synthesis of some of these works has suggested trend differences between cropping areas of Northern hemisphere and areas of Southern hemisphere. These studies have pointed an agreement which many species advanced the spring phenology events (budburst and blooming dates) particularly. According Yu et al. (2010), the large amount of phenology advancements so far registered by research is strongest associated with rising spring temperature than winter warming. This important remark may be coupled with different phenological responses of plants to global warming characterized by Cook et al. (2012) which found around 72-73% of studied plant species categorized as ‘spring-only temperature responders’.

A trend toward blooming early has been reported by several current studies focusing forest and fruit tree species in Northern America (Abu-asab et al., 2001), Europe (Guédon and Legave, 2008; Gordo and Sanz, 2010, 2009) Southern Africa (Grab and Craparo, 2011) and Asia (Primack et al., 2009). In addition, this general synthesis demonstrates be variable according to the species and the region studied (Guédon and Legave, 2008). Situation of advance and delay of phenological events by temperature increase in spring was found according the bud type analyzed (Pope et al., 2013). These authors not consider the advancement of spring phenology as absolute fact in all cases. They also conclude that phenological response to temperature increase may be variable according to the geographic region, the specie and bud type assessed. The consequences of short and long term of blooming date advance in spring may include more vulnerability to frost damage, production irregularity (Domergue et al., 2004) and decrease of fruit quality. Further, the temperature change can effect different varieties of perennial plants with cross-polarization cultivated in the same site by diverging their blooming time (desynchronization) (to search a reference) ensuring the partial or incomplete pollination. These consequences on phenology could not ensure the continuity of economic production of perennial cultures in the future.

The surface land taken account for climate study is an important point to consider when we should study the relationship between temperature and any physiological plant variable. Such climate studies have been developed by considering very large geographical areas (Gordo and Sanz, 2010; Schwartz and Hanes, 2010) where they report this relationship variables by averaging each of these events. However, plant phenology will be greater related to climate variables recorded near the cropping areas of studied plants where the weather has the factual effect on phenology (Schwartz et al., 2006). Moreover, blooming date and blooming duration are two main traits involved in the adaptation of temperate fruit tree species to mild climate regions (Raseira, 2006). Thus, these same traits should preferentially be considered by breeding programs to adapt temperate fruit trees to temperature increase or by studies conducted with this purpose.

The understanding of the dormancy phenomenon and its relationship with temperature is also essential to develop new adapted cultivars to warming. Dormancy in plants from temperate climates has different phases that vary with the species and location of cultivation. Lang et al. (1987) proposed a conceptual standardization of these phases: paradormancy, endodormancy and ecodormancy. Paradormancy is regulated by intrinsic physiological factors of the plant. In this phase, budburst is inhibited by the hormonal action of the leaves and surrounding apical buds. At the end-autumn, there is a decline in physiological activities due to the reduction of the photoperiod and the onset of cold period, which induces the loss of leaves, indicating a transition to another phase: endodormancy (Liew, 2013). During endodormancy, the level of inhibitory agents is elevated, and buds do not develop, even under favorable environmental conditions (Marini, 2009). This mechanism benefits plant survival at low temperatures (Yamane et al., 2008). However, endodormancy is gradually overcome as the chill removes the physiological blocks that inhibit budding and flowering (Dennis, 2003). In the third phase, ecodormancy, the reduced levels of inhibitors allow flowering if environmental conditions are favorable at the end-winter and beginning of spring (Marini, 2009).

During dormancy, various cytological, molecular and metabolic events occur simultaneously, and the investigation of which can aid in a better comprehension of the phenomenon. Qualitative and/or quantitative tests can be performed that permit the verification of the state and/or depth of dormancy of the buds (Mauget, 1987). Recently, various studies have been conducted to determine gene expression (Olukolu et al., 2009; Jiménez et al., 2010;Celton et al., 2011), hormonal action (Horvath et al., 2003) and carbohydrate kinetics (Bonhomme et al., 2005; Leite et al., 2006; Marafon et al., 2011) during dormancy. These tests require specialized knowledge and specific equipment, hindering their application as a tool for the selection of genotypes in breeding programs. Thus, the use of tests with more practical applications permits to study the transition between dormancy phases, the length between then and the variation among different climate conditions.

The study of dormancy in the field is limited by numerous factors that act on trees (Dennis, 2003). Thus, the majority of these studies are performed in a controlled environment, primarily considering temperature; however, these studies show a large variation in methodology. The use of potted trees is difficult due to the space necessary to accommodate adult trees in a greenhouse, being also hard to repeat for several years (Dennis, 2003). Due to these limitations, researchers have opted to use parts of trees structures, such as single bud cuttings and branches removed from plants in the field.

The one-bud cutting test (Pouget, 1963) is normally used for the study of vegetative buds. Their application in apple trees revealed that the lateral vegetative buds of long shoots have different chill requirements (Crabbé, 1968). Additionally, there is evidence that the cuts performed to prepare the cuttings promote budburst due to the production of ethylene (Williams et al., 1979). Additionally, the elimination of a large portion of associated inhibitors contributes to higher and more rapid budburst rates when compared to other tests (Hawerroth et al., 2010). According to Saure (1985), the budburst rate of 50% in temperate climate species can be used as indicator of endodormancy release. However, the numerous characteristics that determine the budburst in this test limit the use of the results for the physiological study of dormancy and to determine the endodormancy release (Dennis, 2003). In the same manner, Champagnat (1983) does not recommend this test for determining chill requirements. However, one-bud cutting is the only test able to reveal the dormancy kinetics and indicate the dormancy depth (Balandier, 1992) by measuring the percentage of budburst, the time to reach a certain phenological stage (Saure, 1985) and the speed of budding (Dennis, 2003). Therefore, this test can be used for the practical study of dormancy (Dennis, 2003), such as in comparative studies among cultivars or among distinct regions. This test has been successfully applied in apple trees cultivated in the subtropical region of Brazil (Putti et al., 2003; Carvalho and Zanette, 2004).

Also, the cut shoots technique has been used in many species (Weinberger, 1950;Richardson et al., 1974; Werner et al., 1988; Hauagge and Cummins, 1991; Herter et al., 1993; Citadin et al., 2002; Valentini et al., 2004; Ruiz et al., 2007; Dantec et al., 2014), mainly to evaluate floral buds. The comparison of cut shoots between adult and juvenile trees of many forest species during dormancy highlighted the existence of important phenological divergences (Vitasse and Basler, 2014). Thus, those authors suggest studying the chill requirements in these species using cut shoots from adult trees since juvenile shoots do not present accurate phenology. However, the use of cut shoots in peach trees revealed several difficulties during forcing, such as bud dropping and low conservation of shoot, preventing an accurate understanding of the real chill requirements in some cultivars (Citadin et al., 2002).

In addition to these limitations, the cut shoots method varies enormously within the same species, such the temperature exposure of shoots during forcing, for example. Although forcing temperature is often omitted (Weinberger, 1950), it was 18-24°C in peach trees (Richardson et al., 1974), 19-21°C (Citadin et al., 2002) and 24-26°C (Ruiz et al., 2007). In apple trees, 11-15°C (Landsberg, 1974), 18-21°C (Shaltout and Unrath, 1983), 18-20°C, 22-24°C (Hauagge and Cummins, 1991) and 25°C (Putti et al., 2000) were used as forcing temperatures. In deciduous forest tree species, the forcing temperature used was 25°C (Dantec et al., 2014).

The flowering threshold is another parameter that varies distinctly in this test, which is used as an indicator of dormancy release (budbreak). In apple trees, flowering above 80% was considered the threshold (Shaltout and Unrath, 1983), and in some cases, the full flowering state was not characterized (Landsberg, 1974). In peach trees, the threshold adopted was 50% of open flowers (Richardson et al., 1974), while a threshold of 25% was satisfactory for Citadin et al., (2002). In a similar study, budbreak in apricot trees was considered when 10 days after forcing, 30% of the flowers were between 51 and 55 stage of BBCH, and floral buds had 30% higher weight than at the previous sampling date (Ruiz et al., 2007). In apricot and peach trees, the threshold adopted was 70% of the shoots with 50% of the flowers opened at 65 stage of BBCH (Valentini et al., 2004).

Although many studies with peach trees consider 50% budburst or flowering as the threshold indicative of endodormancy release, this value may not be a good reference, especially when there are problems with shoot conservation (Citadin et al., 2002). In the same manner, budburst followed by development stagnation due to rosette formation and the opening of flowers poorly developed may not be good indicators of endodormancy release. Thus, in the case of cut shoots, there is a need to evaluate the vigor of the buds and the functionality of the floral structures opened, which are rarely reported (Erez, 1999). However, there are further methodological divergences, such as the mode of conservation of the shoots and the relative humidity and photoperiod during forcing. In many cases, these parameters are ignored or not cited in the studies.

These methodological divergences limit the understanding the length of dormancy phases, which suggests, therefore, a need to standardize the shoot cut method. The main characteristics to be standardized are the collection of branches formed in the previous year, of similar vigor, positioned in the same portion of the tree (Dennis, 2003), specific forcing temperatures for each species and the budburst and/or flowering threshold as indicator of endodormancy release must be adopted for each species.

Another method that permits the evaluation of endodormancy release is the evolution of floral primordia, without forcing (in field conditions) and with forcing (controlled forcing conditions). This test is free from methodological difficulties because it considers in advance the physiological reactions of the floral primordia. The test has been successfully carried out in apricot (Tabuenca, 1964; Legave et al., 2010; Andreini et al., 2014), peach, pear and plum trees (Tabuenca, 1964, 1967). However, the use in apple trees has not been reported in the literature.

Accurate information regarding the dormancy kinetics and the length of each phase has significant practical and economic impacts on the maintenance, production (Fennell, 1999) and management of cultivated deciduous species (Viti et al., 2010). This understanding allows us to calculate the chill requirement to overcome endodormancy and consequently to indicate appropriate cultivars for distinct production locations. This knowledge allows for the differentiation of the physiological behavior of each cultivar being this essential to support the application of chemical treatments to overcome endodormancy (Dennis, 2003) in mild winter regions, in which the chill requirement is partially done. There are two mean limitations when quantifying the chill requirements for endodormancy release (ER): the de-standardization of biological tests and the method for developing the models used to quantify chill.

The mechanisms involved in the physiological control of buds during dormancy are still not understood well. Therefore, a wide variety of methods have been established for the development of phenological models capable of predicting budburst/flowering and exploring variations between species and regions (Fu et al., 2012). Such models constitute an important tool for the investigation of global climate change and its effects on phenology (Zhao et al., 2013). In general, all phenological models are based on three key points: drivers, method of approach and concept model (Cook et al., 2010) (Figure 1).

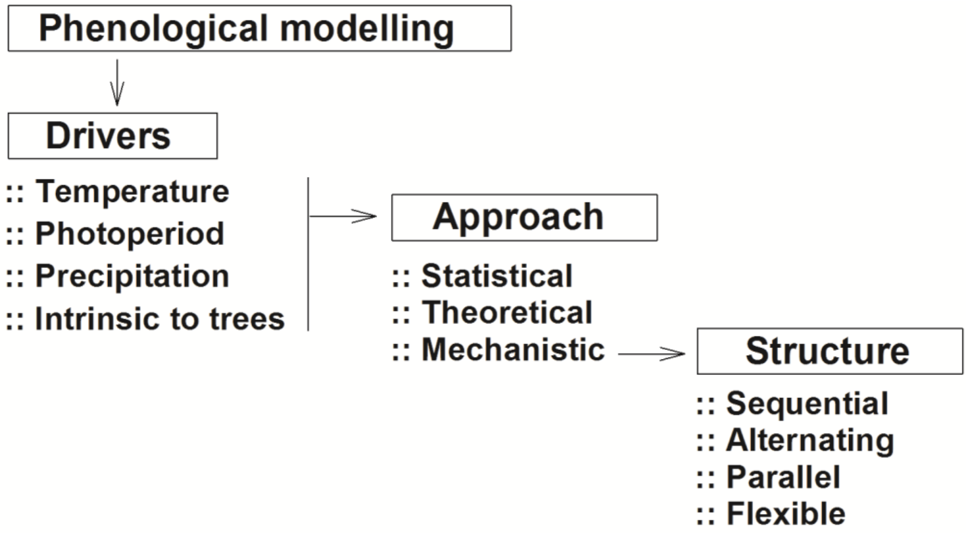

Figure 1. The three categories of phenological models and their subdivisions.

These models reveal plant development as a function of one or more environmental factors or other factor(s) intrinsic to plants. Temperature is considered the main factor that determines plant development (Müller and Braun, 2008), whereas photoperiod is less studied but essential for modeling (Hanninen, 1995; White and Nemani, 2003; Fu et al., 2012). These two factors have an important role in the development of many plant species. Light is also considered an important factor for plant regulation (Cardoso, 2011). These environmental factors are almost always studied separately and used in the same way to parameterize the models, which generates models with low complexity and robustness (Müller and Braun, 2008).

Models are mostly developed for one species or for more than one species belonging to the same biome (Cleland et al., 2007). A majority of the models consider that a species from a temperate climate requires a chill period followed by a warming period to initiate normal development. The development rates of trees are commonly associated with temperature, in each phase, and the end of each phase occurs since accumulation of chill and heat units (Zhao et al., 2013). Also, the chill and heat requirements vary between different cultivars (Müller and Braun, 2008). The simplest concept is based on developing model that expresses the effect of a given factor on a phenological change. Several phenological models have been developed over time, but there is still no consensus on the accurate prediction of phenology in different species (Fu et al., 2012). Three concepts are considered when developing phenological models: statistical, theoretical and mechanistic (Zhao et al., 2013).

Theoretical models predict phenological events from estimated factors, including hormonal balance, temperature, rainfall (Zhao et al., 2013), or plant carbon balance (Caldararu et al., 2014). This information is generally obtained through automated monitoring (for factors intrinsic to the plant) and remote sensing for environmental and phenological factors (Zhang et al., 2007), which allows for the large-scale visualization of phenology and its controlling factors. The main advantages of this type of model are that they consider phenological variations in populations or species and predictions at the global level. The disadvantages include the variation in phenology predictions between regions and limited data series for parameterization and validate models (Zhao et al., 2013). Thus, the development of models with wide applicability should therefore be improved.

Statistical (or empirical) models are also known as one-phase models (Chuine et al., 2014) and are based on mathematical functions that correlate an environmental factor or intrinsic factor to the plant with physiological changes of interest (Zhao et al., 2013). These models relate phenology events with different environmental factors without considering biological processes as a whole. Many of these models assume a simple linear correlation between a physiological event and an environmental factor, and their parameters are usually obtained through the use of different statistical fitting methods (Zhao et al., 2013). Examples of this type of model are the Growing Degree Hours - GDH (Richardson et al., 1975) and Thermal Time (Cannel and Smith, 1983), which describe the development onset in spring by considering the heat accumulation from a fixed calendar date, assuming that the chill requirement has been satisfied (Linkosalo et al., 2008). Therefore, above a certain threshold of heat accumulation, the plants bud/flower. This type of model were also developed to estimate the dates of grapevine flowering and *veraison* (Parker et al., 2011) (similar to GDH), apple tree flowering (Schröder et al., 2014) (based on linear regressions) and cherry tree flowering and fruit growth (Zavalloni et al., 2006) (similar to GDH). The advantages of this type of model are the simplified calculations, and the disadvantaged are the dependence on the correct selection of phenological events and of controlling factors (Zhao et al., 2013). For a large proportion of typically cold regions, the statistical models predict accurately the date of budding/flowering. However, the intensification of global warming and the decrease of chill accumulation in winter, these models may no longer be accurate (Chuine et al., 2014). These models were found not to be efficient in regions with a subtropical climate, when applied to the same species for which they were developed.

Mechanistic models describe cause and effect relationships and are based on an understanding of the physiology of phenological events and their relationship with environmental factors (Zhao et al., 2013). The parameters used to develop these models, such as budding or flowering dates, can be directly obtained through field observations. An understanding of interactions between environmental factors and phenology is important for modeling optimization (Hayhoe et al., 2007). Mechanistic models incorporate well such characteristics, resulting in more accurate phenological predictions. The two main phenological changes considered in mechanistic models are endodormancy release (ER) and budding/flowering dates (Müller and Braun, 2008). However, although mechanistic models have rapidly proliferated for a variety of different species, their development is limited by the difficulty in determining the end of endodormancy (Zhao et al., 2013) and the onset date of chill effect (Müller and Braun, 2008).

The complexity of mechanistic models is composed of three different assumptions, which should always be considered during modeling: (i) type of phenological response to the environmental factor, (ii) dependence of chill and/or heating effects, and (iii) the time window during which the vegetative and/or floral buds are receptive to the effect of chill and/or heat (Chuine, 2000). For this last assumption, the rates of plant development during endodormancy and ecodormancy can be interpreted using alternating, parallel, sequential (Chuine, 2000; Linkosalo et al., 2008), and flexible (Fu et al., 2012) models (Figure 2) concepts. These models, which are also known as two-phase models, consider the effects of temperature on endodormancy and ecodormancy phases (Chuine et al., 2014).

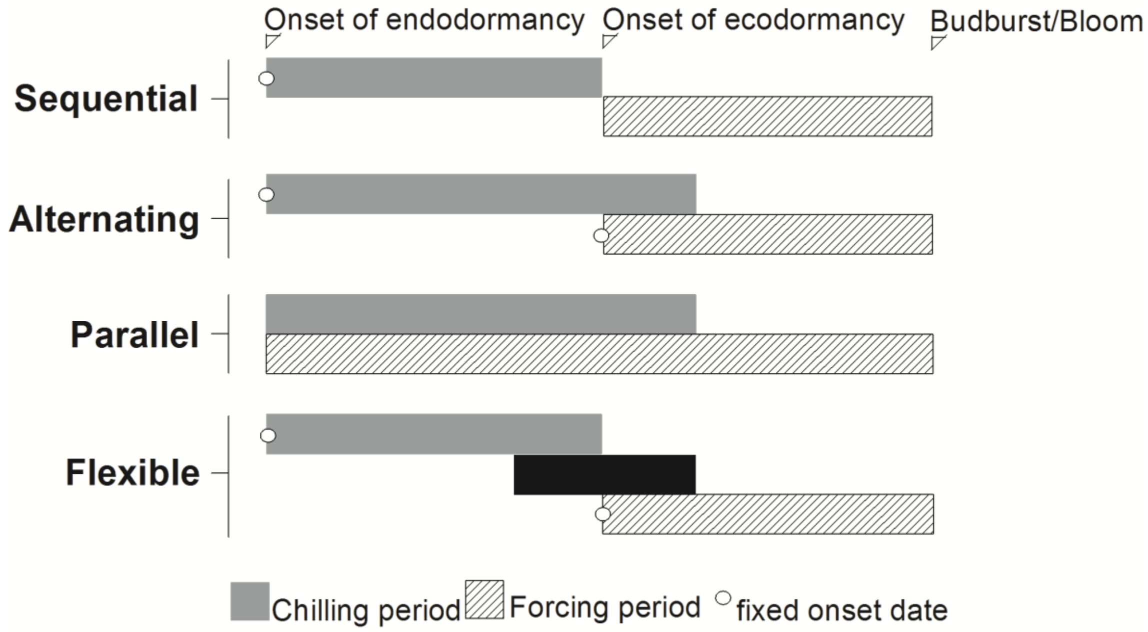

Figure 2. Four hypotheses about the combined effects of "chilling temperatures" and "forcing temperatures" on the development of floral and vegetative buds. Adapted Chuine (2000)and Fu et al. (2012).

The sequential models assume that when the chill requirement is done endodormancy is released, predisposing plants to develop when environmental conditions become favorable (ecodormancy) (Hanninen, 1995). The model therefore assumes that if the required chill accumulation is not done, budburst/flowering will not occur (Linkosalo et al., 2008). However, this is not always true in mild winters because sometimes flowering occur in the field (despite poor quality and low rates) even the models indicate no sufficient chill to overcome endodormancy. Sequential models were developed by Hanninen (1987) and Legave et al. (2013).

Fu et al. (2012) using phenological and temperature data recorded for a cold region, observed that the sequential model was the mechanistic model with highest probability of accurately predicting budding. In future conditions under climate warming, chill requirements may not be met within the currently expected time period. Therefore, the sequential model would be more appropriate for predicting phenological changes because it considers the effect of chill (Fu et al., 2012). This consideration would make sequential models more precise than statistical models, which only consider the effect of heat.

Certain models (Weinberger, 1950; Richardson et al., 1974; Shaltout and Unrath, 1983; Fishman et al., 1987; Linsley-Noakes et al., 1994) developed using mathematical fitting and biological tests estimate only the effect of chill period. These models ponder temperature considering their efficiency to induce budburst or flowering. In this case, these models can only estimate budburst/flowering when associated with one-phase statistical models, such as growing degree hours (GDH). This association of models can also be classified as sequential modeling, and assume a triangular responses to the effect of chill and a sigmoidal, linear (Fu et al., 2012) or exponential (Legave et al., 2013) response to the effect of heat.

The alternating models (Cannel and Smith, 1983; Murray et al., 1989; Kramer, 1994) are similar to statistical because they assume a pre-established onset date to chill and heat accumulation. However, unlike the other model concepts, the satisfaction of the chill requirement of a given species or cultivar may extend beyond the onset date of heat accumulation, resulting in overlapping effects. Usually, alternating models assume a binary effect of chill and a linear effect of heating (Fu et al., 2012).

Parallel models reflect the simultaneous effect of chill and heat on plant development during endodormancy. In this model, the threshold for budburst/flowering is not defined, as it varies greatly due to climate changes in the field, the period in which they occur, and the total chill and heat accumulation (Linkosalo et al., 2008). Thus, theoretically, when there is no chill during winter heat will be responsible for ontogenic developments, and budburst/flowering can occur even chill accumulation has been low. Parallel models have been developed by Landsberg (1974), Hanninen (1990), and Kramer (1994). These models assume a triangular response to the chill effect and a linear response to the heat effect (Fu et al., 2012).

Flexible models (Chuine et al., 2000) are more standardized (Müller and Braun, 2008) because they combine sequential and parallel concepts in a single model. Similarly as sequential model, the duration of the endodormancy period is calculated from a fixed date (as in the sequential model), and measurements of heat accumulation start when the endodormancy period ends. However, this hybrid model has an additional structure that allows a soft transition between chill and forcing phases (Linkosalo et al., 2008).

However, although most of the referenced models were built using reasonable historical temperature series, their application is still restricted to the location for which they were developed (Müller and Braun, 2008). For example, the performance of chill models developed for cold regions by Richardson et al. (1974) and Weinberger (1950) was not satisfactory for subtropical regions such as South Africa (Allan et al., 1993).

Similarly, the application of these models and the North Carolina model (Shaltout and Unrath, 1983) to the subtropical region of Brazil showed disproportionate annual fluctuations in chill accumulation (Table 1).

Table 1. Annual chill accumulation (from 1 April to 31 July) according to four models quantifying chill accumulation in Caçador, Brazil, between 2003 and 2006.

Year	Model			
	Chill hour	Chill unit		
	Weinberger (1950)	Richardson (1974)	Shaltout and Unrath (1983)	Landsberg (1974)
2003	355	126	234	1451
2004	286 V	64 V	609 Λ	1427 V
2005	256 V	0 V	48 V	1336 V
2006	238 V	45 Λ	97 Λ	1386 Λ

Λ and V: increase and decrease of chill accumulation in relation to the previous year, respectively.

Therefore, a given variety in the same year can exhibit differences in the duration of the endodormancy phase depending on the model used. In addition, Chuine (2000) observed that flexible model exhibit good regional performance but resulted in low accuracy for predicting phenology when tested with climate and phenological data over a wider geographical area (Müller and Braun, 2008).

Understanding the effects of temperature on dormancy kinetics and the future impacts of climate on phenology are still limited because there are few phenological models valid over a wide region (Müller and Braun, 2008). When phenological models are selected and applied on macro regions, they can provide precise information on the relationship between the environment and physiological processes (Richardson et al., 2013) and provide an understanding of how plant phenology responds to climate variability.

In future scenarios of climate warming, determining the phenological pattern may still depend on multiple factors, including photoperiod, and not only on the temperature. Today, the action of multiple factors on phenology is not fully understood, and the development of multifactorial models is still restricted (Müller and Braun, 2008). The future accuracy of multifactor models will be dependent of the corrected composition of factors (Hanninen, 1995) and the efficient tools to integrate multiple effects of factors on phenology.

CHAPTER I

Long-term insight into temperature regimes in climate-contrasting apple regions of both hemispheres to understand blooming divergences at world scale

Abstract –The present study was an initial attempt to decipher divergent regional patterns and determinisms of apple blooming traits by using spatially extensive and long-term phenological data. We aimed to highlight future regional vulnerabilities facing continuous warming through large-scale comparisons of blooming phenology in suitable and unsuitable regions for apple cropping. This was a complementary approach of the abundant phenological predictions by modeling. A large database was therefore collected including observation sites in five European temperate regions (suitable) and four mild regions, one in Morocco and three in Southern Brazil (unsuitable). Dates of three blooming stages for three cultivars were analyzed over the last four decades. Two traits were considered from these dates, (i) the blooming time and (ii) the blooming duration. The analysis at world scale supported the independence of these two traits. More complex determinisms are suggested for the blooming duration. A heat control of the blooming time was shown in Europe versus a chill-control in Brazil. An opposing determinism was also suggested for the blooming duration. Thus, marked spring warming in Europe resulted in blooming advances and short durations. By contrast, weak warming in the Brazilian sites resulted in weak phenological changes associated with extended blooming durations. Intermediate temperature and phenological patterns were found in the mild conditions of Morocco. In this context, facing continuous warming, the vulnerability of the French Mediterranean region in near future was outlined through significant changes in blooming patterns as new trends toward extended blooming.

Keywords: Warming vulnerability, Phenology, Regional variability, Fruit trees, Chilling, Heating.

1.1. Hypothesis

The spatiotemporal analyses of temperature and phenology can generate evidences on the determinism of floral phenology. In addition, the comparison of the historical patterns, temperature and phenology trends in Western Europe (cold) with those recorded in the subtropical region of Brazil and mediterranean region of Morocco (warm) could indicate the vulnerability of apple production to temperature warming.

1.2. Objectives

We aimed to identify differences in temporal trends and the regional variability of temperature and blooming dates of many cropping areas in the Southern and Northern hemisphere and their relations. A comprehensive understanding of climate changes on phenology events can be facilitated by using apple tree because this is a species globally cultivated. In this way, we proposed to compare long-term trends of temperature rising during the dormant and blooming phases of apple tree cultivars between cropping areas of both hemispheres and between contrasting cropping areas within each hemisphere.

Furthermore, we focused to compare long-term trends of blooming date and duration between the same cropping areas of both hemispheres and to assess the relationships between the temperature and the blooming trends. Finally, we seek an overview of the spatial and temporal variability of blooming phenology in apple tree at world scale and the binding with temperature changes.

1.3. Materials and methods

1.3.1. Spatial network and temperature data

Table 2 shows a synthetic characterization of the geographical and temperature contrasts within the studied regions. The spatial coverage of apple cropping regions was extensive, extending from latitudes 50ºN to 32ºS, while the altitude of the sites ranged from around 35 m to 1350 m. In the temperate area of Western Europe we collected daily temperatures at five sites suitable for apple cropping which were representative of different temperate climates (continental, oceanic and Mediterranean).

Table 2. Description of the sites and their respective temperature database.

Site characteristics					Temperature data		
Region / Site (country)	Institute	Latitude / Longitude	Altitude (m)	Climate area/ Climate Type	AAT (C°)[a] Max.	 Min.	Collected period
Western Europe				Temperate			
Bonn (Germany)	Inres	50°37’N 6°59’E	179	Continental	14,1	5,6	1958/12
Angers (France)	Inra	47°28’N 0°31’W	38	Oceanic	16,5	7,6	1963/12
Conthey (Switzerland)	Agroscope	46°13’N 7°22’E	504	Continental	15,9	4,7	1958/12
Forli (Italy)	Cra-Frf	44°13’N 12°1’E	34	Mediterranean	18,3	11,1	1966/12
Nîmes (France)	Ctifl	43°45’N 4°27’E	52	Mediterranean	20,0	9,4	1966/12
Northern Africa				Temperate			
Meknès (Morocco)	Inra	33°57’N 5°33’W	499	Mediterranean	23,7	11,4	1972/12
Southern Brazil				Subtropical			
Caçador (SC)	Epagri	26°47’S 51°01’W	960	Continental	22,8	11,5	1961/12
São Joaquim (SC)	Epagri	28°29’S 49°93’W	1353	Oceanic	18,8	9,6	1955/12
Pelotas (RS)	Embrapa	32°42’S 52°24’W	57	Oceanic	23,2	14,0	1961/12

[a] AAT: Annual Average Temperature during the 1973-2012 period.

The annual averages of maximum and minimum temperatures (Tmax and Tmin) varied from around 14°C to 20°C for Tmax and from around 6°C to 9°C for Tmin. The continental sites at Bonn in Germany and Conthey in Switzerland had the coldest temperatures; in contrast, the Mediterranean sites at Forlì in Italy and Nîmes in France had the highest temperatures. While the oceanic site at Angers (France) showed intermediate temperatures. Similarly, we collected daily temperatures at four sites unsuitable to apple cropping, located in different mild areas: one NH site in Northern Africa (Morocco) and three SH sites in Southern Brazil. The annual averages of Tmax and Tmin varied from 19°C to 24°C for Tmax and from 10°C to 14°C for Tmin, i.e. clearly higher temperature ranges compared to those of European sites (for Tmin particularly). The Mediterranean site of Meknès in Morocco showed clearly higher temperatures than those of two European Mediterranean sites. The three Brazilian sites were representative of three different subtropical conditions: although located at the highest latitude, the oceanic site of Pelotas had the mildest temperatures and was the most unsuitable site for apple cropping. The mountainous site of São Joaquim located at high altitude was characterized by less mild conditions, while the site of Caçador showed intermediate mild temperatures. For all nine sites the daily temperatures were collected from weather stations close to the orchards where the phenological data had been recorded. All collected temperature series were checked to ensure that no questionable data were used. In addition, in the case of the Nîmes site, which was the warmest site in Europe during the dormancy and blooming periods, we used temperature values generated by the ARPEGE climatic model (Météo-France CNRM/GMEC) for experiment SCRATCH08 on an 8 km grid (http://www.drias-climat.fr/). We extracted predicted daily Tmax and Tmin through the A1B scenario of the SRES family 1 (IPCC, 2013) from 2013 to 2100 on the grid of Nîmes area.

1.3.2. Phenological data

Blooming dates were accurately collected through assessments, conducted by experienced observers on adult trees growing in long-term orchards. In order to establish the longest blooming series possible, the collection of historical data focused on three main cultivars: Golden Delicious (GD) which is ubiquitously grown or experienced in apple cropping regions; Gala and Fuji which are mainly grown in Southern Brazil but are also grown in European regions. Thus, we gathered data for GD at all nine sites, for Gala at five and for Fuji at four always including European and Brazilian sites for each variety (Table 3). All collected series were checked to ensure that no questionable data were used. No artificial breaking of dormancy was made in the mild sites (Meknès and the three Brazilian sites). The data consisted of assessed dates for three blooming stages: early bloom (approximately 10% of flowers opened), full bloom (at least 50% of flowers opened) and bloom completion (most of petals fallen). These correspond to stages 61, 65 and 67 respectively in the international BBCH code (Meier, 2001). The longest series were collected for the European sites, particularly at Bonn (56 years for the dates of stages 61 and 65) and Angers (51 years for the date of stage 61), while shorter series were collected at the mild sites. In total 47 series were collected to establish the spatial and temporal variability of blooming time (Table 3). Most of these series were also used to estimate the variability of blooming duration (BD) through annual number of days between stages 61 and 65 (BD 61-65) and between stages 61 and 67 (BD 61-67).

Table 3. Description of phenological stages of apple cultivars, and the period that where observed in Western Europe, Northern Africa and Southern Brazil.

World region/ Site (country)	Cultivar	Stage[a]	Observation Period
Western Europe			
Bonn (Germany)	Golden Delicious	61,65	1958/13
Angers (France)	Golden Delicious	61	1963/13
Conthey (Switzerland)	Gala	65	1975/13
	Golden Delicious	65	1970/13
Forli (Italy)	Golden Delicious	61,65,67	1970/13
Nîmes (France)	Gala	61,65,67	1979/13
	Golden Delicious	61, 65, 67	1975/13
	Fuji	61, 65, 67	1980/13
Northern Africa			
Meknès (Morocco)	Golden Delicious	61,65,67	1984/13
Southern Brazil			
Caçador (SC)	Gala	61, 65, 67	1982/13
	Golden Delicious	61, 65, 67	1984/13
	Fuji	61, 65, 67	1982/13
São Joaquim (SC)	Gala	61, 65, 67	1972/13
	Golden Delicious	61, 65, 67	1972/13
	Fuji	61, 65, 67	1976/13
Pelotas (RS)	Gala	61, 65, 67	1983/88
	Golden Delicious	61, 65, 67	1983/88
	Fuji	61, 65, 67	1983/88

[a] 61, 65 and 67 are respectively stages of early, full and complete bloom in the international BBCH code (Meier, 2001).

1.3.3. Calculation of chill and heat accumulation

In order to provide additional data for characterizing the regional differences in temperature and for analyzing the temperature determinisms of blooming traits, we also calculated annual accumulated chill and heat units at all sites. We based these calculations on the usual assumption that chill and heat temperatures have successive effects on the dormancy break and post-dormancy growth that leads to bud break and blooming (Richardson et al., 1974; Rea and Eccel, 2006). A Chill Accumulation period (CAp) and a subsequent Heat Accumulation period (HAp) were defined on the basis of previous results (Legave et al., 2013). In the NH sites, CAp included the months of October, November, December (year *n-1*) and January (year *n*), while HAp included February, March and April of year n. In the SH sites, CAp included the months of April, May, June and July (year *n*), while HAp included August, September and October (year *n*). At all sites, daily chill units were estimated by four chill models: (1) the North

Carolina which is commonly used in Brazil (Shaltout and Unrath, 1983), (2) the Dynamic which has been found to be superior to other chilling models (Fishman et al., 1987; Luedeling et al., 2009), (3) the Positive Chill Unit which has been shown to outperform the original Utah model applied worldwide (Linsley-Noakes et al., 1995), (4) the Unified which is fitted from data in various woody plants (Chuine, 2000).

Daily heat units were estimated by three heat models: (1) the GDH which is applied worldwide and shown to give good predictions in apple (Richardson et al., 1975; Rea and Eccel, 2006), (2) the DTS which was developed in Japan for pear trees (Honjo et al., 2006), (3) the F1Gold1 which is fitted in Western Europe for Golden Delicious (Legave et al., 2013). As most of the utilized models required hourly temperatures, a computational tool was used to estimate daily hourly temperatures from daily Tmax and Tmin.

1.3.4. Statistical analyses

Shapiro-Wilk tests were performed to evaluate the normality of temperature and phenological data. As some of our historical temperature series showed non-normal distributions, the non-parametric Mann-Kendall test was used to detect significant changes both in the temperature series and in the historical chill/heat series. The Mann-Kendall test does not require the data to be normally distributed (Viola et al., 2013). The second advantage of this test is it low sensitivity to abrupt breaks in historical series, such as shifts due to changes in measuring technique (Jaagus, 2005; Mavromatis and Stathis, 2011; Mondal et al., 2012). In addition, the Sen’s test was used to calculate the slope values of trends fitted by the Mann-Kendall tests. These values have been expressed either in *°C per decade* for the temperature trends or in *chill/heat amounts per decade* for the chill and heat trends. The trend analysis was performed over the period 1973-2012 (four decades) in order to compare the different trends over the same years at all nine sites studied (Table 2). The analysis of the blooming date series took into account the different starting years in the data (Table 3).

The usual analysis of phenological series by linear regression (Doi and Katano, 2008; Grab and Craparo, 2011) was therefore discarded because the significance of temporal trends can vary with different starting years in linear regression (Nordli et al., 2008). Additionally, because we observed obviously non-linear trends for some blooming series, when curves of annual data were plotted (not shown), we chose to perform a descriptive analysis of our blooming series by using moving averages of the observed annual dates (expressed in calendar days). This statistical tool more clearly highlighted spatial and temporal variability than by using current curves of annual dates. We tested different time spans when calculating the moving averages and chose three-year averages to highlight temporal trends. Historical series expressed in moving averages have been established for all stages (61, 65 and 67) and cultivars studied (GD, Gala and Fuji).

Further to this, all relationships between the climatic variables (Tmax, Tmean, Tmin, chill amounts and heat amounts) and the phenological variables (dates 61, 65 and 67; BD 61-65 and BD 61-67) were studied using the Spearman's rank correlation coefficient. This was performed from 1984 to 2012 for GD (29 years) and from 1983 to 2012 (30 years) for Gala and Fuji, in order to compare the significance of correlations over the same periods at all nine sites studied (Table 3).

1.4. Results

1.4.1. Regional patterns of temperature, chilling and heating

Figure 3 shows a synthetic characterization of temperature levels and trends within the apple cropping area defined by nine sites studied. Time-course changes in average temperatures have been established at each site for the whole year (annual mean), the chilling accumulation period (mean during CAp) and the heat accumulation period (mean during HAp) over a common long-term period (last four decades). We also characterized each site by levels, established from last four decades, for each of three means.

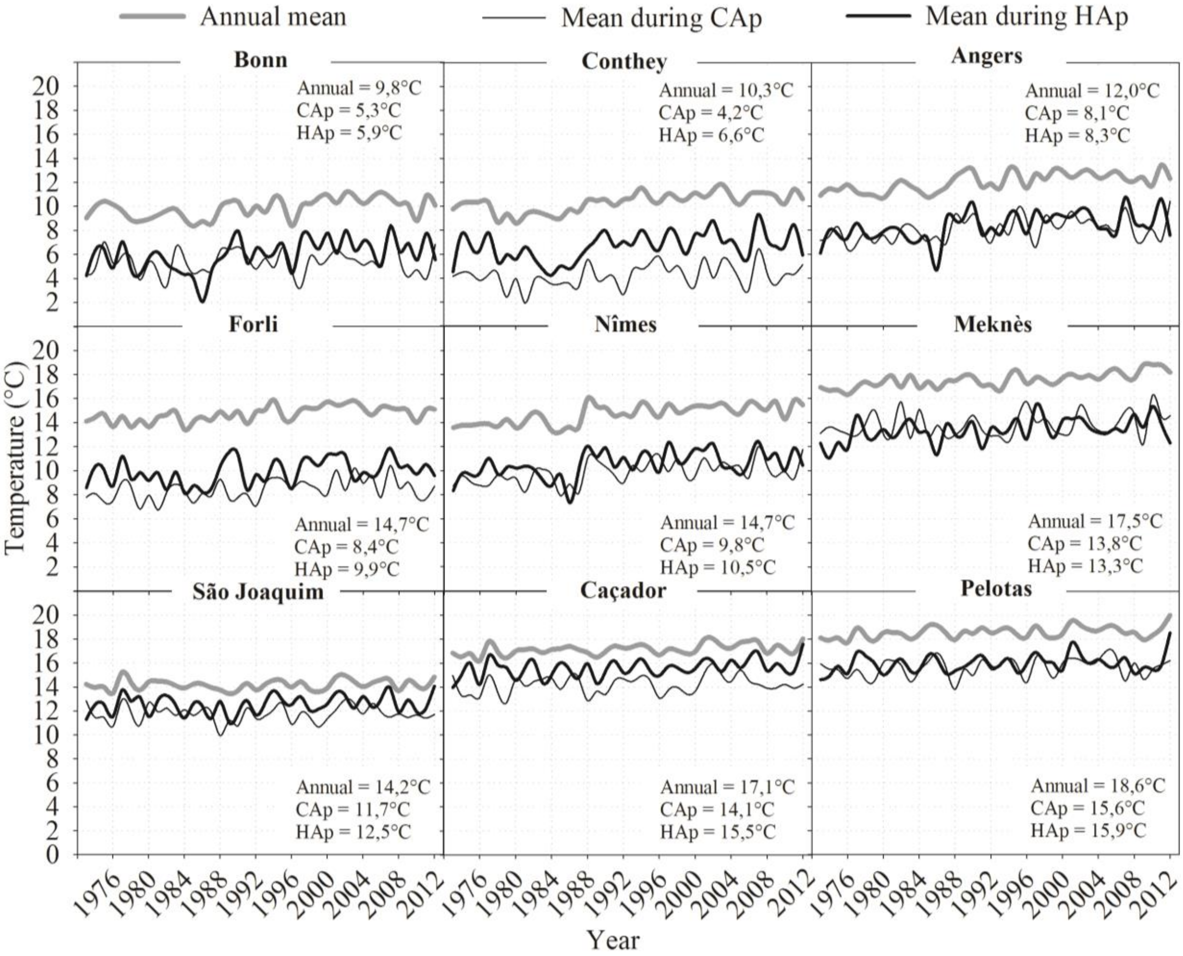

Figure 3. Annual average temperature and mean temperatures during the chill accumulation period (CAp)[a] and the heat accumulation period (HAp)[b] respectively over 40 years (1973-2012) at nine experimental sites of apple cropping. [a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

The temperature level of annual mean showed a large range, round 8,8°C from the coldest site of Bonn to the warmest site of Pelotas. Higher temperature differences were outlined for means during CAp and HAp, round 11,4°C and 10,0°C respectively from the coldest sites of Conthey and Bonn to the warmest site of Pelotas. All the NH sites were characterized by annual means higher than means during CAp and HAp, as clearly at Conthey and in Forlì, while all three Brazilian sites (SH) showed smaller differences between these means.

Moreover, the time trends of annual mean and means during CAp and HAp showed contrasting regional differences. They were evaluated over last four decades through changes in *°C per decade* for Tmax, Tmean and Tmin during each period (whole year, CAp, HAp) (Table 4). In all NH sites annual mean showed significant marked increases (0,32 to 0,52 °C per decade for mean temperature in whole year). Tmax in whole year showed higher increases than increases of Tmin in most of NH sites, as clearly recorded at Bonn. The site of Nîmes showed the highest increase of annual mean due to marked increases both in Tmax and Tmin. The Tmean during CAp was characterized by not uniform changes, regardless the regional temperature level, since significant increases of Tmean were recorded in oceanic and Mediterranean European sites (Angers, Forlì, Nîmes) while not significant increases were recorded either in continental European sites (Bonn, Conthey) or in Morocco (Meknès).

Table 4. Average changes in ºC per decade of maximum, mean and minimum temperatures for the annual period, the chill accumulation period (CAp)[a] and the heat accumulation period (HAp)[b] over 40 years (1973 to 2012) at six sites of the North Hemisphere (NH) and three sites of the South Hemisphere (SH).

Site	Whole year			CAp			HAp		
	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.
NH									
Bonn	**0,62**	**0,35**	0,05	**0,30**	0,07	-0,13	**0,76**	**0,51**	0,18
Conthey	**0,39**	**0,44**	**0,46**	0,13	0,21	0,23	**0,45**	**0,41**	**0,37**
Angers	**0,53**	**0,42**	**0,33**	**0,35**	**0,34**	**0,33**	**0,64**	**0,49**	**0,28**
Forli	**0,44**	**0,32**	**0,21**	**0,36**	**0,32**	**0,24**	**0,48**	**0,30**	0,13
Nîmes	**0,55**	**0,52**	**0,44**	**0,37**	**0,41**	**0,48**	**0,59**	**0,43**	**0,26**
Meknès	**0,50**	**0,39**	**0,22**	0,18	0,22	0,31	**0,54**	**0,39**	0,15
SH									
São Joaquim	0,00	0,06	**0,13**	-0,06	-0,04	0,02	0,10	0,16	0,20
Caçador	**0,17**	**0,21**	**0,22**	0,02	0,11	0,21	0,24	**0,24**	0,28
Pelotas	**0,19**	**0,18**	0,13	0,08	0,17	0,21	**0,20**	0,15	0,11

[a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

Values in bold and bold underlined fonts are significant at P = 0,05 and P = 0,01 respectively by Sen’s test; values in regular font are not significant.

Inversely, the mean during HAp in NH sites was characterized by uniform significant increases mainly due to marked increases in Tmax. Thus, among European sites, Nîmes and Angers were characterized by marked temperature increases both during CAp and Hap for Tmax and Tmin. Figure 3 highlighted marked temperature increases in these two sites since the end of the 1980s. By contrast, the Brazilian sites of Caçador and Pelotas showed low significant increases of Tmean in whole year (0,21 and 0,18 °C per decade respectively), no significant temperature changes during CAp, while only Caçador showed a significant increase of mean during HAp. Nearly constant temperatures characterized the site of São Joaquim, as clearly shown in Figure 3.

A negative correlation was highlighted during HAp, not significant for Tmin but significant for Tmax (P≤0,05) and Tmean (P≤0,01) between the temperature levels and changes in °C per decade (time trends). This is illustrated for Tmean in Figure 4: the three coldest European sites (Bonn, Conthey, Angers) showed the highest increases, the Mediterranean sites (Forlì, Nîmes, Meknès) with intermediate levels of temperature showed intermediate increases, while all three Brazilian sites (São Joaquim, Caçador, Pelotas) showed the lowest increases.

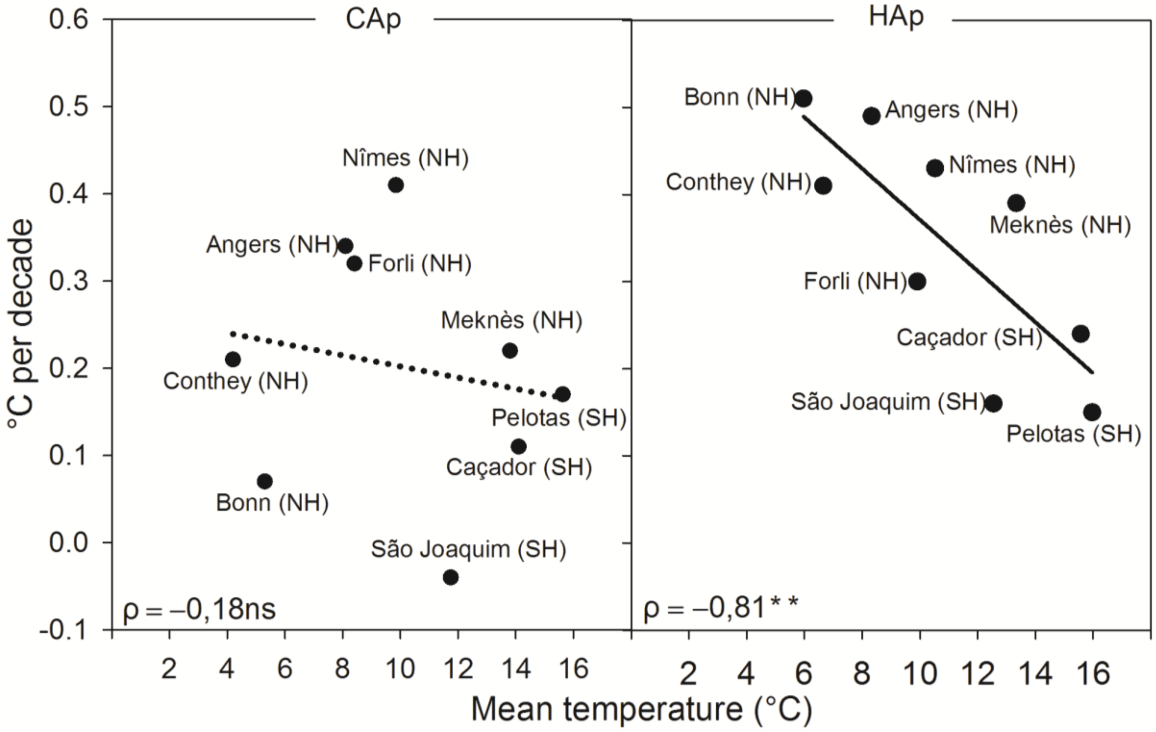

Figure 4. Relationships over 40 years (1973-2012) for the nine sites studied between mean temperature and change in mean temperature (°C per decade) during the chill accumulation period (CAp)[a] and the heat accumulation period (HAp)[b] respectively. Spearman's value (ρ) followed by ** and ns are significant (P= 0,01) and non-significant, respectively. [a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

By contrast, no significant correlation was found during CAp whatever daily temperature. However, within European sites (Figure 4), the relationships for Tmean clearly outlined higher temperature increases in the three warmest sites (Angers, Forlì, Nîmes) and lower increases in the coldest (Bonn, Conthey), with the highest increase at Nîmes (0,41°C per decade) and the lowest at Bonn (0,07°C per decade). The relationships between the temperature levels and increases in Europe appeared more contrasting and in inverse order during CAp compared to relationships during HAp (0,51°C at Bonn versus 0,43°C at Nîmes). Similar relationships were outlined during CAp in Southern Brazil (Figure 4), with the highest increase at Pelotas (the warmest site) and the lowest in São Joaquim (the coldest site). Nevertheless, a global view of whole nine sites outlined quite similar temperature increases during CAp in sites with opposite climates, as shown for continental site of Conthey (0,21°C) and Mediterranean site of Meknès (0,22°C).

Moreover, the effects of temperature changes on the accumulation of chill and heat amounts have been evaluated through average changes in chill and heat amounts per decade during CAp and HAp respectively (Table 5).

Table 5. Changes in chill and heat accumulations per decade according to different chill and heat models during the chill accumulation period (CAp)[a] and the heat accumulation period (HAp)[b], respectively, over 40 years (1973-2012) at six sites of the North Hemisphere (NH) and three sites of the South Hemisphere (SH).

Site	Chill model (CAp)				Heat model (HAp)		
	North Carolina	Dynamic	Positive Chill Unit	Unified	GDH	DTS	F1Gold1
NH							
Bonn	-34,7	-1,49	-47,8	**-28,5**	**938,8**	**1,44**	**5,36**
Conthey	-19,0	**-1,86**	-16,4	**-36,7**	**810,7**	**1,34**	**4,54**
Angers	-53,6	**-2,77**	**-70,4**	**-80,1**	**902,6**	**1,28**	**4,69**
Forli	-27,9	-1,40	-36,6	**-59,0**	**786,1**	**1,68**	**5,44**
Nîmes	**-66,3**	**-2,85**	**-63,1**	**-86,6**	**986,4**	**1,91**	**6,17**
Meknès	-14,9	-0,96	-23,4	-39,8	**773,7**	**2,29**	**6,67**
SH							
São Joaquim	9,22	0,71	14,4	6,21	339,8	1,40	3,70
Caçador	-1,44	0,33	11,2	-6,92	464,1	**1,92**	**5,48**
Pelotas	-43,9	-1,28	-11,9	-31,3	286,9	1,71	4,24

[a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

Values in bold and bold underlined fonts are significant at P = 0,05 and P = 0,01 respectively by Sen's test; values in regular font are not significant.

Globally, these changes appeared in agreement with the regional differences in temperature changes (Table 4). Regarding chill accumulation, modeling outlined contrasting differences both within European sites, and between European sites and mild sites (Moroccan and Brazilian). Within Europe, declines of chill amounts during CAp clearly occurred in the sites of Nîmes and Angers (significant at P≤0,01 for all the models at Nîmes or nearly all models at Angers), while chill declines appeared less pronounced and significant (only for one or two models) in the other sites (Bonn, Conthey, Forlì). By contrast, in mild sites, modeling revealed either relatively low chill declines at Meknès and Pelotas (not significant) or no decline at Caçador and São Joaquim. Regarding heat accumulation during HAp, all the models outlined marked increases, highly significant (P≤0,01), in all sites of the NH, contrasting with relatively low heat increases in Brazilian sites, only significant (P≤0,05) for two models at Caçador.

1.4.2. Regional patterns of blooming time

Correlation coefficients (Spearman's test) calculated between the dates of stages 61 and 65, the dates of stages 61 and 67 and the dates of stages 65 and 67 revealed high correlations for Golden Delicious (GD), which were most often significant at the P≤0,01 level both at the European sites (0,89 to 0,96), in Morocco (0,66-0,75) and at the Brazilian sites (0,80-0,93). Similar correlations were obtained for 'Gala' and 'Fuji'. Thus, only dates for one blooming stage appeared useful for establishing the main features of spatial and temporal variability for blooming time. Therefore, for all three cultivars, Figure 5 shows a representative overview of the data collected through the chronological series for the date 61 at all sites and the date 65 at Conthey (stage 61 was not available). In Europe, a relationship between the spatial variability and latitude (linked to climate type) was found for GD for all blooming stages, as shown by mean dates 61 at the southern Mediterranean sites (Nîmes and Forlì) being earlier than at the northern continental site of Bonn, with intermediate dates at Angers (oceanic site). Over 39 years (1975-2013) GD flowered at Nîmes earlier than at Bonn by an average of around 22 days for stage 61 (mean date on 9 April and 1 May respectively). A similar influence of latitude was recorded for Gala, with dates 65 occurring earlier over 33 years (1981-2013) at Nîmes than at the continental site of Conthey (mean date on 8 April and 27 April respectively).

The temporal variability of the dates 61 of GD highlighted a sudden change toward earlier blooming at the end of the 1980s at all the European sites (Figure 5). Similar trends were also evident for all long-term series for the dates 65 and 67 of GD, as well as for the long series of dates 65 of Gala at Conthey clearly showing earlier blooming over 39 years (1975-2013) (Figure 5). On average, the advance of blooming dates for GD during the period 1975-2013 has been more pronounced at the continental sites (around 8 days for date 61 at Bonn and Conthey) than at the oceanic and Mediterranean sites (5 days at Angers and Forlì and 3 days at Nîmes).

Similarly, mean advances of the date 65 appeared higher for Gala at Conthey (around 5 days) than at Nîmes (2 days) during the period 1981-2013. The influence of latitude on the spatial variability within European and Moroccan Mediterranean sites showed a contrasting pattern: the mean date 61 of GD over 27 years (1986-2012) was earlier at Meknès (4 April, the lowest latitude site) than at Nîmes and Forlì (8 and 9 April respectively), while the mean date 65 was later at Meknès (18 April) than at Nîmes and Forlì (10 and 13 April respectively).

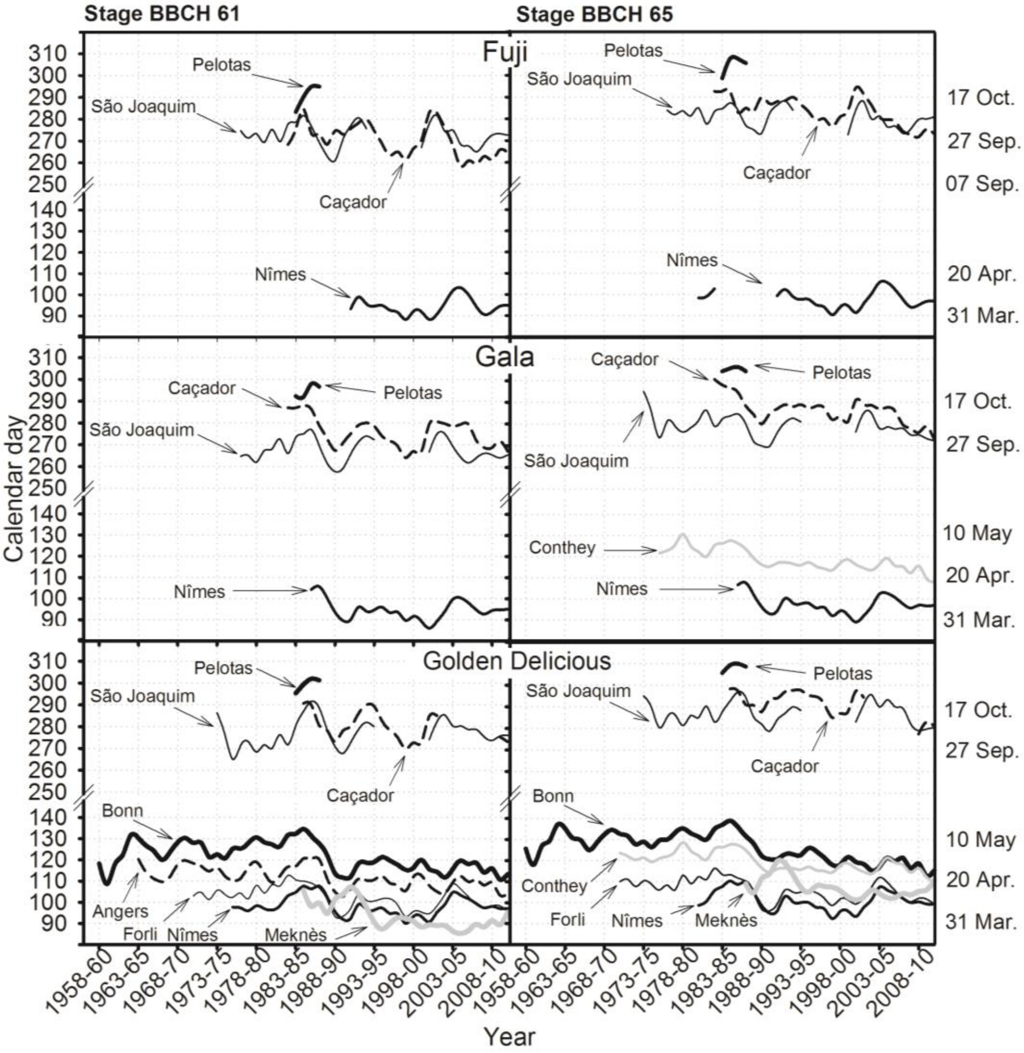

Figure 5. Time-course changes in moving averages (three years) at all studied sites for the annual date of early bloom stage (BBCH 61) (except Conthey) and for the annual date of full bloom stage (BBCH 65) (except Angers) for Fuji, Gala and Golden Delicious apple cultivars. Each average date is expressed in calendar day from 1 January of the flowering year.

Nevertheless, blooming advance has also been observed at Meknès for GD, around 8 days for the date 61 and 5 days for the date 65 during the period 1986-2012, although this occurred later than in Europe (Figure 5). Contrary to Europe, the spatial variability within the Brazilian sites was poorly related to latitude and in an inverse order. For all three cultivars, blooming time was later in the south at the warmest oceanic site of Pelotas (end of October for date 61) than in the north at São Joaquim and Caçador (end of September to early October) for the period 1983-1988. GD and Gala flowered a little earlier at the mountainous site of São Joaquim (the coldest) than at the continental site of Caçador (intermediate temperature). Over 30 years (1984-2013) the mean dates 61and 65 of GD were earlier at São Joaquim than at Caçador by an average of around 2 and 5 days respectively, while this was more marked for the mean date 61 of Gala (around 7 days earlier at São Joaquim), as shown in Figure 5. During the same period, Fuji showed a contrasting blooming pattern (later mean date 61 versus an earlier mean date 65 at São Joaquim).

The varietal ranking in blooming earliness was quite similar for Europe and Brazil, particularly the earlier blooming time of Gala than that of GD, although this difference was more marked in Brazil (for example, around10 days for the date 65 at São Joaquim and only 1 day at Conthey during the period 1984-2013). With regard to temporal variability of blooming dates, no change was observed at São Joaquim whatever the cultivar and stage. At Caçador, slight trends toward earlier dates were observed for all thee cultivars, but not as suddenly as in Europe (Figure 5).

1.4.3. Regional patterns of blooming duration

Figure 6 shows a synthetic overview of the number of days for BD 61-65 at all sites where dates 61 and 65 were available. The greater amount of data collected for GD highlighted two contrasting patterns between (i) short BD 61-65 within European sites either continental (Bonn) or Mediterranean (Forlì and Nîmes) and (ii) clearly higher BD 61-65 within both the Mediterranean site of Meknès and all three Brazilian sites. In the same way, Gala and Fuji showed a short BD 61-65 at Nîmes and clearly higher values at the Brazilian sites. Similar results were obtained for the whole of the blooming duration, with a relatively low number of days for BD 61-67 in Europe (around 7 days at Nîmes and 10 at Forlì for GD) in contrast to significantly higher numbers in Morocco (around 21 days) and in Brazil (19 days at São Joaquim and 20 at Caçador).

Moreover, Figure 6 suggests that no relationship was evident between the annual variations of the blooming duration and blooming time, since short and extended BD 61-65 were outlined between Nîmes and Meknès respectively, although GD flowered at these two sites over a similar period (expressed in calendar day; Figure 6). Inversely, similar values for BD 61-65 were recorded at the European sites in spite of differences in blooming earliness. This was also observed within the Brazilian sites (later blooming at Pelotas with similar BD to those of two other sites).

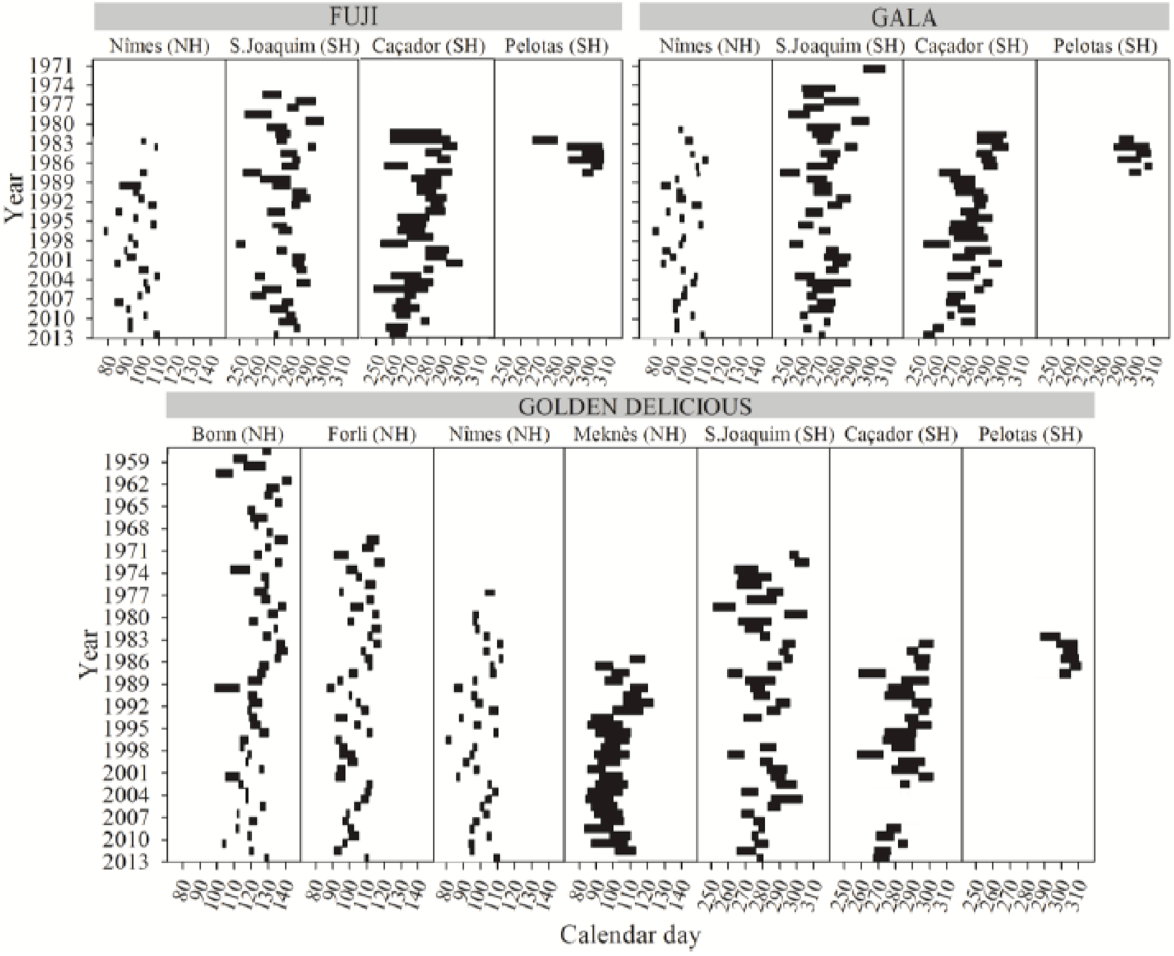

Figure 6. Annual blooming duration from the date of early bloom stage (BBCH 61) to the date of full bloom stage (BBCH 65) for Fuji, Gala and Golden Delicious apple tree cultivars in sites of the North Hemisphere (NH) and South Hemisphere (SH).

1.4.4. Determinisms of blooming time

Table 6 synthesized correlations between the annual dates 61 for GD (except dates 65 at Conthey) versus annual chill and heat amounts cumulated during the CAp and HAp periods. Similar results were obtained for the dates 61 of Gala and Fuji, as well as the dates 65 and 67 for the three cultivars. Great negative correlations were observed during HAp for all models at the European sites (Table 6; -0,57 to -0,93;

P≤0,01) indicating that higher post-dormancy heat accumulation could determine earlier phenological stages. Thus, this may clearly explain blooming advances since the end of the 1980s at European sites (Figure 5), where marked warming was recorded during HAp (Tables 4 and 5) and particularly since this time (Figure 3). By contrast, no correlation with chill accumulation during CAp, whatever the model, suggests that the chill satisfaction in Europe was not a determining factor of blooming time.

Table 6. Correlation values (Spearman's test) calculated over 29 years (1984-2012) between annual dates of bloom stages[1] of Golden Delicious and annual chill or heat amounts, according to different models, cumulated during the chill accumulation period (CAp)[a] and heat accumulation period (HAp)[b] respectively, at six sites of the North Hemisphere (NH) and two sites of the South Hemisphere (SH).

Site	Chill model (CAp)				Heat model (HAp)		
	North Carolina	Dynamic	Positive Chill Unit	Unified	GDH	DTS	F1Gold1
NH							
Bonn	0,06	0,10	0,07	0,05	**-0,93**	**-0,85**	**-0,90**
Conthey	-0,17	-0,06	-0,19	0,16	**-0,84**	**-0,71**	**-0,65**
Angers	0,19	0,19	0,20	0,12	**-0,62**	**-0,57**	**-0,59**
Forli	0,02	0,12	0,04	0,12	**-0,85**	**-0,73**	**-0,77**
Nîmes	0,01	0,09	-0,06	0,00	**-0,71**	**-0,69**	**-0,71**
Meknès	-0,21	-0,22	-0,21	-0,25	-0,18	-0,31	-0,28
SH							
São Joaquim	**-0,55**	**-0,68**	**-0,67**	**-0,67**	-0,24	-0,22	-0,18
Caçador	**-0,68**	**-0,60**	**-0,70**	**-0,69**	-0,20	-0,10	-0,14

[1] Early bloom stage (BBCH 61) at all sites except Conthey and full bloom stage (BBCH 65) at Conthey site. Values in bold underlined fonts are significant at P = 0,01; values in regular font are not significant.

[a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

Correlations for São Joaquim and Caçador highlighted opposing determinisms in Brazil: low and non-significant negative correlations with heat accumulation during HAp suggested a low influence of post-dormancy temperatures on blooming time, while great negative correlations during CAp, recorded for all models (-0,55 to -0,70; P≤0,01), indicated that chill satisfaction was inversely a determining factor of blooming time. This may explain later blooming dates at Pelotas (Figure 5) where warmer temperatures were recorded during CAp (Figure 3). Moreover, at the Moroccan site of Meknès intermediate relationships were observed since relatively low and non-significant negative correlations were obtained between the dates 61 and chill or heat amounts during CAP and HAp, respectively (Table 6). Nevertheless, some significant negative correlations were found between the dates 65 and heat amounts for the DTS and F1Gold1models (-0,42 and -0,38 respectively at P≤0,05; data not shown), suggesting that higher temperatures during HAp could determine earlier blooming in Morocco as in Europe.

To further investigate the temperature determinisms of blooming time, we analyzed correlations for each month of CAp and HAp. Table 7 contains synthesized correlations for GD regarding the dates 61 at all sites except at Conthey (dates 65). They are representative of correlations obtained for other stages and cultivars. In Europe, significant negative correlations during HAp (Table 7) were mainly due to high correlations (P≤0,01) for February (-0,29 to -0,68) and more so for March (-0,51 to -0,82). As expected, the earliest blooming sites (Forlì and Nîmes) showed correlations only for February and March while the latest blooming sites (Bonn and Conthey) showed correlations also for April. Similarly to the results during CAp, no clear correlation was found for each of four months involved in CAp. Although not obtained for all models, some unexpected significant positive correlations (for December at Nîmes and January at Bonn) could be due to modeling defects or express both chill and heat requirements during intermediate months from bud dormancy to active growth. At Brazilian sites (Table 7), significant negative correlations for September (-0,41 to -0,56) indicated that higher temperatures, a short time before blooming (Figure 5), could determine earlier phenological stages. This could explain slight trends toward earlier dates at Caçador where slight warming was recorded during HAp (Tables 4 and 5). Moreover, the great negative correlations during CAp (Table 6) were mainly due to high correlations for June (-0,41 at P≤0,05 to -0,62 at P≤0,01).

This indicated that higher chill satisfaction during June could determine earlier blooming dates in Brazil, although chill satisfaction in April, May and July appeared to also have an influence on blooming earliness particularly at Caçador. At the Moroccan site, significant negative correlations were found between the dates 61 and heat amounts when accumulation was considered over March and April (-0,32 to -0,38 at P≤0,05 for GDH and F1Gold1, respectively (data not shown). This may explain clear blooming advances at the beginning of the 1990s (Figure 5) at Meknès where marked warming during HAp was recorded (Tables 4 and 5). Furthermore, significant negative correlations were also found at Meknès between the dates 61 and chill amounts for

November (-0,38 to -0,40 at P≤0,05) (Table 7), suggesting that chill satisfaction could be also a determining factor of blooming dates in Morocco.

Table 7. Correlation values (Spearman's test) calculated over 29 years (1984-2012) between annual dates of bloom stages[1] of Golden Delicious and annual chill or heat amounts, according to different models, accumulated during each month of the chill accumulation period (CAp) and heat accumulation period (HAp), respectively, at six sites of the North Hemisphere (NH) and two sites of the South Hemisphere (SH).

Site	Chill model (CAp)												Heat model (HAp)					
	North Carolina				Dynamic				Unified				GDH			F1Gold1		
	Oct. Apr.	Nov. May	Dec. Jun.	Jan. Jul.	Oct. Apr.	Nov. May	Dec. Jun.	Jan. Jul.	Oct. Apr.	Nov. May	Dec. Jun.	Jan. Jul.	Feb. Aug.	Mar. Sep.	Apr. Oct.	Feb. Aug.	Mar. Sep.	Apr. Oct.
Bonn (NH)	0,12	0,16	0,22	-0,14	-0,02	0,16	0,34	-0,13	0,07	0,02	-0,21	**0,61**	**-0,56**	**-0,69**	**-0,46**	**-0,51**	**-0,67**	**-0,44**
Conthey (NH)	0,04	-0,09	-0,09	-0,26	0,13	-0,01	0,00	-0,29	0,05	0,21	-0,12	-0,02	**-0,49**	**-0,69**	-0,30	**-0,51**	**-0,51**	-0,24
Angers (NH)	0,19	0,09	0,08	0,13	0,14	0,12	0,08	0,16	0,16	0,02	-0,11	0,25	-0,31	**-0,69**	-0,18	-0,29	**-0,67**	-0,15
Forli (NH)	-0,03	0,18	0,22	-0,12	-0,01	0,23	0,23	-0,24	0,00	0,12	0,14	0,12	**-0,68**	**-0,82**	0,04	**-0,57**	**-0,82**	0,09
Nîmes (NH)	0,31	-0,33	**0,41**	-0,13	0,27	-0,22	**0,44**	-0,22	0,22	-0,32	0,22	0,16	**-0,59**	**-0,78**	-0,02	**-0,55**	**-0,80**	-0,02
Meknès (NH)	**-0,38**	**-0,39**	-0,13	0,14	-0,26	**-0,40**	-0,23	0,26	-0,10	**-0,38**	-0,21	0,11	0,04	-0,22	-0,23	0,04	-0,29	-0,26
São J. (SH)	-0,06	-0,31	**-0,50**	-0,28	-0,17	-0,31	**-0,60**	-0,24	-0,08	-0,33	**-0,57**	-0,26	-0,28	**-0,41**	0,31	-0,23	**-0,42**	0,29
Caçador (SH)	**-0,48**	**-0,42**	**-0,41**	-0,32	-0,23	-0,21	**-0,62**	-0,22	-0,39	**-0,45**	**-0,56**	-0,27	-0,22	**-0,56**	0,35	-0,20	**-0,56**	0,29

Values in bold and bold underlined fonts are significant at P = 0,05 and P = 0,01 respectively; values in regular font are not significant.

[1] Early bloom stage (BBCH 61) at all sites except Conthey and full bloom stage (BBCH 65) at Conthey site.

[a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

1.4.5. Determinisms of blooming duration

Relationships between the annual variations of BD 61-65 and BD 61-67 and the annual variations of mean temperature during these periods showed significant negative correlations at the cold site of Bonn (-0,46;P≤0,05), while this was not significant at Forlì (-0,21) (and non-existent at Nîmes, where no BD variability was recorded; Figure 6). Similar results were obtained for correlations involving heat amounts during BD (-0,43 to -0,55 at Bonn according to the model). By contrast, at the Moroccan and Brazilian sites characterized by relatively extended BD (Figure 6), no clear relationship was obtained between the annual BD and (i) the annual mean temperature or (ii) heat amount during BD. Moreover, correlation analyses between the annual BD versus the annual chill and heat amounts accumulated during CAp and HAp respectively did not show any relevant results for both the Moroccan site and all three Brazilian sites. Particularly, no clear negative correlations were found between BD and chill amounts cumulated during CAp, contrary to the results found for the blooming time (see 1.4.4). Nevertheless, interesting relationships were outlined when the means of BD annual values were correlated to the means of chill and heat annual amounts during CAp and HAp respectively, i.e when relationships were investigated at the geographical scale of all sites studied (Figure 7).

Thus, significant negative correlations between BD and chill amounts were obtained (P≤0,01), as shown by representative results shown in Figure 5 for BD 61-65 and BD 61-67 using the Dynamic model to estimate chill amounts and the GDH model to estimate heat amounts. This highlighted a global determinism of blooming duration, i.e. the less chill units are accumulated and then the more blooming duration is extended. Another global determinism was outlined by significant positive correlations between BD and heat amounts during HAp (P≤0,01), i.e. the more heat units are accumulated and then the more blooming duration is extended (Figure 7).

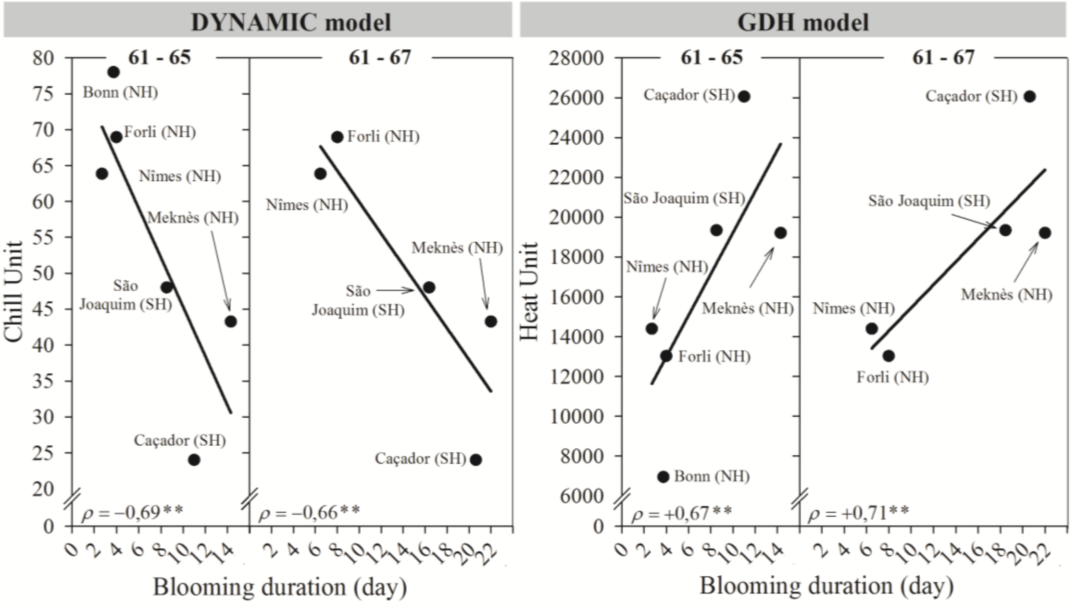

Figure 7. Spearman's correlation values (ρ) calculated between blooming duration (BBCH 61 - 65 and BBCH 61 - 67) of Golden Delicious and the accumulation of chill and heat quantities during chill accumulation period (CAp)[a] and heat accumulation period (HAp)[b] during 1986-2012 period, according Dynamic and GDH models, respectively. Spearman's value followed by ** are significant at P = 0,01. [a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

1.5. Discussion

Many studies have documented climate and phenological changes in the Northern hemisphere (Menzel and Fabian, 1999; Rutishauser et al., 2009) but few have studied these changes between regions of both hemispheres. We took advantage of the worldwide cropping area of apple tree to decipher the patterns and determinisms of blooming traits, in a woody plant, induced by contrasting temperature and warming patterns of both hemispheres.

1.5.1. Temperature, chill and heat accumulation patterns

The analysis of mean temperatures revealed strong temperature gradients between cold sites of Western Europe and mild sites both in Morocco and Southern Brazil (see AAT in Table 2). However, the warmest European sites (Forlì and Nîmes) showed annual temperatures close to those of the mild site of São Joaquim in Southern Brazil (Figure 3). The annual means were slightly higher in Forlì and Nîmes than in São Joaquim, although the mean temperatures remained lower in the two

Mediterranean European sites during the CA and HA periods, that are essential for analyzing the spring phenology. Particularly, the mean temperatures during the CA period in Forlì and Nîmes remained below the level of 12°C, while this mean was close to 12°C at São Joaquim and 14°C at Meknès (Figure 3). Fishman et al. (1987) fixed an upper threshold of 13°C to estimate the chill effects in the dynamic model, and Bonhomme et al. (2010) found 12°C as optimal temperature to obtain efficient chill estimates by the Utah model (Richardson et al., 1974) in peach tree. The mean temperatures found during the HA period showed also noticeable differences between the warmest European sites of Forlì and Nîmes (round 10°C)and the mild sites of Meknès and São Joaquim (round 13°C), while this temperature was clearly higher in the mildest sites (round 16°C at Caçador and Pelotas). This suggests that the mild sites are characterized by intense rates of heat accumulation during the bud growth phase (ecodormancy) in comparison with temperate sites.

However, as warming is likely to exceed around 1,5°C for the end of the 21st century (IPCC, 2013), our results supports to focus in near future on temperature comparisons between European Mediterranean sites, as Nîmes especially, and mild sites of both hemispheres (as Meknès and São Joaquim). Our results also outlined not generalized and uniform temperature trends within the studied sites. Synthetically, three contrasting warming patterns (WP) were observed: (i) WP1 prevailing both in continental European sites and in Morocco characterized by marked temperature and heat accumulation increases (mainly Tmax) during HAp (ecodormancy) but without significant changes during CAp (dormancy); (ii) WP2 prevailing in oceanic and Mediterranean European sites characterized by trends during HAp similar to those of WP1, but also temperature increases and chill declines during CAp; (iii) WP3 prevailing in Southern Brazil characterized by nearly no significant changes in temperature and chill/heat accumulations during CAp and HAp.

The strong warming recorded for the whole year over the last four decades in the NH sites (Table 3) was similarly outlined from analyses over longer observation periods (Viola et al., 2013) and also related to increases in Tmax (Rebetez and Reinhard, 2007). We recorded a low warming for the Brazilian sites, although Marengo and Camargo (2008) observed a more intense warming in Southern Brazil, but this was not recorded in all studied sites. Most of time, different warming patterns were identified through the analysis of Tmax and Tmin seasonal trends in specific regions

(Martínez et al., 2010; Sansigolo and Kayano, 2010; Cordero et al., 2011). But the climatic analyses more rarely compared temperature changes between contrasting world regions during distinct chill and heat periods, essential for the flowering of woody plants (Schwartz and Hanes, 2010). With this approach, using contrasting models, we obtained divergent regional responses during the chill period. Both cold European and mild sites of Morocco and Southern Brazil showed no significant chill decline (WP1 and WP3) versus chill declines in oceanic and Mediterranean European sites (WP2). Such opposing patterns suggest the emergence of divergences in warming impacts during dormancy (CAp) within the apple cropping regions of the NH and between regions of both hemispheres, regardless of temperature levels (Figure 3). If trends toward chill decline were also reported in cold sites of the NH (Sunley et al., 2006) and mild sites of the SH (Darbyshire et al., 2011), the need of multi-model analysis and model improvement was recently pointed out to assess their relevance (Luedeling et al., 2009b; Darbyshire et al., 2011).

Furthermore, “spring” warming during the heat period was more rarely detailed despite its determining impacts on spring phenology advance (Fujisawa and Kobayashi, 2010; Legave et al., 2013). Thus, strong “spring” warming was found in temperate and mild sites of the NH (WP1 and WP2) and was rarely found in the mild sites of Southern Brazil (WP3). Such contrasting patterns also suggest the emergence of divergences in “spring” warming impacts during ecodormancy (HAp) between the apple cropping areas of both hemispheres, particularly between mild regions of both hemispheres (Morocco versus Brazil). When warming during dormancy and spring warming are globally considered, this outlines a marked vulnerability to warming impacts over last 40 years in temperate Mediterranean (Nîmes) and Oceanic (Angers) regions of the NH (Figure 4).

1.5.2. Blooming time patterns and their relationships with thermal patterns

Due to the large panel of our historical data for same apple cultivars and the extensive geographical area of the study, four different blooming time patterns were synthetically observed, whatever the phenological stage, in response to different temperature conditions in space and time:(i) a heat-control during ecodormancy in continental regions of Western Europe, mainly driven by the temperature conditions of February, March or even April (Table 7) suggesting a relatively long period of heat control; Thus, marked spring warming during ecodormancy (WP1) explained blooming advances since the end of the 1980s (Figure 5). (ii) A similar heat-control in Mediterranean and oceanic regions of Western Europe. But marked spring warming during ecodormancy added to chill declines during dormancy (WP2) resulted in lower mean blooming advances since the end of the 1980s (see 1.4.2). (iii) A heat-control in Northern Africa less clear than in the European regions, since relatively weak relationships were found with heat accumulation during the period March-April (see 1.4.4 and Table 6). As spring warming also occurred in Morocco (WP1), although later than in Europe (Figure 3), this may explain blooming advances since the beginning of the 1990s (Figure 5). The relationships found with the chill accumulation in November and also in October and December suggested an additional chill-control (Table 7).(iiii) A chill-control in Southern Brazil, mainly driven by the temperature conditions of June but also by those of April, May and July (Table 7) suggesting a relatively long period of chilling-control in mild conditions. No significant or weak warming during dormancy and ecodormancy (WP3) may explain unchanged blooming time trends, except possible slight advances at Caçador (Figure 5) driven by increases in mild temperatures of September (Table 7).

A chill-control of bud phenology in mild regions was currently associated with phenological disorders, commonly so-called symptoms of inadequate chilling. They are many and vary with species. Generally, they appear as excessive delays in bud break and anthesis, apparent as extended periods of blooming time leading to irregular fruit set (Atkinson et al., 2013). In Morocco (Oukabli and Mahhou, 2007) symptoms of inadequate chilling in apple are currently observed for chilling requirements like those of GD at sites as Meknès where the mean temperature during the chill period was around 14°C (Figure 3). In Brazil (Petri and Leite, 2004), similar symptoms can be observed in the main apple cropping area around the Caçador site also characterized by a mean temperature around 14°C during the chill period, while the area of Pelotas was deserted for apple cropping due to excessive mild conditions during the chill period (mean temperature around 16°C). These symptoms are less observed in the mountainous cropping area of São Joaquim where the satisfaction of chilling requirements was more a marginal issue (mean temperature during CAp around 12°C). Despite symptoms of inadequate chilling, weak blooming advances in Caçador or no change in São Joaquim over the last four decades may be correlated to weak or no significant spring warming during ecodormancy. This could be due to the fact that the critical chill and heat periods (CAp and HAp) were poorly affected (Table 3) by the global warming mentioned in Southern Brazil (Marengo and Camargo, 2008). Similarly, weak blooming advances were found in SH sites of Southern Africa (Grab and Craparo, 2011) and Australia (Darbyshire et al., 2013). Increasing minimum air temperature by 1°C from May to September in Southern Brazil would lead to a reduction of the apple areas with adequate chilling, while a 3°C temperature increase would result in a dramatic increase of areas with inadequate chilling (Wrege et al., 2010). Nevertheless, such predictive impacts of future warming should be carefully considered since chill declines can greatly differ among the chill models (Darbyshire et al., 2013). In addition, concerning Southern Brazil, our results show that the chill period could start from April and not include September.

Moreover, the relationships between symptoms of inadequate chilling and changes in blooming trends remain unclear as poorly investigated (Campoy et al., 2011). Our results on that issue outline different determinisms for (i) extended blooming periods commonly associated with inadequate chilling (Atkinson et al., 2013) and for (ii) blooming time changes determined by different changes in the satisfaction rates of chilling and heat requirements (Legave et al., 2013). This was supported by no close relationship between blooming duration and blooming time when the regional patterns of these two traits are compared (see 1.4.3). This can explain similar extended blooming periods in Moroccan and Brazilian sites showed (Figure 6) despite divergent blooming time trends (Figure 5).

1.5.3. The blooming duration in mild regions, critical issue to better understand

The duration of blooming time in cold European regions was correlated to heat accumulation during the blooming phase (see 1.4.5) and tended to decrease in the context of spring warming (April, March). This resulted in similar short durations in cold and Mediterranean regions since the beginning of the 2000s (Figure 6) despite chill declines during dormancy in the Mediterranean regions (see 1.4.1). Inversely, extended blooming durations observed in the mild sites (Morocco and Brazil) can induce crucial consequences on fruit production (Petri and Leite, 2004; Oukabli and Mahhou, 2007). Heterogeneous satisfaction of the chilling requirement within the floral buds could result in heterogeneous blooming in the field. The first buds release from dormancy could immediately grow and rapidly reach the released stage. This could inhibit other buds by correlative inhibitions and lead to extended periods of blooming time despite high temperatures favorable to bud growth (Petri and Leite, 2004).

Nevertheless, such determinism linked to inadequate chilling and commonly related (Atkinson et al., 2013), was not in agreement with our results at the scale of each mild sites studied. However this influence of inadequate chilling on blooming duration was supported at a global scale, i.e. considering all sites studied. At this scale, our results also highlighted an unexpected influence of the heat accumulation during ecodormancy, suggesting that excessive heat effects would induce extended blooming durations in the mild sites (Figure 7).Diverse combinations of successive inadequate chilling and heat effects might explain the annual variation of the blooming duration under mild conditions. Such combinations of chill and heat effects were also suggested for the dormancy breaking (Campoy et al., 2011). We can hypothesize that mean temperatures of 10-11°C during CAp and 11-12°C during HAp could correspond to temperature thresholds above which the unfavorable pattern of extended blooming durations is induced. Indeed, these temperatures reached respectively 11.7°C and 12.5°Cat São Joaquim where extended blooming durations were observed (Figure 6) under the lowest mild conditions during CAp and HAp (Figure 3).

1.5.4. Can São Joaquim or Meknès phenology depict the near future of Nimes?

Although the noticeable warming during dormancy in Nîmes area did not significantly impacted blooming time (Table 6) and increased blooming duration (Figure 6), this Mediterranean cropping region appears as the most vulnerable among European regions facing continuous warming. The mean temperature during the chill period at Nîmes over the last four decades was lower than the one of Meknès by 4°C, but lower than the one of São Joaquim by only 1,9°C, and this difference was reduced since the end of the 1980s (Figure 3). Similarly, the mean temperature at Nîmes during the heat period was lower than the one of Meknès by only 2,8°C and lower than the one of São Joaquim by only 2°C.

The temperature simulations by the A1B scenario of the SRES family (Figure 8) suggest that the temperature difference between Nîmes and São Joaquim for the chill period could be achieved from the decade 2040, which appears as a short time for adapting the apple industry to warming. Thus, the chill decline during dormancy already initiated at Nîmes (Table 4) could increase up to a critical level in Southern France in near future for cultivar like Golden Delicious. Chill decline up to the mild conditions of Meknès appears less probable because this will suppose a mean increase at Nîmes around 4°C during the chill period. The simulation concerning the heat period also suggest that the mean temperature at Nîmes during ecodormancy would be similar to the present temperature at São Joaquim from 2040 and similar to the present temperature at Meknès from 2070.

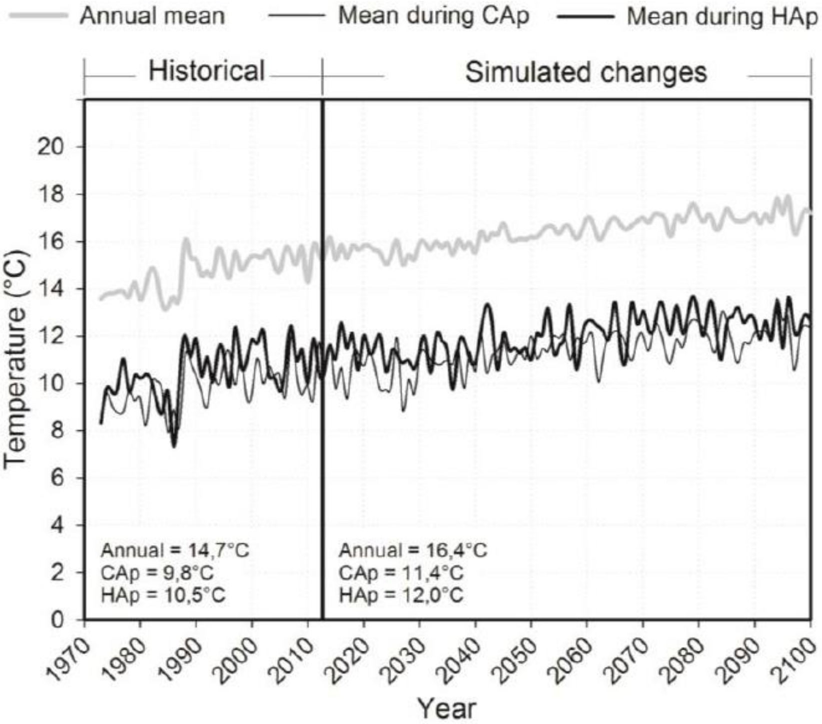

Figure 8. Historical (from 1973 to 2012) and simulated changes (from 2013 to 2100) in the annual mean temperature, mean during the chill accumulation period (CAp)[a] and the heat accumulation period (HAp)[b] at Nîmes (NH) by A1B scenario from temperatures projected by ARPEGE model. [a] North Hemisphere: October, November, December (year *n-1*) and January (year *n-1*); South Hemisphere: April, May, June and July (year *n*). [b] North Hemisphere: February, March and April (year *n*); South Hemisphere: August, September and October (year *n*).

The phenological consequences in near future (decade 2040) for apple cropping in Mediterranean regions would be new patterns of blooming time and blooming duration. The present trend at Nîmes toward slight mean blooming advances, lower than in European continental regions (Legave et al., 2013), could be changed according to new ratios between the opposite effects of chill decline during dormancy and heat accumulation increase during ecodormancy (Schwartz and Hanes, 2010), as this was already observed in Walnut tree (Pope et al., 2013). In the same way, the short blooming phase at Nîmes could progressively change toward extended periods of blooming time due to the overtaking of temperature thresholds during both the chill and heat periods.

As this could induce crucial consequences on fruit production in apple orchard, new cultural strategies should be investigated in European Mediterranean regions to avoid as possible the displacement of apple cropping in colder regions. Although the current strategy for adapting bud phenology involves the application of dormancy-breaking chemicals (Petri and Leite, 2004), increased awareness of the negative environmental effects has mainly resulted in breeding strategies (Celton et al., 2011). Nevertheless, the complex nature of the processes involved in dormancy release and bud growth claims for greater knowledge of the physiological mechanisms and temperature controls (Campoy et al., 2011; Atkinson et al., 2013).

1.6. Conclusions

Our results showed a large variability of blooming patterns in space and time in response to contrasting temperature levels and climate warming in a woody plant as apple tree. Over the last four decades, divergent patterns were in particularly highlighted between Brazilian mild regions and temperate cropping regions of Western Europe. They were based on opposing temperature determinism for the blooming time, a chill-control in Brazil versus a heat control in Europe. Our results also suggested opposing determinism for the blooming duration, a chill and heat-control in Brazil versus a heat-control in Europe. The combination of such opposing determinisms with no significant or weak warming in Brazil during the chill and heat periods versus marked warming in Europe during the heat period resulted in no significant blooming time change and extended durations in Brazil versus blooming advances and short durations in Europe. In this context, the French Mediterranean region (Nîmes area) experienced a marked warming since the end of the 1980s associated with a relatively high temperature level in the chill period. Thus, our study outlined its warming vulnerability in near future by experiencing extended blooming durations, as observed in the mountainous mild region of São Joaquim in Southern Brazil.

Our results supported that blooming time and blooming duration are two independent traits. More complex determinisms are suggested for the blooming duration. We also hypothesized on the levels of temperature thresholds above which extended durations unfavorable to apple cropping could be regularly observed. The current assumption of two successive chill and heat periods for analyzing the blooming traits would be not the best in some mild conditions since the analysis of Moroccan data showed less clear temperature determinisms of blooming time. All these conclusions claim for developing new research on dormancy and bud growth to improve the objectives and strategies for adapting apple industry to climate warming especially in the warmest regions of Southern Europe.

CHAPTER II

Kinetics of dormancy in apple tree cultivars grown under temperate and mild climates reveal contrasted status of buds and impose a new interpretation of classical tests

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Abstract –The intensification of warming and recent advances in phenology of apple trees in Europe requires the physiological understanding when grown under unfavorable temperature regime during the dormancy period. The aim of this study was to compare the dormancy kinetics of vegetative and floral buds between cold and mild climates. Attempt was also made to describe the variability of dormancy between cultivars at each site. Long shoots bearing vegetative buds and short shoots bearing floral buds (spurs) were collected regularly during two successive cycles in Palmas, Brazil (mild winter) (2012, 2013) and Marsillargues, France (cold winter) (2011/12, 2012/13). The kinetics of dormancy in vegetative buds was evaluated by “one-bud cutting test” and in floral buds by “Tabuenca’s test” (forcing’s test) monitoring the floral primordia weight, under forcing and field conditions. The first test highlighted important differences in the dormancy kinetics comparing both sites, and the depth level of dormancy was correlated with winter temperature. Thus, the coldest site highlighted variable dormancy depth during the beginning (shallow), middle (deep) and end (shallow) of the observed period, while in the mild site they appeared less variable and shallow over time. Distinct temporal changes between both sites were found for flower primordia weight. Moreover, the transition between endodormancy release and strict ecodormancy establishment could be estimated by significant increase of fresh and dry weight. Also, the establishment of ecodormancy was linked to significant changes in dry weight and the capacity to reach rapidly higher water content (77%) according to the Tabuenca test. High temperatures in Brazil allow a quick transition between endo- and ecodormancy relating to France. The weak endodormancy associated with a quasi-absence of ecodormancy could explain the strong spatiotemporal heterogeneity of budburst and flowering under mild climate. This may be useful for understanding the future phenology under global warming conditions and for the selection of adapted varieties.

Keywords: vegetative buds, MTB, budburst, ecodormancy, endodormancy, floral primordia, fresh weight, dry weight, water content.

2.1. Hypotheses

It is suspected that the physiological behavior of apple cultivars during winter depends on the climate conditions to which they are subjected. The evolution of floral primordia weight under field conditions and under forcing during the winter may be a good indication of the date of endodormancy release in floral buds. Furthermore, it is believed that mild winter conditions provide superficial dormancy kinetics that is different from cold regions where the kinetics may be deeper.

2.2. Objectives

This study aimed to compare the kinetics of dormancy of vegetative buds from different apple cultivars in contrasting climates (Western Europe and Southern Brazil) using the ‘one-bud cutting test’. Furthermore, we aimed to verify which temporal evolution of the weight and water content of the floral primordia during the winter can be used as an indicator of endodormancy release date.

2.3. Materials and Methods

2.3.1. Characterization of the study location

The experiments were conducted during two successive years in Brazil (2012 and 2013) and in France (2011/12 and 2012/13). In Brazil, adult plants of ‘Eva/Maruba’, ‘Fuji/M9’ and ‘Gala/M9’ apple cultivars were used. These were cultivated at Palmas, Paraná (51°59’W, 26°29’S; 1356 m a.s.l.) in the farmer of Mr. Geraldo Lovo. The local soil was classified as an ALIC CAMBISOIL under subtropical climate. The annual mean temperature is 17,3°C, ranging from a mean of 14,5°C from April to July and 15,8°C from August to October. The mean annual precipitation is 2110 mm (Figure 9).

In France, the experiment was conducted using adult apple trees from the cultivars ‘Gala/M9’, ‘Golden Delicious/M9’ and ‘Granny Smith/M9’, in an orchard belonging to the *Centre Expérimental Horticole de Marsillargues* (CEHM) [Marsillargues Experimental Horticultural Center] – Languedoc-Roussillon (04°09’E; 43°37’N, 2 m a.s.l.). The soil of the region was classified as SILTY CLAY under temperate Mediterranean climate. The annual mean temperature is 14,7°C, with means of 10,1°C from October to January and 10,5°C from February to April, with an annual mean precipitation of 629 mm accumulated primarily during the winter (Figure 9).

Figure 9. Details of the orchards of the common variety Gala, cultivated in Palmas, Paraná, Brazil (a) and in Marsillargues, Languedoc-Roussillon, France (b), in the months corresponding to June and December, respectively.

2.3.2. Primordia weight dynamics and endodormancy release date

To observe the temporal evolution of the weight of the floral primordia, we used the Tabuenca's test (Tabuenca, 1964). A total of 30 short shoots bearing terminal flower buds were sampled weekly in Palmas (from May to September) and in Marsillargues (from November to April). At every sampling, the shoots were separated into two sets: in the first one (field condition), 30 floral buds were peeled to obtain the fresh and dry weight of the primordia (six groups of five primordia each; dehydration at 90ºC); the second one was stored in a forcing chamber (24°C±1; 16-hr photoperiod) with a regular supply of water. After seven days, 30 floral primordia forced were used to obtain the fresh and dry weight (Figure 10).

The date of endodormancy release was defined by observing the first significant increase of weight of the floral primordia after forcing, in relation the unforced primordia (field condition). Additionally, the fresh weight and dry weight of the floral primordia in each sampling were used to calculate the water content (%) according to the equation $\%H_2O=[(FW-DW)*100]*FW^{-1}$, where FW = fresh weight, and DW = dry weight.

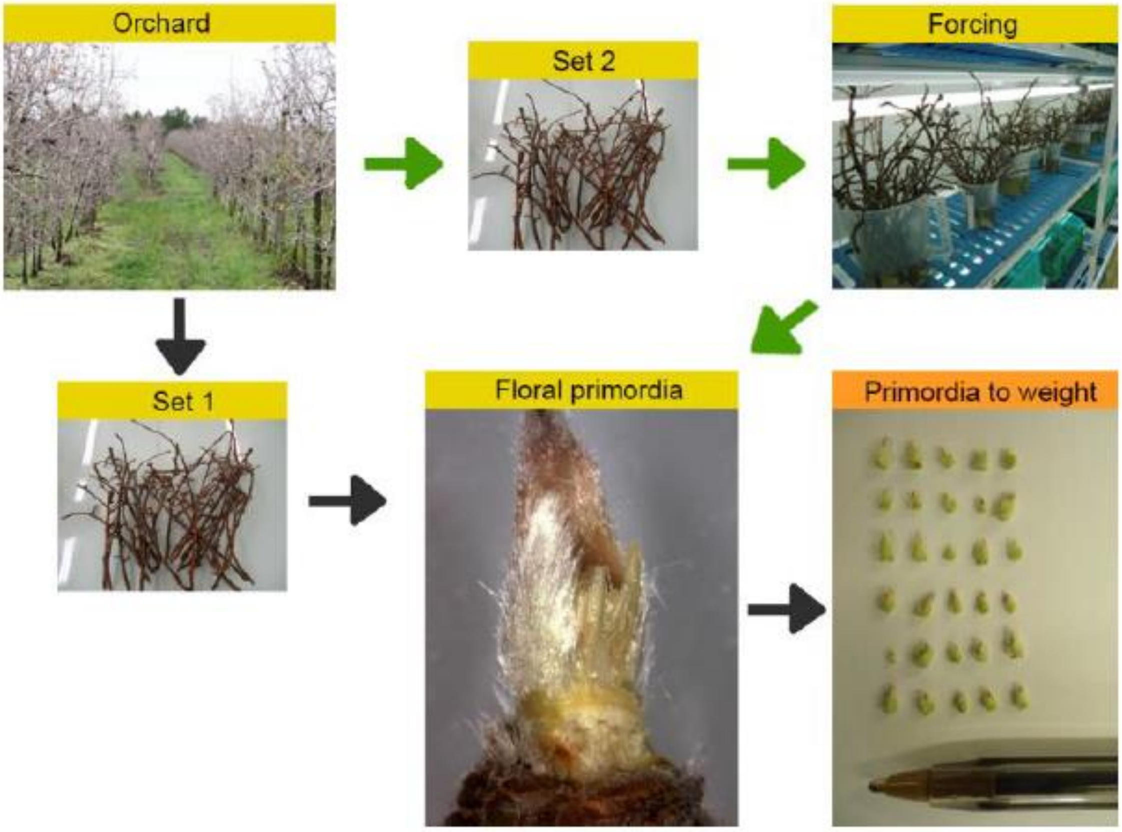

Figure 10. Procedure performed for extracting and weighing the floral primordia.

2.3.3. Budburst capacity and dormancy phases

For evaluate this capacity, the Single-node cutting test (Pouget 1963; Rageau 1982; Dennis 2003) was applied to vegetative buds bearing by long shoots (45 to 55 cm) were randomly sampled from the trees. In Palmas, the samplings were performed every two weeks from March to September and weekly from July to August. In Marsillargues, samplings were performed every two weeks from September to March, and weekly from December to January. From the intermediate portion of the shoots, cuttings of 7cm were prepared containing only one bud, for a total of 40 and 80 cuttings per sampling in Palmas and Marsillargues, respectively. The cuttings were stored in a forcing chamber (24°C±1; 16hr photoperiod) with a regular supply of water (Figure 11).

In Palmas, phenolic foam was used to fixate the cuttings in 2012 and 2013. In Marsillargues, rock wool was used in 2011/12, and plastic screens to suspend cuttings in 2012/13. At each sampling date, observations were performed every two days during two months to determine the occurrence date of the ‘green-tip’ stage (BBCH 07) (Meier, 2001). After this period, the percentage of budburst and the Mean Time of Budburst (MTB) were determined for each sampling date (Figure 11).

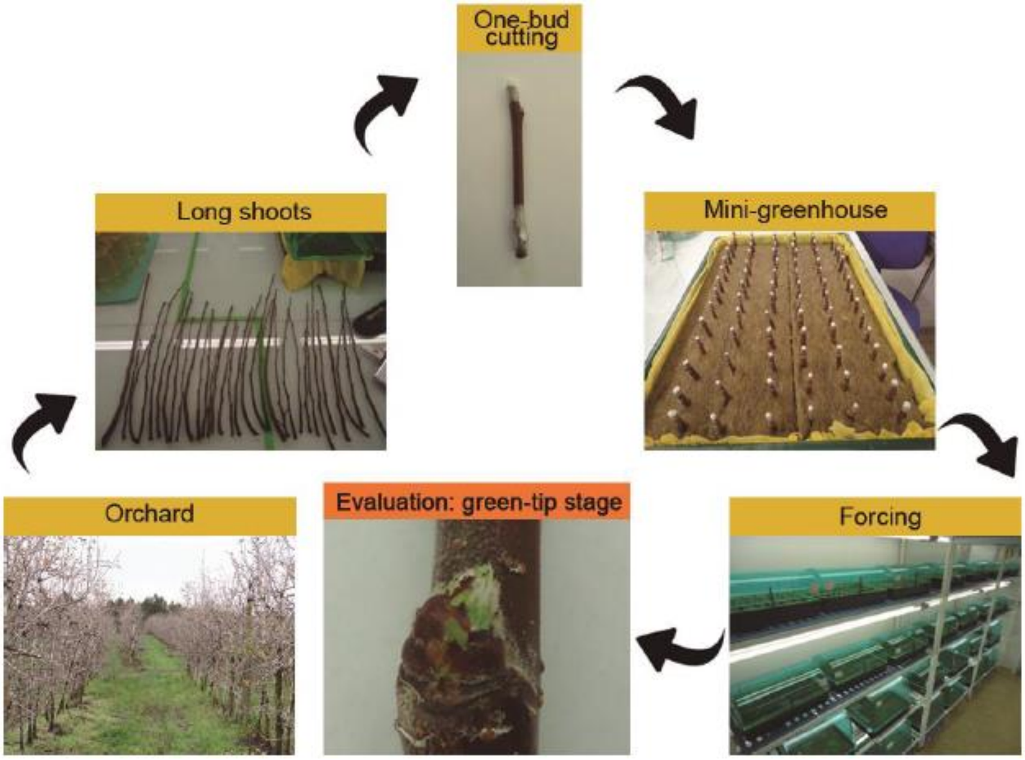

Figure 11. Procedure performed for the preparation of the one-bud cuttings.

2.3.4. Statistical procedures

The data were initially subjected to the Shapiro-Wilk test to verify the normality of the data. The data obtained for all samplings were not-normal. Consequently, the temporal variations of the data related to the two forcing tests were evaluated using the confidence interval with the bootstrap procedure with 5000 resampling ($p \leq 0,05$) using the software BioEstat 5.0®. The dormancy kinetics of the vegetative buds and the temporal changes of the fresh and dry weight of the floral primordia (with and without forcing) were represented graphically.

The chill accumulation between October (of year *n-1*) and April (of year *n*) in Marsillargues and between April and October (of year *n*) in Palmas were also calculated using the Weinberger (Weinberger, 1950), Bidabe (Bidabe, 1967), Landsberg (Landsberg, 1974), Utah (Richardson et al., 1974), North Carolina (Shaltout and Unrath, 1983), Dynamic (Fishman et al., 1987b), Positive Chill Unit (Linsley-Noakes et al., 1995), DVI (Developmental Stage Index) (Sugiura and Honjo, 1997, cited by Yamamoto et al., 2010), Unified (Chuine, 2000), and F1Gold1 and F1Gold2 (Legave et al., 2013) models. The daily variations in chill accumulation were represented graphically according to the Dynamic and Unified models; furthermore, they indicate the total chill accumulated until the endodormancy release date estimated by fresh weight of the floral primordia.

2.4. Results

2.4.1. Winter changes in bud weight – dynamic of floral primordia fresh weight during winter

The fresh and dry weight of the floral primordia during the dormancy period showed distinct patterns between Marsillargues and Palmas. However, the pattern of weight evolution was similar during the two cycles evaluated and important variations were found among cultivars in each site. When analysing the fresh weight of the floral primordia in the first cycle at Marsillargues, the first significant increase in weight after forcing, relative to the primordia in field conditions, was observed early, on January 10th for Granny Smith (GS) (Figure 12). In contrast, this same change occurred later in 'Gala', on January 31st. The Golden Delicious (GD) cultivar showed a significant increase in weight slightly earlier than 'Gala', on January 24th. During 2012/13, the same pattern was observed for GS, with an early differentiation in weight, on January 9th. An inverse pattern was observed in 2012/13 when GD showed a change in weight on January 30th and 'Gala' one week earlier, on January 23rd. The fresh weight also revealed important differences among cultivars. 'Granny Smith' appeared to require less chill to overcome endodormancy than 'Gala' and GD, which required the same amount of chill. However, the absolute weight values indicated that the significant increase of the fresh weight in GD after forcing occurred with relatively low values (always below 9 mg) in both cycles of dormancy. For 'Gala' and GS, this always occurred with values above 12 mg (Figure 12).

For the dry weight, it was observed that the first significant change after forcing in 2011/12 occurred on February 14thfor all three apple cultivars; this date was later than that recorded for fresh weight during the same period. Similarly, in 2012/13, the change in weight occurred on February 6th, approximately one week earlier than in the previous year; however, this change also occurred at a later date compared with the fresh weight. In addition, the time elapsed between the significant change in fresh weight under forcing conditions and the increasing of weight in the field was seven weeks for GS and four weeks for GD and 'Gala', for both dormancy cycles. Considering the dry weight, this difference was three weeks for all three cultivars (Figure 12).

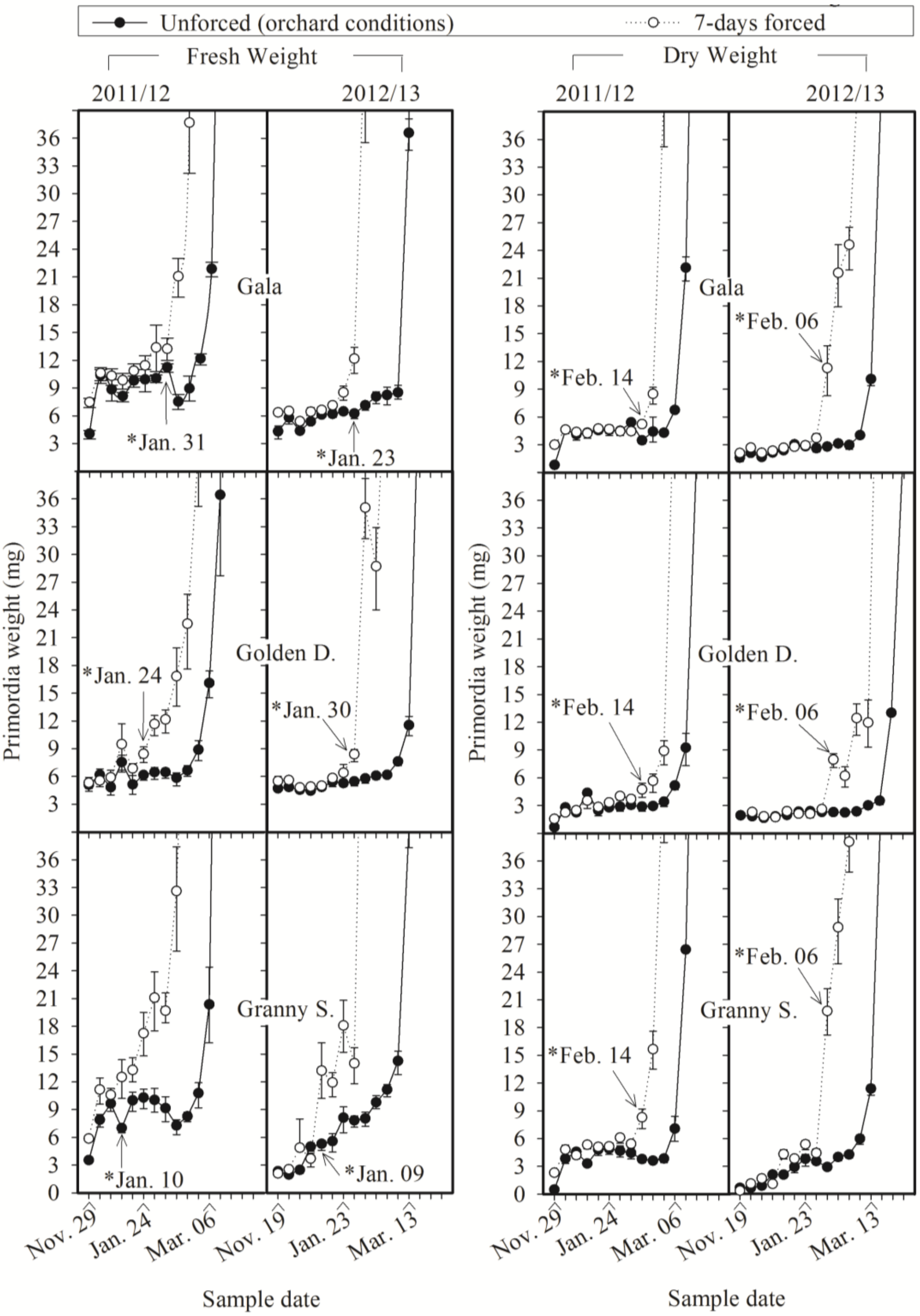

Figure 12. Fresh and dry weight of floral primordia, before and after forcing, for 'Gala', 'Golden Delicious' and 'Granny Smith' apple cultivars, during 2011/12 and 2012/13 in Marsillargues, France. * Dates followed by an arrow indicate a significant change (*$p<0.05$*) in weight.

Regarding the evolution of the fresh weight in Palmas, a similar evolution was observed for the 'Fuji' and 'Gala' cultivars for both dormancy cycles. During the first cycle, the significant weight increase for 'Fuji' occurred on August 23rd, while it occurred one week later for ‘Gala’, on August 30th. During the second cycle, however, an inverse pattern for these same cultivars was observed, with a change in weight in ‘Gala’ on September 7th, while in ‘Fuji’ it was one week later, on September 14th For ‘Eva’, the increase in weight occurred early, on July 12th in 2012, and very early, on June 8th in 2013 (Figure 13).

Considering the changes in the dry weight, ‘Fuji’ and ‘Gala’ showed similar patterns, with a significant increase on September 6th in 2012 and, later, on September 14th in 2013. During the two cycles, the significant change in dry weight occurred later for ‘Gala’ compared with the same response shown for the fresh weight. An exception was observed for ‘Fuji’ in 2013, as the significant increase of the fresh and dry weight occurred at the same time on September 14th. For ‘Eva’, the first significant change in dry and fresh weight occurred on the same date; however, this change happened long before that of ‘Fuji’ and ‘Gala’, on July 12th in 2012 and quite early in 2013, on June 8th. The early endodormancy release followed by the rapid development of buds predisposes the ‘Eva’ cultivar to frost damage, as observed in 2012 (Figure 13).

In general, the results indicate a slow temporal progression of the fresh and dry weights, from the beginning of the observations until endodormancy release, for all three cultivars evaluated in Palmas, with it being most evident for ‘Fuji’ and ‘Gala’. However, sudden increases to the fresh and dry weight under field conditions were recorded at the same time or one week later than the significant increase to the fresh and dry weights under forcing conditions, for all three cultivars evaluated (Figure 13). For ‘Gala’, the common cultivar studied at Palmas and Marsillargues, the absolute values of fresh and dry weight followed similar patterns; however, the weight under field conditions at Palmas increased rapidly after endodormancy release.

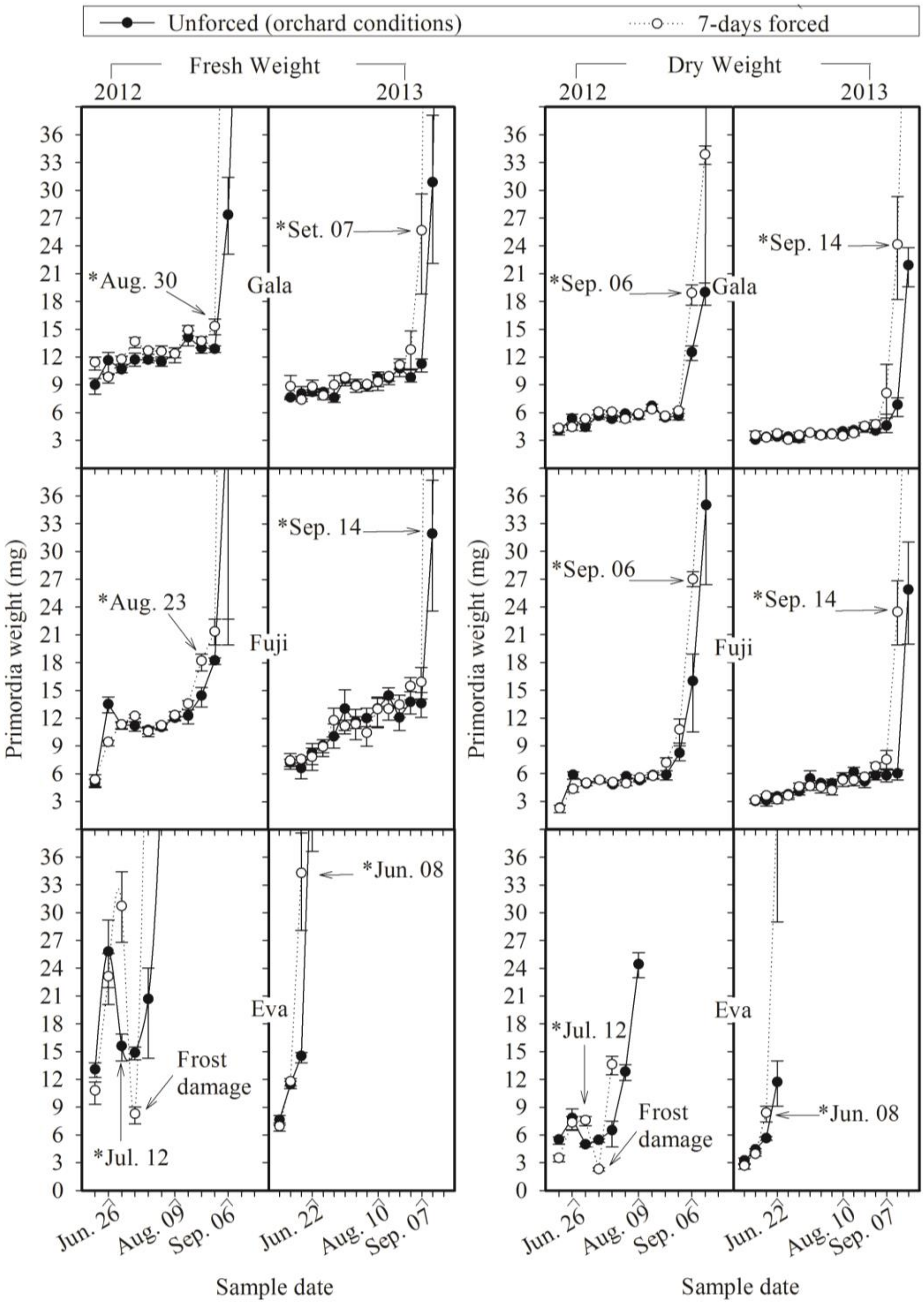

Figure 13. Fresh and dry weight of floral primordia, before and after forcing, for ‘Eva’, ‘Fuji’ and ‘Gala’ apple cultivars during 2012 and 2013 in Palmas, Brazil. * Dates followed by an arrow indicate a significant change ($p<0.05$) of weight.

2.4.2. Water content of floral primordia during the dormancy period

The results highlighted temporal variations with notable differences between Marsillargues and Palmas, among the cultivars and between both dormancy cycles (Figure 14).

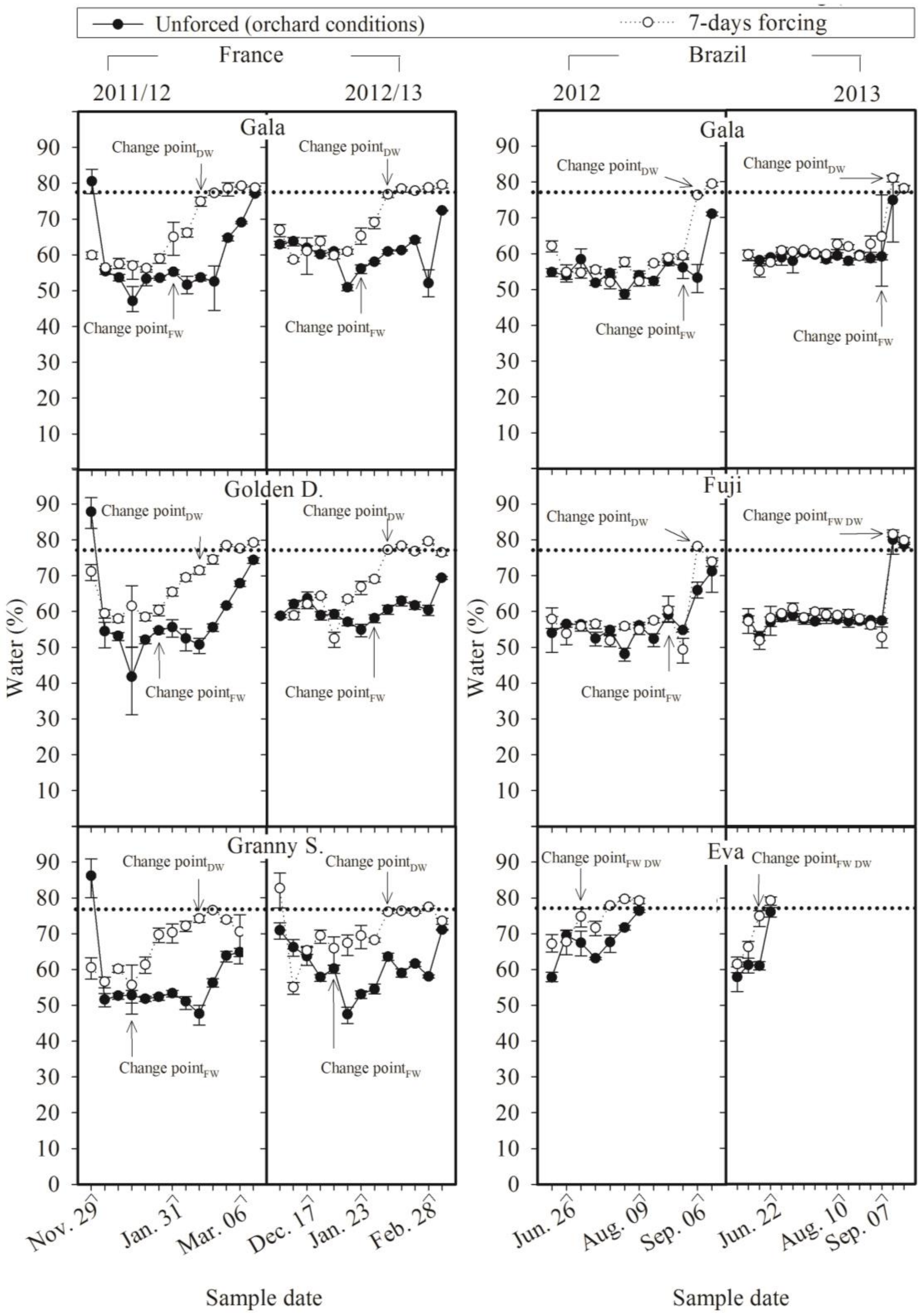

Figure 14. Water content (%) in floral primordia, before and after forcing, for ‘Gala’, ‘Golden Delicious’ and ‘Granny Smith’ in Marsillargues, France, in 2011/12 and 2012/13, and for ‘Eva’, ‘Fuji’ and ‘Gala’ in Palmas, Brazil, in 2012 and 2013. * The arrow shows the date when the fresh weight (FW) and dry weight (DW) of floral primordia increased significantly under forcing conditions relative to field conditions, according to figures 12 and 13.

At Marsillargues, the water content of the floral primordia under field conditions remained stable around 50% during 2011/12 and was slightly higher during 2012/13, from initial samplings until the significant increase of the dry weight (Change point$_{DW}$). After this period, a progressive increase was evident, especially during 2011/12, for all tree cultivars. On the other hand, the water content under forcing conditions remained near 60% (70% for GS in 2012/13) from the initial samplings until the significant increase to the primordia fresh weight (Change point$_{FW}$). Subsequently, a progressive increase was observed followed by stabilization close to 77% for all cultivars and both dormancy cycles (Figure 14).

At Palmas, significant changes to water content between ‘Change point$_{FW}$’ and ‘Change point$_{DW}$’ occurred within one week. Therefore, before these changes the mean water content recorded was 50-60% for ‘Gala’ and ‘Fuji’, close to Marsillargues values, and 60-70% for ‘Eva’. Later, an abrupt increase was observed reaching a mean value of 77% for the primordia under forcing conditions, as observed at Marsillargues. Under field conditions, the abrupt increase occurred (except for ‘Fuji’ in 2013) after ‘Change point$_{FW}$’, as observed at Marsillargues.

An interesting finding was that the floral primordia ‘Change Point$_{DW}$’ coincided both at Marsillargues and Palmas with the initial stabilization of floral primordia water content (approximately to 77%) under forcing condition, for all cultivars. In addition, ‘Change Point$_{DW}$’ also coincided with the beginning of the progressive increase of floral primordia water under field conditions. Furthermore, the water content curves under both forcing and field conditions were very close at Palmas during the dormancy period. In contrast, the difference between curves was larger, and took longer to equalize for all of the cultivars studied at Marsillargues (Figure 14).

2.4.3. Date of endodormancy release and duration of the ecodormancy and pre bloom period

The analysis of the time elapsed between the date of endodormancy release (given by Tabuenca’s test) and blooming date in the field showed, at Marsillargues, 47 and 71 days for ‘Gala’, respectively, in 2011/12 and 2012/13. This same period was also long in duration for GD, lasting for 48 days in 2011/12 and 73 days in 2012/13. For GS, the length of this period was 47 days in 2011/12 and 71 days in 2012/13 (Table 8).

Table 8. Dates of endodormancy release[a], BBCH 61 (beginning of blooming) date, and variation in days between these dates at Marsillargues, France, and Palmas, Brazil, for two cycles of dormancy.

Apple cultivar	Marsillargues - France			Palmas - Brazil			
Year	Date of end. Release	BBCH 61 date	Variation (days)	Year	Date of end. Release	BBCH 61 date	Variation (days)
Gala				Gala			
2011/12	Feb. 14th	Mar. 31st	47	2012	Sep. 06th	Sep. 20th	15
2012/13	Feb. 06th	Apr. 16th	71	2013	Sep. 14th	Oct. 10th	27
Golden				Fuji			
2011/12	Feb. 14th	Apr. 01st	48	2012	Sep. 06th	Sep. 20th	15
2012/13	Feb. 06th	Apr. 18th	73	2013	Sep. 14th	Oct. 10th	27
Granny				Eva			
2011/12	Feb. 14th	Mar. 31st	47	2012	Jul. 12th	Jul. 26th	14
2012/13	Feb. 06th	Apr. 16th	71	2013	Jun. 08th	Aug. 03rd	56

[a] date given by floral primordia dry weight in the Tabuenca's test.

At Palmas, floral primordia weight under field and forcing conditions increased quickly after the change point FW. As a result, short periods were recorded for 'Gala' (15 days), 'Fuji' (28 days) and 'Eva' (14 days) in 2012. In the last year, the late occurrence of chilling temperature (T<12°C) delayed the endodormancy release for 'Gala' and 'Fuji', extending the period (27 days) in comparison with the previous year. For 'Eva', the low temperatures in July and August delayed the F1 date

2.4.4. Winter changes in budbreak capacity

The kinetics of dormancy in vegetative buds of apple tree cultivars were assessed in the northern (cold climate) and southern (mild climate) hemispheres. The cycles of dormancy in Marsillargues (France) and in Palmas (Brazil) were compared to assess the existence of annual variations and environmental effects in each location. The curves shown in Figures 9 and 10 correspond to the state of dormancy or the kinetics of dormancy of the vegetative buds of apple trees collected in the field from the end of the harvest until the beginning of budburst the following cycle. During the first winter, at Marsillargues, several distinct phases were observed during dormancy and this was most evident for 'Golden Delicious' (GD) (Figure 15).

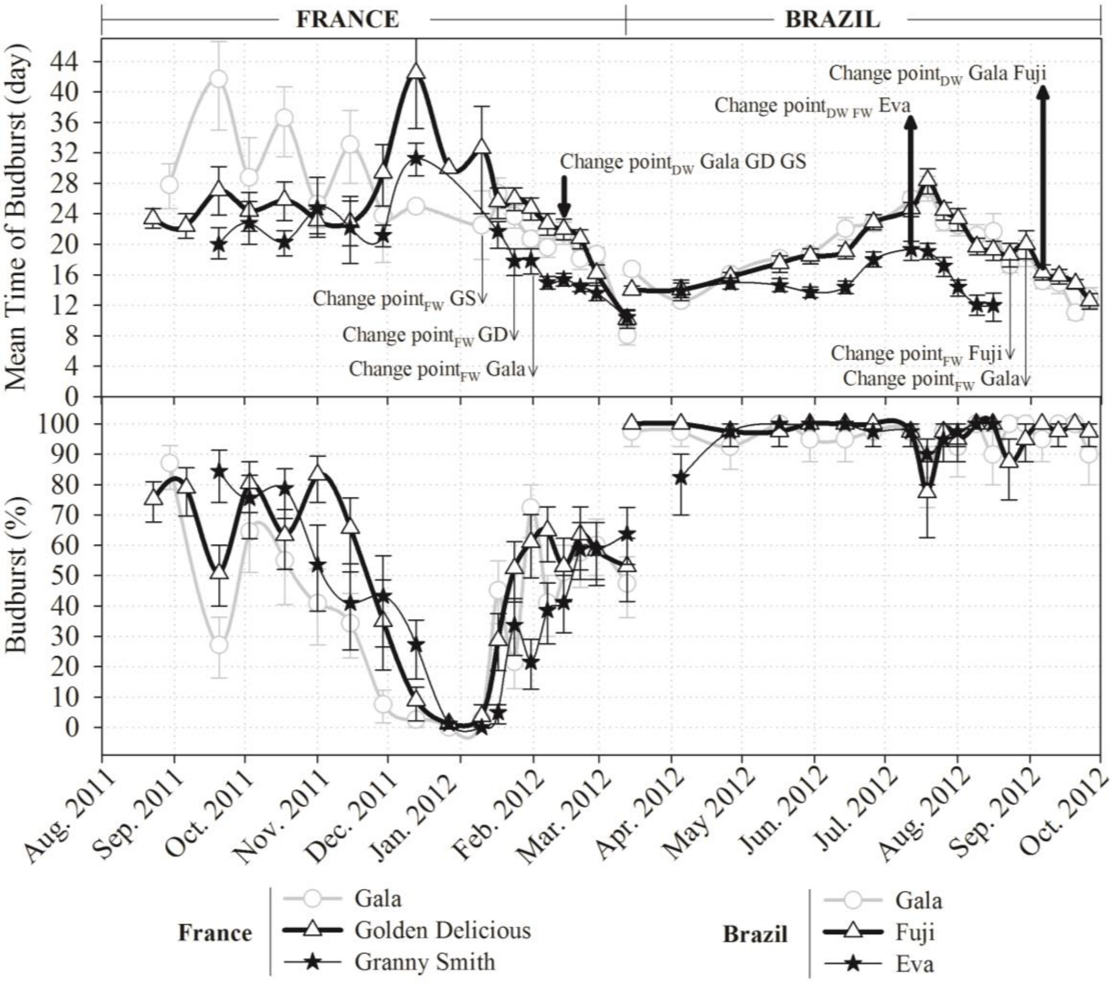

Figure 15. Time-course changes in Mean Time of Budburst (MTB) and budburst rates, during winter 2011/12 at Marsillargues, France for ‘Gala’, ‘Golden Delicious’ and ‘Granny Smith’ apple cultivars and at Palmas, Brazil (2012) for ‘Gala’, ‘Fuji’ and ‘Eva’ apple cultivars. The vertical bars represent the confidence interval at 5% of significance. The arrows indicate the date where the significant change of fresh and dry weight of floral primordia in Fuji, Gala, Golden Delicious and Granny Smith cultivars were recorded after 7-days forcing, according Tabuenca’s test.

A first phase occurred between the end-August and mid-November with stable Mean Time of Budburst (MTB) (close to 24 days) and mean of 70% of budburst (Figure 15). Then, between the end-November and beg-January, a second phase was recorded expressing elevated MTB values (peak) and a strong reduction of budburst rate to levels close to zero. This change coincided with the decrease in temperatures during a long period under 15°C. From the mid-January to mid-February, a third phase was characterized by a slow decrease of MTB values and an increase of budburst rate up to 60%. Finally, a rapid decrease of MTB occurred until mid-March. However, the budburst rates remained lower than those recorded in the first phase. Similar kinetics of MTB and budburst rates was observed for ‘Granny Smith’ (GS). However, for ‘Gala’, the MTB values recorded did not correspond well to this kinetics and only the third and fourth phases follow the GD one. In the first phase, Gala showed large MTB fluctuations and no clear peak of MTB, similar to that for GD was observed. Regarding GS, the MTB values were always lower over the dormancy period (Figure 15).

In Palmas the cultivars ‘Fuji’ and ‘Gala’ showed similar pyramid-shaped MTB curves. Initially, lower MTB values (15 days) were found, increasing slowly up to the end of May, followed by a stronger increase until an MTB peak (28 days) at mid-July. A strong decrease of these values was recorded until the beg-August followed by a sort of plateau until end of august. Then a rapid decrease was observed. In contrast, ‘Eva’ cultivar showed lower MTB values than ‘Fuji’ and ‘Gala’ over the period evaluated. A clear peak of MTB but with low value (19 days) was also observed in mid-July. Regarding the budburst rates, for the three cultivars, the values were always close to 100%, with a slight decrease to 80% in the mid-July, coinciding with the maximum MTB. Another small decrease appeared mid-August, and both coincided with drop of temperature under ten degrees (Figure 15).

During the second year (2012/13), in Marsillargues, the MTB and the budburst kinetics showed a trend very close to that observed in 2011/12, for the three cultivars. The main differences concern the lower values of MTB especially during the first phase, the longer and more precocious period of high MTBs (Figure 16). The period of high MTBs seems to begin when temperatures passed under 15°C. For ‘Gala’, unlike the previous year, the MTB kinetic was very similar to the other cultivars, and the peak was observed at mid-December. For GS, the MTB values were lower than GD and ‘Gala’ over time and the MTB peak was recorded later (January 7th) compared to the other cultivars and to the first cycle. Regarding the budburst rate values of GD and ‘Gala’, the decrease correspond with the phase of increasing MTB. For GS we found an early decrease (between the end-August and end-October) relating to 2011/12 and others cultivars. Low rates of budburst during winter were recorded during a longer period than in the previous year for all cultivars. Furthermore, budburst rates remained low (Figure 16).

At Palmas, the dormancy kinetics in 2012, did not clearly define the phases observed in Marsillargues in the two dormancy cycles (Figure 10) and considerable oscillations in the budburst values were recorded, mainly for ‘Gala’ and ‘Fuji’ that showed similar MTB curves over time. Only the plateau and the last phase of strong decrease of MTB in September appeared clearly. Regarding ‘Eva’ cultivar, lower MTB values and higher budburst rates were always showed over time, and the abrupt decrease in MTB values occurred two months earlier, comparing with ‘Fuji’ and ‘Gala’ (Figure 16).

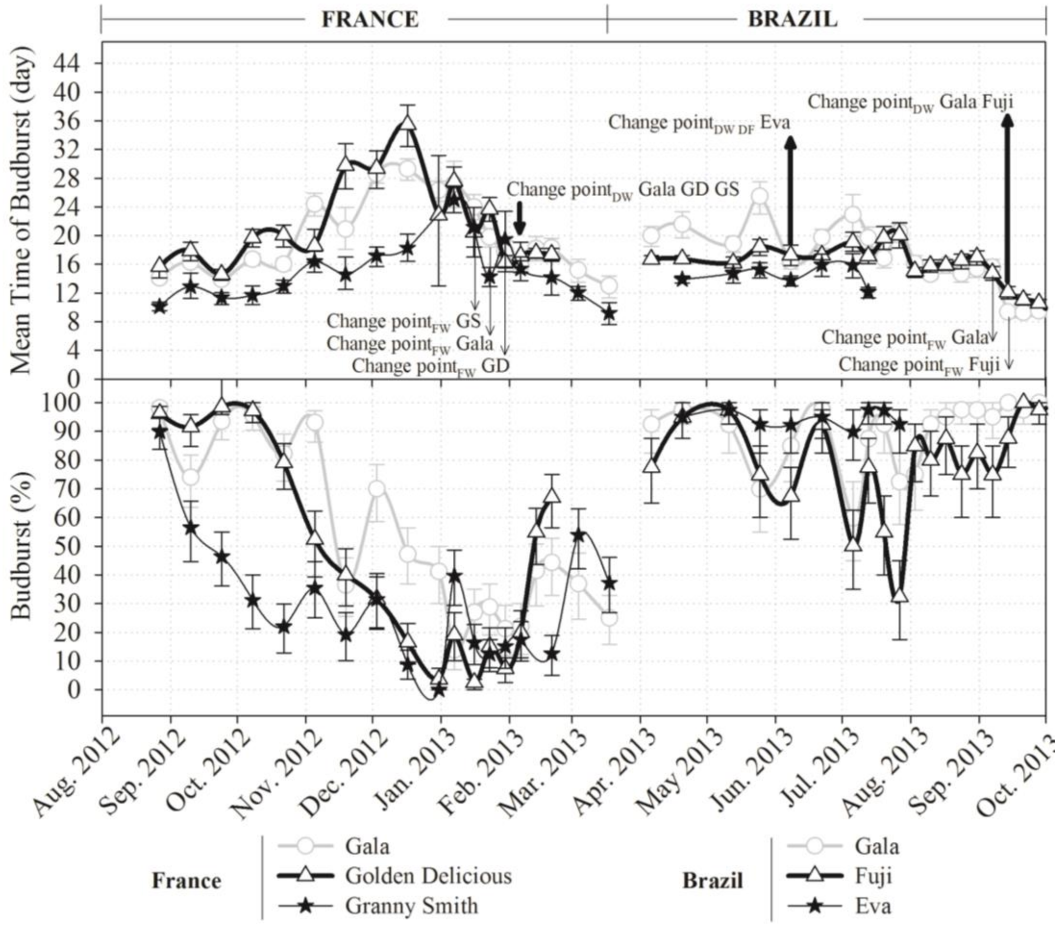

Figure 16. Time-course changes Mean Time of Budburst (MTB) and budburst rates during winter (2012/13) at Marsillargues, France for ‘Gala’, ‘Golden Delicious’ and ‘Granny Smith’ apple cultivars and at Palmas, Brazil (2013), for ‘Gala’, ‘Fuji’ and ‘Eva’ apple cultivars. The vertical bars represent the confidence interval at 5% of significance. The arrows indicate the date where the significant change of fresh and dry weight of floral primordia in Fuji, Gala, Golden Delicious and Granny Smith cultivars were recorded after 7-days forcing, according Tabuenca’s test.

2.4.5. *Main changes in mean winter temperatures and chill accumulation during both years and sites*

At Marsillargues the mean temperatures (approximately 20°C) in October began to decrease progressively until December, and then oscillated between 0 and 10°C. The decrease seem to be more rapid in 2012 than in 2011 and this could be related to the more precocious increase of MTB. Later (end-February) the temperatures began progressive increase until flowering. In 2011/12, an abrupt decrease in mid-February led to negative temperatures during several days (Figure 17). At Palmas, three cold period with temperatures below 12°C occurred in 2012, between April and the end-July. Such drop appeared later (mid-July) in 2013. Except these periods, the temperatures in Palmas remained close to 15°C and always higher than 12°C.

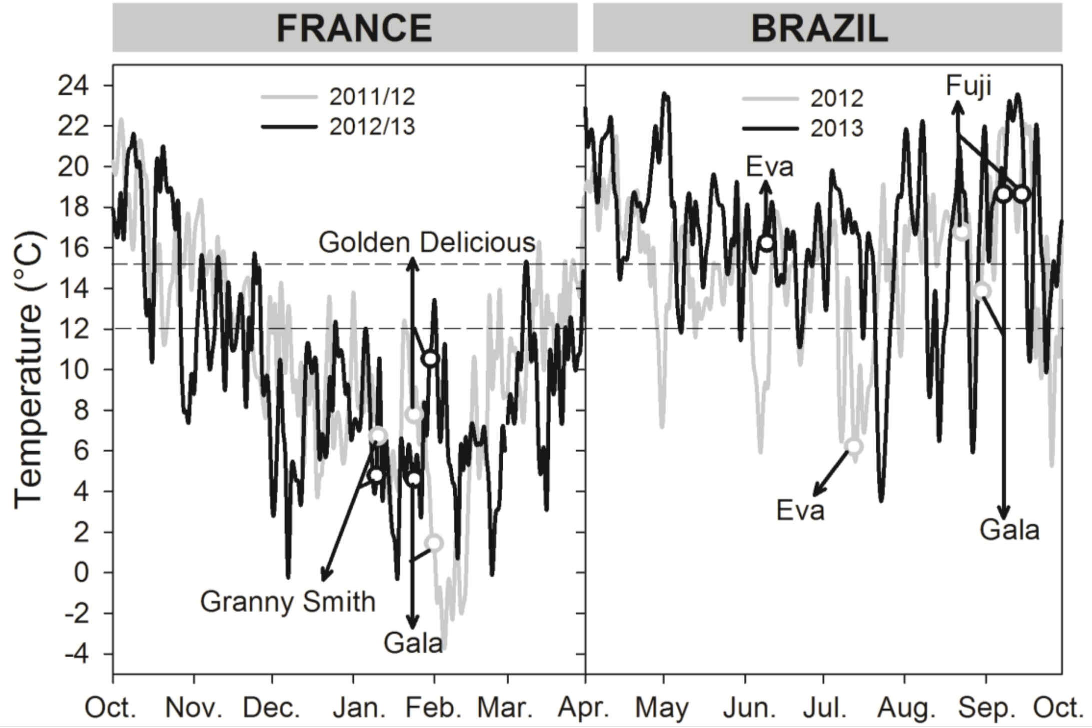

Figure 17. Mean temperatures recorded during the dormancy cycle in Marsillargues, France, during the 2011/12 and 2012/13 periods, and in Palmas, Brazil, during the 2012 and 2013 periods. The circles indicate the moment when endodormancy was released.

To better understand the effect of chill on the previously observed variation of flowering dates, the chill accumulation was estimated. For the establishment of a relationship, the significant changes of the fresh weight of the floral primordia were considered as a marker of endodormancy release date as explained in the section 4.2. In Marsillargues, a constant pattern of accumulation was observed over the dormancy period. The late endodormancy release in Marsillargues in 2011/12 compared to 2012/13 was possibly a reflection of the low chill accumulation between October and mid-November, according to the Dynamic and Unified models (Figure 18).

In 2012/13, the constant low temperatures recorded between November and the end-February favoured the more rapid chill accumulation, which allowed an early endodormancy release for the three cultivars compared to 2011/12. The total chill accumulated between October and the estimated date of endodormancy release (EDER) (dry weight of floral primordia – Feb 14th) in 2011/12, according to the Dynamic model was 58,3 portions for all three cultivars. In 2012/13 there was accumulated 66,8 portions also for all cultivars. In contrast, the chill accumulation in 2011/12 according to the Unified model was 2022,7 chill units for all cultivars. In 2012/13, this same model indicated a chill accumulation of 2064,6 chill units (Figure 18).

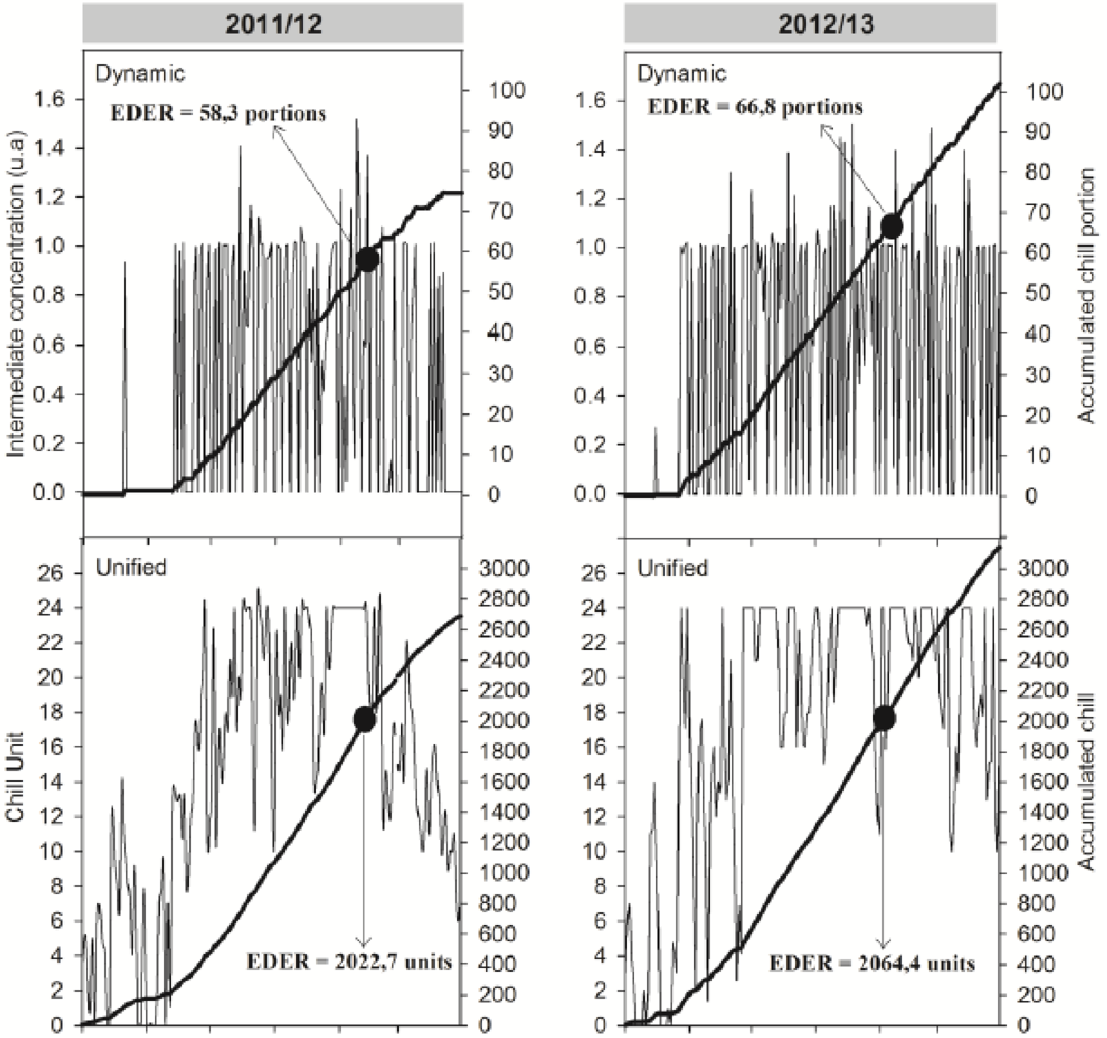

Figure 18. Variations in the intermediate concentration (--) and accumulated chill portion (▬) by the Dynamic model and chill unit variation (--) and accumulated chill (▬) by the Unified model as a function of hourly temperatures in Marsillargues - France, between October and March, in the periods 2011/12 and 2012/13. a.u.: arbitrary unit.

In contrast, in Palmas, a considerable daily fluctuation in chill accumulation was observed in 2012 and 2013 with much lower accumulated totals between April and

October compared to the period from October to April in Marsillargues (Figure 19). In 2012, the chill accumulation was concentrated between May and the end-July, and the accumulated chill between April and EDER (September 06th)was 30,7 portions and 1581,8 chill units for ‘Fuji’ and ‘Gala’, according Dynamic and Unified models, respectively. For ‘Eva’ this was estimated as 20,9 portions and 1017,2 chill units (from April to July 12th (EDER)) (Figure 19).

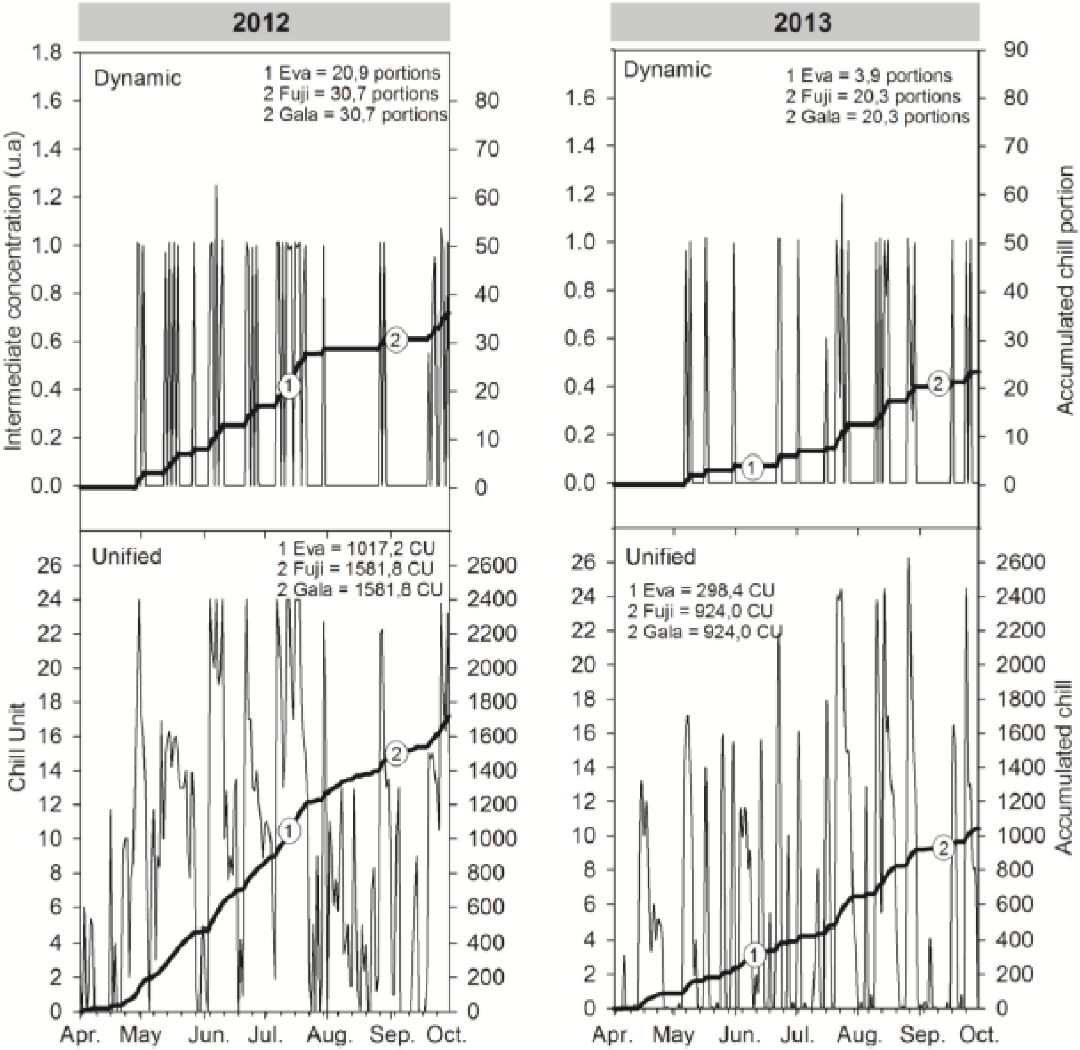

Figure 19. Variations in the intermediate concentration (--) and accumulated chill portion (▬) by the Dynamic model and chill unit variation (--) and accumulated chill (▬) by the Unified model as a function of hourly temperatures in Palmas, PR - Brazil, between April and September, in the period 2012 and 2013. a.u.: arbitrary unit.

In 2013, however, the chill accumulation was concentrated between July and end-September, totalling a cumulative amount lower than those recorded in the previous year. The late endodormancy release observed in ‘Fuji’ and ‘Gala’ in 2013 was possibly related to the low quantity of accumulated chill during the winter. The quantities of chill accumulation recorded in the EDER were also relatively lower than in 2012. For instance, 'Gala' and 'Fuji' accumulated 20,3 portions and 924,0 chill units. In relation to 'Eva', an accumulation of only 3,9 portions and 298,4 chill units was observed in 2013. The disparity between the total amounts of chill accumulated in the two years of the study may indicate that 'Eva' does not effectively enter endodormancy during the winter. Thus, the early chill in 2012 delayed the beginning of floral development of this cultivar, and the subsequent warm accelerated flowering. In 2013, the warm at the beginning of winter accelerated floral development and the late chill contributed to the delay in flowering (Figure 19).

2.5. Discussion

2.5.1. Dormancy release, bud weight and water content

Few works relate the possibility of marking the endodormancy release by monitoring the development of floral primordia and water content (Tabuenca 1964, Legave and Garcia, 1982 on apricot; Bonhomme et al., 1997; Leite et al 2006 on peach). Our results showed that significant increases to floral primordia fresh and dry weight under forcing conditions in comparison with unforced (field conditions) can be linked to endodormancy release, the establishment of ecodormancy and the end of ecodormancy in floral buds. Indeed, the initial increase to fresh weight was the result of water accumulation in the floral primordia probably through cell-cell transport and parietal pathway, since the vascular system is still not differentiated by this phase (Aloni, 1987). This is in agreement with the observations done by van der Schoot and Rinne (1999, 2011), Rinne and Van der Schoot (2004) on the isolation of the meristematic zone during endodormancy and the retrieval of symplastic pathway with endodormancy release (Rinne et al., 2001). It is also in agreement with the possible closure of the plasmodesmata observed by Gamalei (1985) for temperatures below 10°C. A similar increase in the fresh weight of buds with endodormancy release (but without comparison with under field conditions) was obtained by forcing peach tree buds (Sugiura et al., 1995) and including this comparison by Bonhomme et al (1997) and Leite et al. (2006). The capacity of rehydration probably revealed the end of endodormancy and a transition into ecodormancy. Nevertheless, when append, in temperate zone, the transition is not rapid and the capacity of full rehydration needs the time laps between fresh change point and dry change point. This seems dependent of the temperatures which are not high enough to permit an active metabolism. The metabolic activity would be more and more active according to the progressive increase of the temperature in the field leading to permit the detection of a significant increase of dry mass after seven days of the forcing test, revealing the recovery a full growth capacity.

Under mild climate as in Palmas, the recovery of full capacity of rehydration and growth is very fast, due to the level of temperatures (above 15°C). So the question is: what should be considered as the end of endodormancy? Is the beginning of rehydration capacity (fresh weight change point) or the full rehydration capacity and detectable dry matter accumulation? A possible hypothesis would be that, since the isolation of the meristematic zone is broken, the influx and metabolic activity could react in function of the level of the temperatures, increasing the osmotic potential of the cells and their sink strength. For us, the second step showing a full capacity of rehydration indicates the end of endodormancy and the ecodormancy state, but we agree that this needs further investigations and especially with histological approaches.

The water state of floral and vegetative buds was also investigated in apple trees (Faust et al., 1991, 1995) using magnetic resonance imaging to determine the water state of the buds with the aim of linking these images to the dormancy state. It was found that the conversion of bound water to free water within the cell structures of the buds occurred progressively during the period of chill accumulation (but without biological tests in parallel). Thus, during endodormancy, the water remained bound to the cellular structures at low quantities for the maintenance of basic reactions only. However, in buds that had their chilling requirement satisfied the water became free in the cells and could be detected but not quantified.

The increase of dry weight of peach tree floral buds after forcing was related to the establishment of ecodormancy (Sugiura et al., 1995). It is well known that carbohydrate metabolism changes progressively during endodormancy (Marafon et al., 2011) but the largest changes occur during ecodormancy (Bonhomme et al., 2005). Leite et al. (2006) observed an increase in respiration, changes in the carbohydrate metabolism and an increase in the ATP/ADP ratio in the buds during ecodormancy as previously showed by Bonhomme et al.(2000). Furthermore, in this phase, a larger volume of water appears to migrate to the buds due to progressive vascular differentiation (Sugiura et al., 1995) even if Bartolini and Giorgelli (1994) showed that complete vascular connection are useful only a short time before bloom. In contrast, the transition from endodormancy to ecodormancy was characterized by the active transport of sorbitol from the xylem to the floral buds in *Pyrus pyrifolia*. Thus, the increase of the dry weight and water content of the floral primordia may have been the result of the translocation of a larger volume of solutes, as has been reported for other temperate climate species (Essiamah and Eschrich, 1986). Therefore, the stabilization of the water content after the significant increase to the dry weight of the floral primordia may be related to the higher carbohydrate metabolism activity in apple trees during ecodormancy. Thus, favourable conditions for development in this phase will results in flowering. In this situation, a water content value of 77% after forcing may be considered a threshold that is indicative of the effective establishment of ecodormancy in apple tree floral primordia.

However, for some temperate climate species, this pattern does not appear to occur, and the water state of the buds is not related to endodormancy release. Studies have shown late changes to the water state of buds which have been related to ecodormancy. In *Acer* spp., *Betula* spp., *Alnus*spp., *Fagus* spp. and *Fraxinus* spp. the water content increased late, occurring in the spring when the trees were in ecodormancy (Essiamah and Eschrich, 1986). Likewise, the increase in water activity was observed late in *Fraxinus excelsior*, occurring in the mid-April, one week before budburst (Cottignies, 1990).

2.5.2. Budburst capacity, MTB and budbreak rate

The One-bud cutting test has been employed to study dormancy in many deciduous species (Balandier, 1992; Putti et al., 2003) but was rarely used in apple trees (Mauget and Rageau, 1988; Zguigal et al., 1999; Carvalho and Zanette, 2004) and never used to compare responses under mild and temperate climate. We found that the MTB and budburst kinetics of apple trees cultivated under temperate climate (Marsillargues) were distinct from those cultivated under mild climate (Palmas). Additionally, this test revealed ‘Granny Smith’ and ‘Eva’ developed precociously at Marsillargues and Palmas, respectively, by both exhibiting low MTB values during the entire dormancy period.

Our results highlighted distinct phases during dormancy in the cold winter of Marsillargues that may correspond to the three phases of dormancy described by Lang et al. (1987): paradormancy, endodormancy and ecodormancy. The classical interpretation (Mauget and Germain, 1980; Rageau, 1982; Mauget and Rageau, 1988; Dennis 2003) this first phase correspond to paradormancy, the phase with the peaks of MTB correspond to the endodormancy and the following phase (since beg-February) to ecodormancy. The first part of this supposed ecodormancy phase (slow decrease of MTB or plateau) is questionable, especially if in parallel, we look at the results of Tabuenca's tests under mild winter situation. The beginning of this phase correspond to the fresh change point indication the start of rehydration capacity but not sufficient to reach the critical level of 70-75% and still a to slow metabolism for accumulate rapidly significant dry mass at 25°C during the forcing test in floral buds. The end of this period correspond the situation in which floral buds are able to rehydrate fully and quickly and accumulate dry mass during the test at 25°C but not in the field where temperatures are lower. So it is only at this point that the situation corresponds strictly to the ecodormancy definition, growth blocked by to low temperatures. If we consider such a situation, of slow decrease or plateau this period appeared to be a transition phase between endo- and ecodormancy that could be under the control of temperature (lower the temperature, longer the phase) but not only because at 25°C buds are not able to fully rehydrate. Such an interpretation is also in agreement with the dynamics in mild winter in which the plateau exists (for Gala and Fuji) but where fresh and dry change points appeared at the end of the plateau. More during this period in 2012, field-mean temperatures are higher than 15°C.

It seems also clear than peaks of MTB, interpreted as endodormancy, are induced by low temperatures in both situations and the correspondence with low budburst rates during the test is also clear. Indeed, in Palmas, the absence of temperatures below 12°C up to mid-July in 2013 did not induce a real increase of MTB contrary to the situation in 2012. The duration of this "endodormancy period" seems also strictly correlated with the duration of such temperatures. The existence and duration of this phase could be under genotype (i.e. chilling requirement level) x environment control.

Another argument in favour of this hypothesis is the absence of such a response very low chilling 'Eva' cultivar, which never reach high MTB level (and real endodormancy?) and did not present such a plateau. ‘Eva’ seems immediately able to growth when temperatures remained around 15°C and above. Nevertheless, the budburst rates during the test could not be by itself a real marker of the dormancy state as it is always high in mild climate (Palmas 2012) although the existence of endodormancy revealed by peak of MTB and the decrease of this rate is not always in correspondence with MTB curves phases. Thus, the time course of budburst rate could only “help” to the interpretation of the MTB curves. All these result highlight also the strong imbrication of dormancy entrance and acclimation to low temperatures and the difficulties linked for correlate these two phenomena.

At Palmas, superficial dormancy was observed and was characterized by constant high rates of budburst and relatively low MTB values compared with Marsillargues, throughout the dormancy period but mainly during the second cycle. A similar study conducted in the subtropical region of Brazil found similar results but with a more superficial dormancy for ‘Imperial Gala’, which displayed a maximum MTB (dormancy peak) of 15 days (Carvalho et al., 2004). The contrast of MTB kinetics for both dormancy cycles have probably been the result of the distinct climate conditions observed and the distinct period with temperatures below 12°C during these two cycles.

We hypothesize that the mild temperature during the winter at Palmas was not sufficient to induce deep (or real) endodormancy. Thus, this latency condition predisposes the plants to develop immediately during warm peaks into winter. This is not suitable because a sudden occurrence of frost can compromise budburst and productivity. For ‘Eva’ which did not display an effective dormancy, the strong temperature fluctuations under mild climate could induce more serious consequences to the phenology.

2.5.3. Connection between development processes and relationships with climate

Another important point of our results is related to the speed of growth of the floral structures after endodormancy release. In Palmas, the period between endodormancy release and flowering was shorter than in Marsillargues in both dormancy years. This observation is explained by higher temperatures recorded in Palmas after endodormancy release, which drive a quick heat accumulation and the restoration of metabolic activities i.e. the ecodormancy is quite inexistent. Regarding water content, a very close evolution under forcing and field conditions was observed at Palmas. At Marsillargues, the water content on field conditions increases slowly so the transition between endo- and strictly ecodormancy seems to take time, function of the increase of temperature. Climate determines these distinct situations and the cold and dry winter at Marsillargues hindering the hydration of the tissues on the field. At Palmas, the mild winter is also more humid, favouring the maintenance of bud hydration under field, which is always higher in the very low chilling 'Eva' cultivar. Could it correspond to a lower isolation of the meristem during endodormancy?

This means that, for a given variety, the lower temperature during autumn induce higher MTB values contributing to a stronger isolation of the meristem (and consequently also, a better frost resistance). Then the satisfaction of the chilling requirement occurs under intense chill (temperature below 12°C) and that the plants remain in ecodormancy for relatively long periods under cold winter (corresponding to period with temperature below 15°C). Contrary, in mild winter relatively the high temperatures favour a fast development of buds.

So the development process could be described as follow: under temperate climate, paradormancy is early installed. The first temperatures below 15 or 12°C induce a very rapid transition into endodormancy and the endodormancy is released by chilling and a transition phase during which the capacity of rehydration and metabolic activity is progressively restored. This is follow by a strict endodormancy period while the temperature remained below 15°C. Under mild winter climate, paradormancy is installed followed by an increase of inhibitions process that could be associated to a weak endodormancy when temperature below 12°C occurred. This weak endodormancy is rapidly release by some chilling temperatures (almost for the low chilling varieties) resulting in a rapid growth resumption if temperature remained above 15°C. If not, a very small period of ecodormancy appeared. For the very low chilling varieties as 'Eva', it seems that only paradormancy exists. The absence of ecodormancy process could explain some strong heterogeneity of budburst and flowering, enhancing the heterogeneity of status existing among the bud population of the tree.

This fast development culminates in a temporal heterogeneity of blooming during two or three weeks (Figure 20a) that extended to later stages of development process (Figure 20b). This phenomenon was described also by Petri and Leite (2004) under the mild climate of Southern Brazil. This situation may be drastic due the occurrence of peaks of low temperature and/or late frosts in high elevation orchards in Brazil and in the next future in south Europe.

Figure 20. Details of heterogeneous phenology during blooming time (a) and during later stages of development in Palmas, PR – Brazil.

The chill accumulated until the dates of endodormancy release varied considerably in the two years evaluated both in Marsillargues and in Palmas, according to the Dynamic and Unified models. These differences were also observed applying Weinberger, Bidabé, Landsberg, Utah, North Carolina, Positive Chill Unit, DVI, F1Gold1 and F1Gold2 models. However, the variations were lower in Marsillargues compared to those of Palmas.

The disparities between the total chill accumulated in Palmas and Marsillargues until endodormancy release, especially for 'Gala', can be a result of the deficiency of the models in estimating the real chilling requirement of the species cultivated in mild locations, as was expected. This situation was also found by Allan et al. (1993) in the subtropical conditions of South Africa. Certainly, the empirical development of these models and validation based on unstandardized biological tests contributed to the lack of efficacy in correctly estimating the chilling requirements. However, this appointment cannot be made considering the dormancy pattern of 'Eva' because this cultivar seems does not effectively enter in endodormancy during winter. The soft MTB and budburst curves for vegetative buds and the accelerated resumption of floral development revealed by Tabuenca's test confirm this hypothesis. Therefore, the development of parameterised phenological models based on historical series of temperature and phenology data and validated by biological data obtained from biological tests with low physiological interference must be recommended.

2.5.4. *Are floral and vegetative apple buds so different regarding dormancy?*

The mixed structure of the floral bud and associated particularities, as described by Pratt (1990) or Lauri et al (2008), seems to induce a similar response during dormancy to that observed for leaf buds. This similarity of response was also described in situation of cold deprivation (Herter, 1992) during which strictly floral buds undergo necrosis while vegetative apple buds survive. Indeed, dry weight change point that could be considered as the end of endodormancy corresponds well with the end of the transition phase observed in vegetative buds and the strict entrance into ecodormancy. In apple, the determination of the endodormancy release date seems to be easier by the weight measurement (before and after 7 days of the forcing process) as well under temperate than under mild winter climate.

2.6. Conclusions

The dormancy kinetics of vegetative buds was linked to the temperature patterns at each site. Thus, the cold climate induced deeper dormancy and slower resumption of bud development after winter. Contrarily, mild winter induces superficial dormancy and fast resumption of development under warming. At Marsillargues, ‘Granny Smith’ was the precocious cultivar, while ‘Golden Delicious’ and ‘Gala’ cultivars had similar dormancy dynamics. Similarly at Palmas, ‘Eva’ developed earlier and its bloom date seems inversely correlated with cold chilling temperature received, while ‘Fuji’ and ‘Gala’ developed similarly late.

Used for the first time on ‘mixed’ buds and under contrasted climate for the same variety, the dynamics of fresh and dry weight of the primordia (with and without forcing i.e. Tabuenca’s test) appeared efficient to define the date of endodormancy release in apple floral buds under temperate and mild winter climate. In addition to this, the capacity to increase their dry weight and water content (threshold of 77% during the test) appears to be a strong indication of the effective establishment of ecodormancy in buds and a complete endodormancy release. The development in the mild winter region (Palmas) was characterized by the abrupt restart of development after endodormancy release, with a very short time between endodormancy release and the effective real ecodormancy when compared with the cold region (Marsillargues). The accuracy of Tabuenca’s test in defining the date of endodormancy release can be applied to validate phenological models. The exception occurred when this test was applied to the ‘Eva’ cultivar, as it gave evidences that its cycle did not pass through a real endodormancy phase.

Our results revealed also the key role of rehydration processes and the urgent necessity to better analyse and understand the physiological processes involved in water and nutrient transport to the primordia before the full development of vascular connections and budburst.

Understanding these distinct response mechanisms under contrasting climates (especially the climate observed in Brazil) allows more accurate decisions to be made regarding the implementation of strategies for the adaptation and management of apple crops under the warming scenario, which is becoming increasingly evident in the Northern hemisphere.

CHAPTER III

Application of phenological models for the prediction of flowering in apple trees grown in contrasting climate conditions

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Abstract –Current changes to the climate have increased the number of phenological studies conducted on apple trees that focus on defining measures of adaptation, new agricultural zoning and predictions of the effects of global warming. In this context, phenological modeling, based on a historical data series, can be used to predict future changes. The main goal of the present study was to select and validate temperature-based sequential phenological models that are capable of precisely predicting flowering dates of apple cultivars for a wide region of cultivation in the Northern (Western Europe and North Africa) and Southern (southern Brazil) Hemispheres. Historical series of temperature and flowering dates, collected at five locations in the Northern Hemisphere (NH) (four in Western Europe and one in North Africa) and two locations in the Southern Hemisphere (SH), were utilized for the mixed parameterization of the models. For the NH, models NHGoldenF1 and NHGoldenF2 were validated for the F1 (beginning of blooming) and F2 (full blooming) flowering stages of the Golden Delicious apple cultivar at four locations in Western Europe, but not for Meknès, Morocco. The models were validated, explaining more than 70% of the data variability for all of the locations. Chill sub-models were based on triangular and parabolic functions according to the effects of minimum and mean temperatures with optimum temperatures of 1,0 and 7,0°C, respectively. The heating sub-models were based on exponential and sigmoidal functions. These models were useful for predicting phenology trends by the end of this century. For the SH, the models selected for the Fuji, Gala and Golden Delicious cultivars were not validated for the studied locations. The results of the present study revealed the difficulty in validating phenological models for subtropical regions and indicated a requirement for more complex modeling. New modeling concepts that are more mechanistic and incorporate multiple factors should contribute to overcoming this limitation.

Keywords: sequential model, chill accumulation, heat accumulation

3.1. Hypothesis

It is hypothesized that the parameterization of phenological models using a wide variability of temperature and phenology data will enable the development of robust models able to predict phenological events in a large region.

3.2. Objectives

This study focused on select sequential phenological models for annual predictions of phenology, using large-scale parameterizations based on historical temperature and phenology data. The models were selected for specific apple cultivars, as Fuji, Gala and Golden Delicious grown in southern Brazil and Golden Delicious grown in Western Europe and Morocco. We also tried to predict phenological pattern form the current situation to the end of this century, using the selected models and scenarios of project temperatures for the same period.

3.3. Material and Methods

3.3.1 Temperature data

The historical temperature series (maximum, mean, and minimum) described in chapter 1 were used. The characterization of locations and respective temperature series are presented on the first chapter. The temperature series were collected at weather stations from research centers at six locations in the Northern Hemisphere and four locations in the Southern Hemisphere. A preliminary analysis of the temperature records was performed to detect inconsistencies. For the Northern Hemisphere (NH), the temperature series were collected in Angers and Nîmes (France), Bonn (Germany), Conthey (Switzerland), Forlì (Italy) and Meknès (Morocco). The longest temperature series was recorded in Bonn and Conthey (1958-2012: 54 years), and the shortest series was recorded in Meknès (1972-2012: 40 years). For the Southern Hemisphere (SH), temperatures were obtained in Pelotas, Caçador and São Joaquim (Brazil) and Las Brujas (Uruguay). This last location has an oceanic temperate climate and was humid with a mean annual temperature of approximately 16°C. The longest temperature series was recorded in São Joaquim (1955-2012: 57 years) and the shortest was recorded in Las Brujas (1991-1998: 7 years).

The studied locations of Southern Brazil provide good conditions for the cultivation of apple trees with few restrictions. The studied sites represent a range of variable patterns of temperatures, which are important characteristics for the development of flexible and robust phenological models.

3.3.2 Phenological data

The phenological data were obtained from observations performed in apple tree orchards that were in close proximity to the weather stations where the temperatures were recorded. The long phenological series, which were also studied in the first chapter (see table 3 of chapter 1), were composed of dates indicating the beginning of flowering (approximately 10% of flowers open) and full flowering (approximately 50% of flowers open). These two stages correspond to stages 61 and 65 of the BBCH scale (Meier, 2001), which are commonly designated F1 and F2, respectively. Annual flowering dates were observed for adult plants (>3 years) by experienced evaluators. Flowering dates for the cultivars Golden Delicious, which is more commonly grown in the NH, and Fuji and Gala, which are more commonly grown in the SH, were used for modeling. The Golden Delicious cultivar was also considered for the SH as historical data was available. For the NH, the longest flowering data series was recorded at Bonn (55 years) for Golden Delicious, and for the SH, the longest series was recorded at São Joaquim (41 years) for Gala and Golden Delicious.

3.3.3 Fitting and validation of phenological models

Phenological modeling that considers the successive and independent effect of temperature for endodormancy release (ER)and ecodormancy in floral buds of apple cultivars is known as a sequential model (Chuine, 2000). It uses a combination of chill and heat accumulation sub-models for the estimation of annual dates of endodormancy and ecodormancy release of vegetative and/or floral buds, in temperate fruit species.

Individual phenological models were parameterized for Fuji, Gala and Golden Delicious apple cultivars grown in the SH and Golden Delicious grown in the NH. Seven different mathematical functions were tested and combined between the chill and heating (forcing) sub-models: binary, linear, exponential, sigmoidal, triangular, parabolic and normal. The associated functions and parameters were those used by Legave et al. (2013) (Table 9).

Table 9. Mathematical models and associated parameters tested to model F1 date of apple cultivars in south and north hemisphere.

Sub-model	Temperature (T) function	Function parameter
Chilling	Chilling function (Fc)	
Binary	Fc (T)=1 if T<Tc, Fc (T)=0 if T>Tc	Tc: threshold T[a]
Linear	Fc (T)=Tc-T if T<Tc , Fc (T)=0 if T>Tc	Tc: threshold T[a]
Exponential	Fc (T)=exp (-T/Tc)	Tc: specific T[a]
Sigmoidal	Fc (T)=1 / (1+exp [(T-Tc) / Sc])	Tc: specific T[a]; Sc[b]
Triangular	Fc (T)=1-($\lvert T-Tc \rvert$ /Ic) if Tc- Ic<T<Tc+Ic, Fc (T)=0 if not	Tc: optimal T[a]; Ic[c]
Parabolic	Fc (T)=1-$[(T-Tc) / Ic]^2$ if Tc-Ic<T<Tc+Ic, Fc (T)=0 if not	Tc: optimal T[a]; Ic[c]
Normal	Fc (T)=exp [- $(T-Tc)^2$ / Ic]	Tc: optimal T[a]; Ic[c]
Heating	Heating function (Fh)	
Binary	Fh (T)=1 if T<Th, Fh (T)=0 if T> Th	Th: threshold T[a]
Linear	Fh (T)= Th -T if T< Th , Fh (T)=0 if T> Th	Th: threshold T[a]
Exponential	Fh (T)=exp (T/Th)	Th: specific T[a]
Sigmoidal	Fh (T)=1 / (1+exp [(T-Th) / Sh])	Th: specific T[a]; Sh[b]
Triangular	Fh (T)=1-($\lvert T-Th \rvert$ /Ih) if Th- Ih<T<Th+Ih, Fh (T)=0 if not	Th: optimal T[a]; Ih[c]
Parabolic	Fh (T)=1-$[(T-Th) / Ih]^2$ if Th-Ih<T<Th+Ih, Fh (T)=0 if not	Th: optimal T[a]; Ih[c]
Normal	Fh (T)=exp [- $(T-Th)^2$ / Ih]	Th: optimal T[a]; Ih[c]

[a] temperature parameter (°C) depending on the function

[b] slope parameter of the sigmoidal function around Tc or Th (temperature for which 50% of maximum daily effect is reached)

[c] temperature interval (°C) defining the range of efficient temperatures around Tc

Other parameters were also considered for the parameterization of the chill and heating sub-models, such as temperature (maximum, mean, and minimum), optimum temperature, temperature range (above and below the optimum temperature), chill period onset, total chill accumulation (between the start of the chill period and estimated date of ER), and heat sum accumulation (between the estimated date of ER and flowering) (Table 9).

The range of values for each parameter was pre-defined to maximize the fit of the models. These values were included in the data platform of Pollenoscope software (Legave et al., 2008), and the parameters were combined using the Metropolis algorithm (Chuine et al., 1998) to maximize the coefficient of determination (R^2). Concurrently, Microsoft Excel files containing the annual temperature data (maximum, mean and minimum) were prepared and automatically inputted into the software. The dates of flowering that corresponded to each year of temperature records were also automatically inputted. The hypothesis that the best and worst fit of a model depended on the year selected from the historical series was considered for the file preparation. Therefore, the year combination resulting in the parameterization of models with the highest fit was initially attempted. From the total available years for each historical temperature series, five new subseries were randomly formed, and they were each composed of approximately 75% of the years available in the original series.

The F1 and F2 flowering stages of Golden Delicious in the NH were parameterized using the temperature and phenology data from series of 129 and 119 years and selected from a total of 202 and 203 years, respectively. For the SH, the models selected to estimate the F1 and F2 flowering stages were parameterized using data from series of 41 and 42 years and selected from a total of 64 and 67 years for Golden Delicious and Fuji applies, respectively. For the Gala cultivar, the models selected to estimate the F1 and F2 dates were parameterized using data from a series of 44 and 46 years and selected from a total of 69 years (Table 10).

Table 10. Total number of years available in each historical series, used to fit and validate models for F1[a] and F2[b] date of Golden Delicious, Gala and Fuji apple cultivars.

Apple cultivar	F1 date			F2 date		
Site (Country)	Years available	Years used to fit models	Year used to validate the models	Years available	Years used to fit models	Year used to validate the models
Golden Delicious						
Bonn (Germany)	38	24	14	56	24	14
Angers (France)	51	32	17	-	-	-
Conthey (Switzerland)	-	-	-	42	29	13
Forli (Italy)	44	28	15	44	28	15
Nîmes (France)	39	25	13	33	20	13
Meknès (Morocco)	30	20	10	28	18	10
Total	202	129	69	203	119	65
Golden Delicious						
Caçador (Brazil)	25	16	9	25	16	9
São Joaquim (Brazil)	39	25	13	39	25	13
Total	64	41	22	64	41	22
Gala						
Caçador (Brazil)	32	21	10	32	21	10
São Joaquim (Brazil)	37	23	13	37	25	13
Total	69	44	23	69	46	23
Fuji						
Caçador (Brazil)	32	20	12	32	20	12
São Joaquim (Brazil)	35	22	13	35	22	13
Total	67	42	25	67	42	25

[a] beginning of blooming = BBCH 61 stage; [b] full blooming = BBCH 65 stage.

A computer network was used for six consecutive months to test a total of 27.720,00 and 24.024,00 models in the SH and NH, respectively. The highest range of possible fits was obtained by parameterizing all of the temperature sub-series for each location and cultivar (simple parameterization). The results were then thoroughly analyzed to determine the subseries that resulted in the model with the best fit (highest R²). Finally, the subseries selected for each location, cultivar, and hemisphere were mixed to form a composite series, which was again subjected to parameterization (mixed parameterization) (Figure 21).

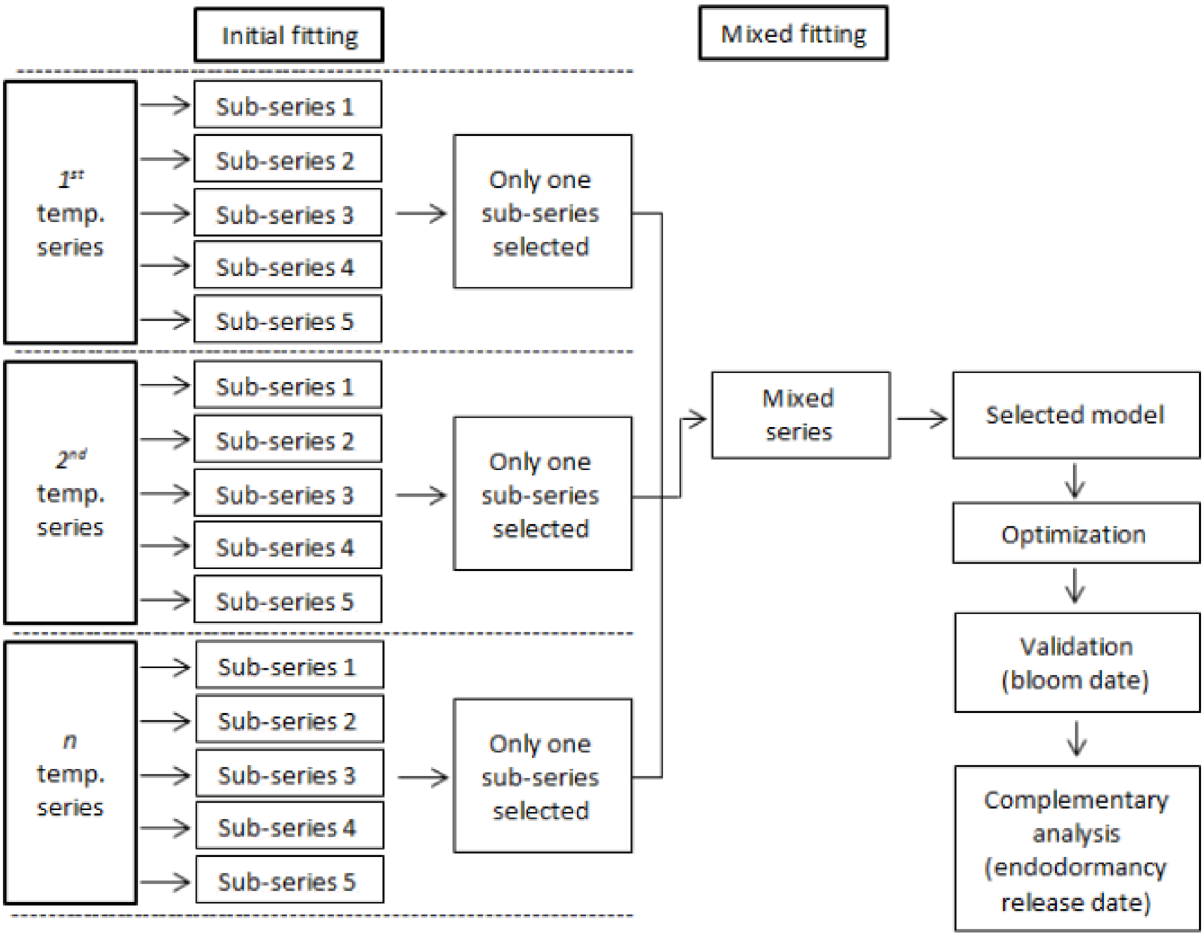

Figure 21. Scheme to illustrate the procedure outlined to the parameterisation.

Following the mixed parameterization, the model with the best fit was selected based on the highest R² value and biological consistency of the defined parameters. The parameters of each model were then optimized by maximizing the R² value by widening the range of values close to the previously selected for each model.

Finally, the selected models were validated for the cultivars of each hemisphere using the significance of data and a root mean-square error (RMSE) ≤ 7 and R² ≥ 0,65. These values were calculated based on the difference between the observed and estimated flowering dates using the temperature and phenology data from the years (approximately 25%) that were not considered for the parameterization of each model (internal validation) (Table 9). In addition to the internal validation, the models selected for Gala and Fuji (F1 and F2, SH series) of the SH were also validated based on the temperature and phenology data from Pelotas and Las Brujas (external validation) that were not used for the parameterization.

Additional evaluations of the efficiency of the NHGoldenF1 and NHGoldenF2 models were performed by comparing the observed dates of ER and F1/F2 flowering stages observed for Marsillargues, France (see chapter 2), with the dates estimated using the model. For comparison, a similar evaluation was performed using the sequential effect of the chill sub-model (North Carolina) developed by Shaltout and Unrath (1983) and the heating sub-model GDH developed by Richardson et al. (1975). The threshold for Golden Delicious for ER was 1,050 chill units (Hauagge and Cummins, 1991) and to break ecodormancy the threshold was 10,000 GDH (Seeley et al., 1996).

To study the evolution of the physiological behavior of the Golden Delicious cultivar, dates for ER and the F1 flowering stage were calculated using the NHGoldenF1 model for Bonn, Angers, Nîmes and Forli and using 53-, 49-, 38- and 43-year series, respectively. Future predictions (2015-2100) of the dates of ER and flowering (F1 and F2) of Golden Delicious were obtained for Angers and Nîmes using temperatures (Tmax and Tmin) simulated by the ARPEGE climatic model platform (Météo-France CNRM/GMEC) (http://www.drias-climat.fr/) based on the 4,5 (intermediate optimism) and 8,5 (less optimistic) scenarios of the Representative Concentration Pathways (RCPs) series developed for the 1950-2005 reference period (IPCC, 2013). The simulated temperatures (Tmax and Tmin) were obtained using the mean temperatures recorded at four geographical coordinates (latitude and longitude) close to each location. The annual variation between the dates of ER and flowering (days) was then calculated for the past and future scenarios.

The data did not exhibit a normal distribution and were subjected to the Mann-Kendall non-parametric test at $p<0.05$ for trend detection. In parallel, the magnitude of the trends was calculated using Sen's test, which expressed the rates as days per decade [d 10 y^{-1}]. Both analyses were performed using the XLStat® software.

3.4. Results

3.4.1. Model selection

The models that were selected and optimized for both hemispheres were classified as sequential, and the flowering (F1 or F2) date was estimated based on the effect of temperature according to the chill and heating sub-models (Table 11). For the NH, the phenological model used to estimate the F1 flowering date for Golden Delicious was selected using the temperature and phenology data from 129 contrasting years. This model, NHGoldenF1, explained 87% of the data variability and exhibited a 4,13 day precision for the flowering date. The cold sub-model was based on a triangular function and a 1,0°C optimum mean daily temperature (To). The selected temperature interval was 24,0°C (Ti), and the chill requirement was 98 units starting on October 5th (Figure 22a). The heating sub-model was based on an exponential function and a 9,6°C specific mean daily temperature (Th), and the heating requirement to break ecodormancy was 67,2 heat units (Figure 22b).

Table 11. Features of sequential models selected for F1 date of apple trees cultivars in South and North Hemispheres.

Parameters of model	Models			
	NHGoldenF1	SHFujiF1	SHGalaF1	SHGoldenF1
Chilling sub-model				
Temperature function	Triangular	Normal	Triangular	Sigmoidal
Daily temperature involved	Minimum	Minimum	Mean	Mean
Optimal temperatures (˚C) (To)	1,0	4,0	5,3	10,0
Temperature interval (˚C) (Ti)	24,0	19,0	18,0	6,0
Chilling period onset	October 05	April 10	April 16	March 26
Chilling amount required (C)[a]	98	84	62	100
Heating sub-model				
Temperature function	Exponential	Exponential	Exponential	Exponential
Daily temperature involved	Mean	Mean	Mean	Maximum
Specific temperature (˚C) (Th)	9,6	12,0	12,0	14,0
Slope parameter (Ts)	\	\	\	\
Heating quantity required (H)[a]	67,2	58,5	66,5	88,9
R^2	0,87	0,67	0,77	0,73
RMSE (days)	4,13	6,30	5,02	5,76

[a] Sum of daily effects expressed in units depending on the temperature function.

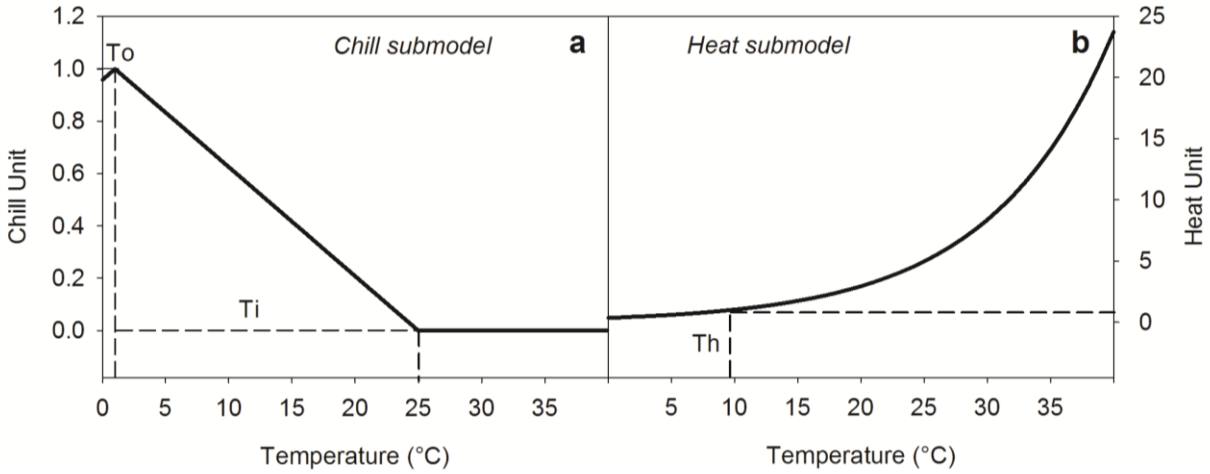

Figure 22. Temperature response functions and the parameters of the chill submodel (triangular) (a) and heat submodel (exponential) (b) of the sequential model NHGoldenF1.

For the SH, three phenological models, SHFujiF1, SHGalaF1 and SHGoldenF1, were selected to estimate the onset of flowering (F1 stage) for Fuji, Gala and Golden Delicious cultivars, respectively. These models were selected based on the parameterization of 42, 44 and 41 years of contrasting temperature and phenology data, and they explained 67,0; 77,0 and 73,0% of the data variability (R^2) and exhibited a precision of 6,30; 5,02 and 5,76 days, respectively. The chill sub-models were based on normal, triangular and sigmoid functions. The function defined for the SHFujiF1 model was based on the minimum temperature, whereas the SHGalaF1 and SHGoldenF1 models were based on the mean temperature. The onset date for chill accumulation for the SHFujiF1 and SHGalaF1 models were close at April 10th and 16th, respectively, whereas SHGoldenF1 estimated a later date (March 26th). Considerable variation among the chill sub-models was also observed for the optimum temperature, temperature interval and chill requirement for endodormancy release (ER). The optimum temperature corresponds to the maximum chill accumulation, whereas the temperature interval represents the range of temperatures considered effective for chill accumulation. For example, the 4°C minimum daily temperature for the SHFujiF1 model will result in the maximum chill accumulation (1 unit), whereas the temperatures recorded for the extremes of the 19°C interval (-5,0°C and 13,0°C) will have the smallest effect. Moreover, the effect of the temperatures recorded within this range will be conditioned to the defined normal function (Table 11). All of the heating sub-models of the SH series models were based on exponential functions. A 12°C mean daily temperature was considered optimum by the models selected for Fuji and Gala. For

Golden Delicious, the optimum maximum temperature considered was 14°C. The heat requirement varied between the models (Table 11).

In addition, phenological models were selected to estimate the date of the F2 flowering stage for both the hemispheres (Table 12). The model selected for Golden Delicious for the NH (NHGoldenF2) explained 91,0% of the data variability with a 3,51 day precision. The chill sub-model was based on a parabolic function, 7,0°C optimum mean daily temperature (To), and 28°C temperature interval (Ti). The estimated chill requirement was 127 units accumulated after October 12th (Figure 23a). The heating sub-model was based on a sigmoid function and 12,0°C specific maximum daily temperature (Th), with a heat requirement of 67,2 units (Figure 23b).

Table 12. Features of sequential models selected for F2 date of apple trees cultivars in South and North Hemispheres.

Parameter of model	Models	
	NHGoldenF2	SHGoldenF2
Chilling sub-model		
Temperature function	Parabolic	Sigmoidal
Daily temperature involved	Mean	Mean
Optimal temperatures (˚C) (To)	7,0	4,0
Temperature interval (˚C) (Ti)	28,0	10,0
Chilling period onset	October 12	April06
Chilling amount required (C)[a]	127	82
Heating sub-model		
Temperature function	Sigmoidal	Exponential
Daily temperature involved	Maximum	Maximum
Specific temperature (˚C) (Th)	12,0	16,0
Slope parameter (Ts)	4,0	\
Heating quantity required (H)[a]	67,2	71,5
R^2	0,91	0,70
RMSE (days)	3,51	5,51

[a] Sum of daily effects expressed in units depending on the temperature function;

A model was not selected for Fuji and Gala cultivars for the SH because the best fits for the mixed parameterization were lower than 65%. However, the model SHGoldenF2 was selected for Golden Delicious, and the chill sub-model was based on a sigmoid function. In this case, the model explained 70% of the data variability (R^2) and resulted in a 5,51-day precision based on a 4°C optimum mean daily temperature and start of chill accumulation on April 6th. The results indicated a 10,0°C ideal temperature interval and a requirement of 82 chill units for ER. The heating sub-model was based on an exponential function, 16,0°C daily maximum temperature, and a requirement of 71,5 heat units.

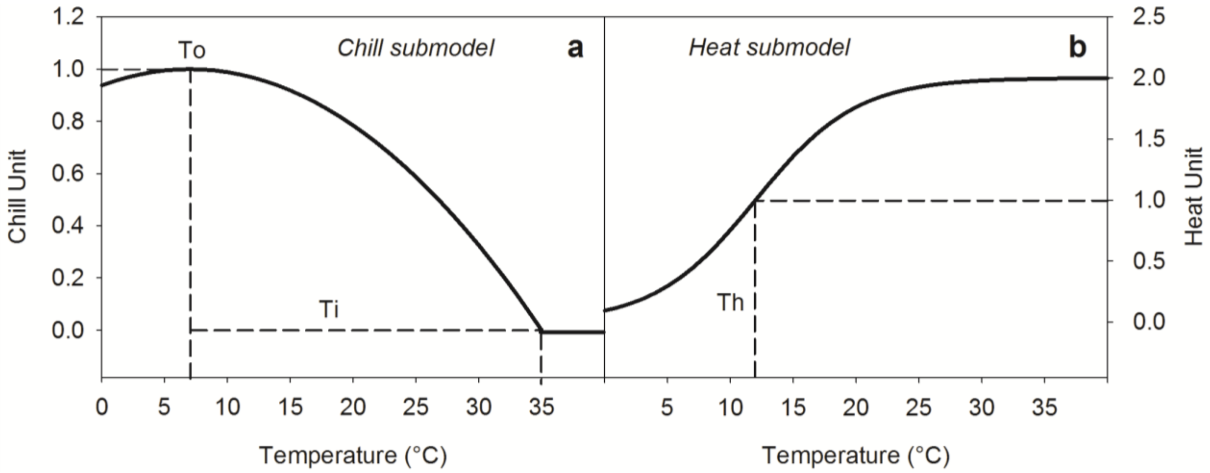

Figure 23. Temperature response functions and the parameters of the chill sub-model (parabolic) (a) and heat sub-model (sigmoid) (b) of the sequential model NHGoldenF2.

3.4.2 Validation of the selected models

To validate the models selected to predict the dates of the F1 flowering stage for all cultivars studied, the annual deviations of the observed and estimated dates were evaluated. For the NH, the NHGoldenF1 model was not valid for the data from Meknès, Morocco because it exhibited a non-significant estimation precision and an absolute error of 9,2 days between the estimated and observed flowering dates (Figure 24). However, the model was capable of accurately estimating the dates of the F1 flowering stage of Golden Delicious for the Western Europe locations with 81, 70, 87 and 71% precision for Bonn, Angers, Nîmes and Forli, respectively. The annual mean absolute errors obtained through the differences between the observed and estimated F1 dates were under 4.4 days for these locations (Figure 24).

The models selected for the Fuji, Gala and Golden Delicious cultivars for the SH were not internally or external validated for their estimations of the date of the F1 flowering stage. The models SHFujiF1, SHGalaF1 and SHGoldenF1 exhibited low precision in their estimations of the F1 date for all locations. Although not significant, the precision was highest for the SHFujiF1 model for Fuji at Las Brujas (37%) and for the SHGalaF1 and SHGoldenF1 models for Gala (47%) and Golden Delicious (68%), respectively, at São Joaquim. The absolute mean errors between the locations of 11,7; 9,6, and 9,5 days were observed for the models selected for Fuji, Gala, and Golden Delicious, respectively. In addition, absolute errors were higher for Brazilian locations, especially for warmer locations such as Caçador and Pelotas. In general, the models selected for the SH exhibited a slight tendency to underestimate the dates of flowering, especially for São Joaquim and Caçador. For Pelotas, this tendency varied between models, whereas for Las Brujas, the estimates were closer to the real values (Figure 24).

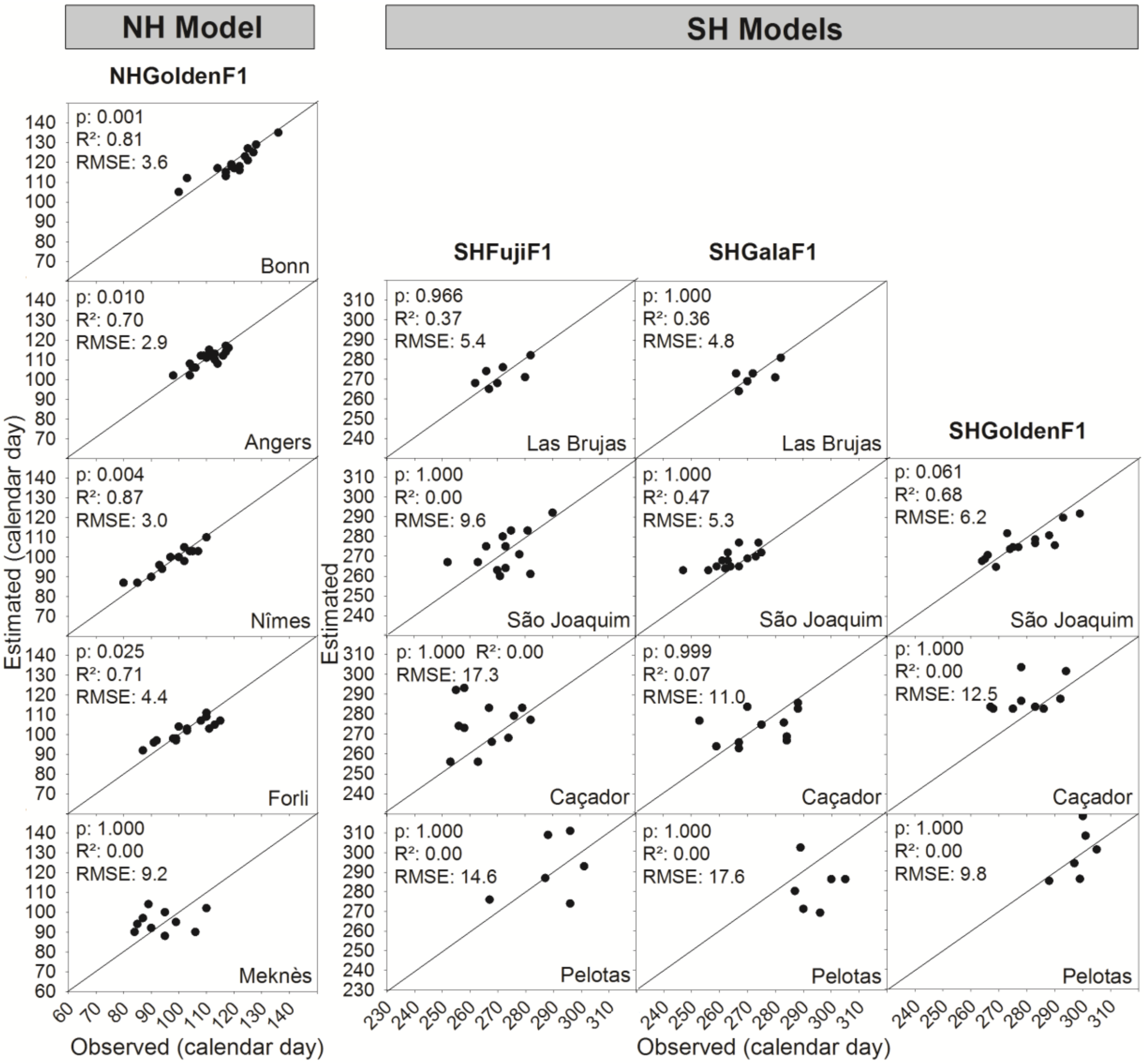

Figure 24. Deviation (in days) between observed and simulated F1 dates of Golden Delicious in the Northern Hemisphere and Fuji, Gala and Golden in the Southern Hemisphere according to selected NH and SH models. The continuous line in each graph represents the expected precision. p: value recorded by Fisher test; R²: determination coefficient; RMSE: root mean square error.

For the F2 flowering stage of Golden Delicious, the NHGoldenF2 model was only validated for Bonn, Conthey and Nîmes with a 5% error margin and for Forli with a 7% error margin. This model explained 84% of the variability for Bonn, Conthey and Nîmes and 70% for Forli (Figure 25).

The mean variation between the estimated and observed dates was 3,8 days for Forli, 3,2 days for Bonn and Conthey, and 2,6 days for Nîmes. For Meknès, where the model application was not valid, the fit of the model was null and the mean absolute error between the observed and estimated dates was 19,7 days (Figure 25).

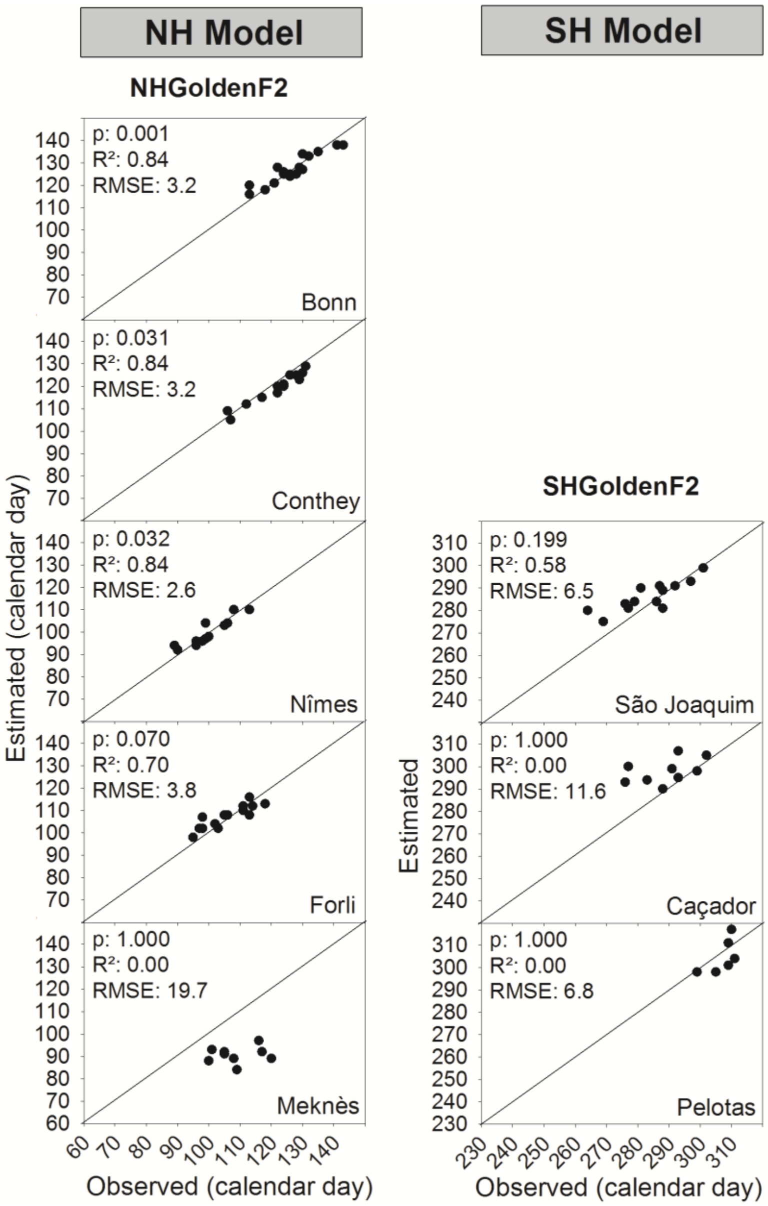

Figure 25. Deviation (in days) between observed and simulated F2 dates of Golden Delicious in the Northern and Southern Hemispheres according to selected models. The continuous line represents the expected precision. p: value recorded by Fisher test; R²: determination coefficient; RMSE: root mean square error.

The SHGoldenF2 model, which was selected for the SH, exhibited a non-significant 58% precision for São Joaquim and a null precision for Caçador according to the internal validation. A lack of precision was also observed for the model through external validation using data from Pelotas. Overestimated mean deviations between the estimated and observed flowering dates of 6.5 and 11.6 days were observed for São Joaquim and Caçador, respectively, whereas an underestimated mean deviation of 6.8 days was found for Pelotas (Figure 25).

3.4.3 Complementary efficiency analysis of the NHGoldenF1 and NHGoldenF2 models

The efficiency of the NHGoldenF1 sequential model was compared with a version of the thermal model, which was selected according to the same parameters defined for the sequential model. The thermal model only considered the effect of springtime heat for the phenological predictions, assuming that the chill requirements were met before the heat sum accumulation starting date. The comparison of the estimated and observed dates of the F1 flowering stage for Bonn (53 years), Angers (49 years), Nîmes (49 years), and Forli (43 years) confirmed the better fit of the sequential model compared with the Thermal model (Table 13).

Table 13. Efficiencies of modeling assumptions (Sequential and Thermal) to estimate the F1 date for Golden Delicious in Bonn, Angers, Nîmes and Forli.

Site	Period	Sequential model[a]		Thermal model[b]	
		R^2	RMSE (days)	R^2	RMSE (days)
Bonn	1960-2012	0,79	4,17**	0,76	4,41**
Angers	1964-2012	0,68	4,30**	0,63	4,64**
Nîmes	1975-2012	0,82	2,96**	0,53	4,77**
Forli	1970-2012	0,79	3,49**	0,73	3,95**

[a] chill sub-model + heat sub-model of 'NHGoldenF1' model using parameters presented in Table 1; [b] the best model fitted: sigmoidal model involving men temperature and the following parameters: Th: 8°C; Ts: 4; H: 64,2 units; heating onset: 19th February. ** significant value (*$p<0.01$*)

The sequential model efficiency was obvious for the Nîmes temperate region because it explained 82% of the data variability, whereas the thermal model only explained 53%. Similarly, the absolute errors (difference between estimated and observed dates) decreased from 4,77 days for the thermal model to only 2,96 days for the sequential model. For the remaining locations, the efficiency of the sequential model was slightly higher than that of the thermal model. The thermal model explained 76, 63, and 73% of the data variability for Bonn, Angers, and Forli, respectively, whereas the sequential model explained 79, 68, and 79%, respectively. Similarly, the absolute errors for the thermal model were 4,41; 4,64, and 3,95 days and for the sequential model they were 4,17; 4,30, and 3,49 days for Bonn, Angers, and Forli, respectively.

The efficiency was studied for the models NHGoldenF1 and NHGoldenF2 for the temperature data and observed dates of ER and F1/F2 flowering stages that were recorded for the Golden Delicious cultivar at Marsillargues, France, for cycles 2011/12 and 2012/13 (see chapter 2). These data were subjected to the parameters of the validated models and the dates of ER and F1/F2 flowering stages were estimated; concurrently, a comparison was conducted of the dates observed by the Tabuenca test and direct field observations (Figure 26).

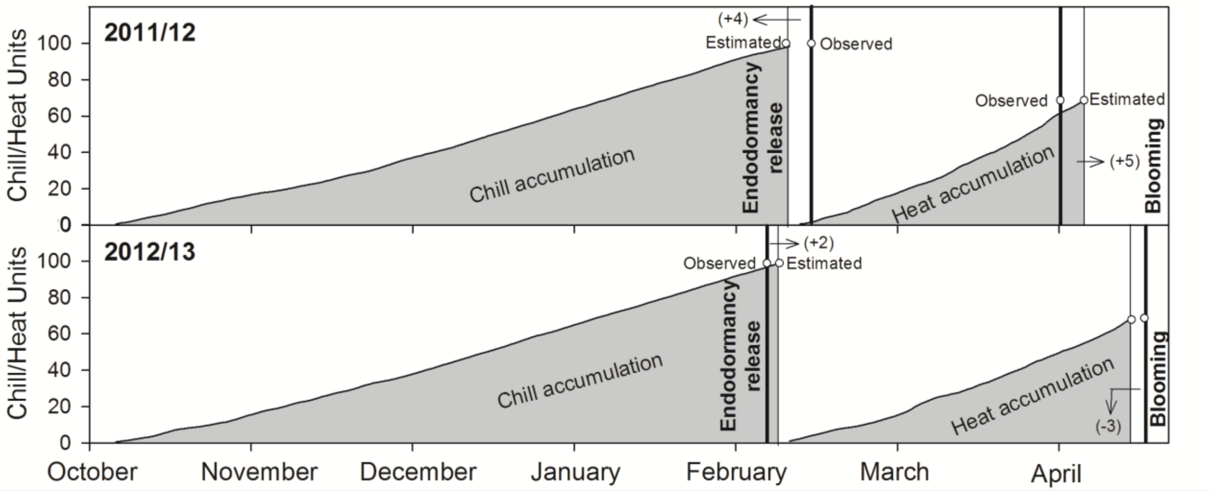

Figure 26. Evolution of chill and heat accumulation in Marsillargues, according to the 'NHGoldenF1' model during 2011/12 and 2012/13.

In general, the critical phases for ER and the F1 flowering stage that were estimated by the NHGoldenF1 model exhibited relatively smaller variations in 2012/13 than in 2011/12. The date of ER in 2011/12 was observed on February 14th but was underestimated by 04 days (10th February) by NHGoldenF1. In 2012/13, the observed date of ER was on February 6th, whereas the estimated date by the chill sub-model was February 8th, which indicates a 2-day overestimation (Figure 26).

For the F1 flowering stage, the variation between the observed (April 1st) and estimated (April 6th) dates was only 5 days in 2011/12 according to the exponential heat sub-model. In 2012/13, this difference was only 3 days (observed April 18th; estimated April 15th). Although the model explained 74% of the data variability with 4,3 days absolute error between the observed and estimated dates of the F1 flowering stage, the short series of observations did not allow the model for Marsillargues to be validated with significant precision (Figure 26).

The precision of the estimations for the limits of each physiological stage of the NHGoldenF1 model was compared with that of the sequential matching of the North Carolina chill sub-model (Shaltout and Unrath, 1983) and the GDH heating sub-model (Richardson et al., 1975) (Figure 27).

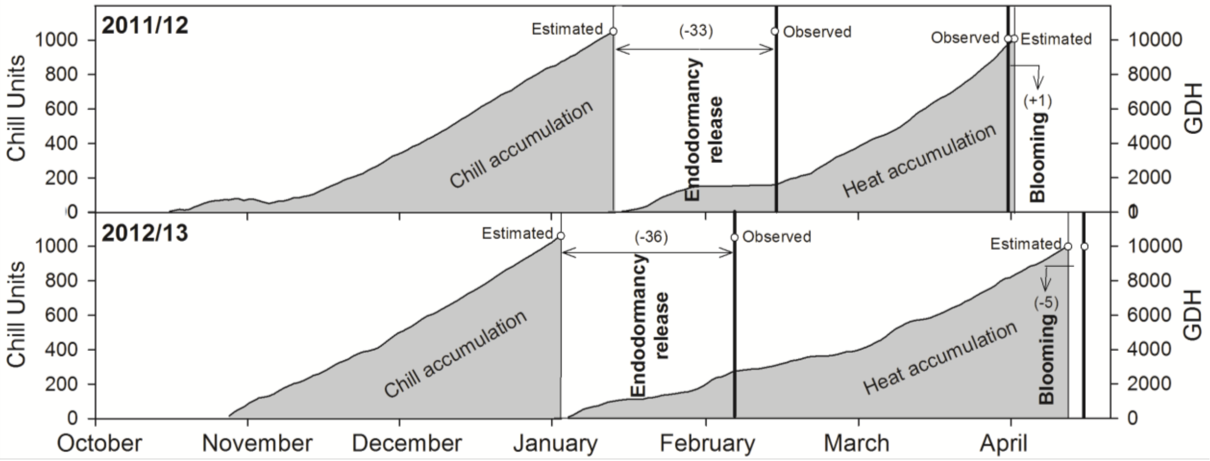

Figure 27. Evolution of chill and heat accumulation in Marsillargues, according to the 'North Carolina' model during 2011/12 and 2012/13.

Based on a 1,050 hour chill threshold, the date of ER was estimated as January 13th for 2011/12. This model underestimated the date of ER by 33 days compared with the observed date (February 14th). For the following cycle (2012/13), the difference between the estimated (January 2nd) and observed (February 6th) date was 36 days. In turn, the estimated date of the F1 flowering stage for the GDH heat sub-model (Richardson et al., 1975) was April 2nd and 13th for 2011/12 and 2012/13, respectively. The variation relative to the observed dates April 1st and 18th were 1 and 5 days, respectively, which were close to the variations observed for the NHGoldenF1 model (Figure 27).

The variations between the observed and estimated dates of ER for the NHGoldenF2 model were more pronounced than those of the NHGoldenF1 model by up to 10 days for 2011/12 (observed date February 14th; estimated date February 24th) and 16 days for 2012/13 (observed date February 6th; estimated date February 22nd). The chill sub-model overestimated the date of ER for both years. The heating sub-model estimated the dates of the F2 flowering stage for 2011/12 and 2012/13 as April 5th and 15th, respectively, representing mean deviations of 0 and -6 days relative to the dates of flowering observed in the field, which were April 5th and 21st for 2011/12 and 2012/13, respectively. Finally, the NHGoldenF2 explained only 54% of data variability and showed an absolute error of 4.5 days at Marsillargues. This model did not show a significant precision, therefore could not be considered valid for Marsillargues (Figure 28).

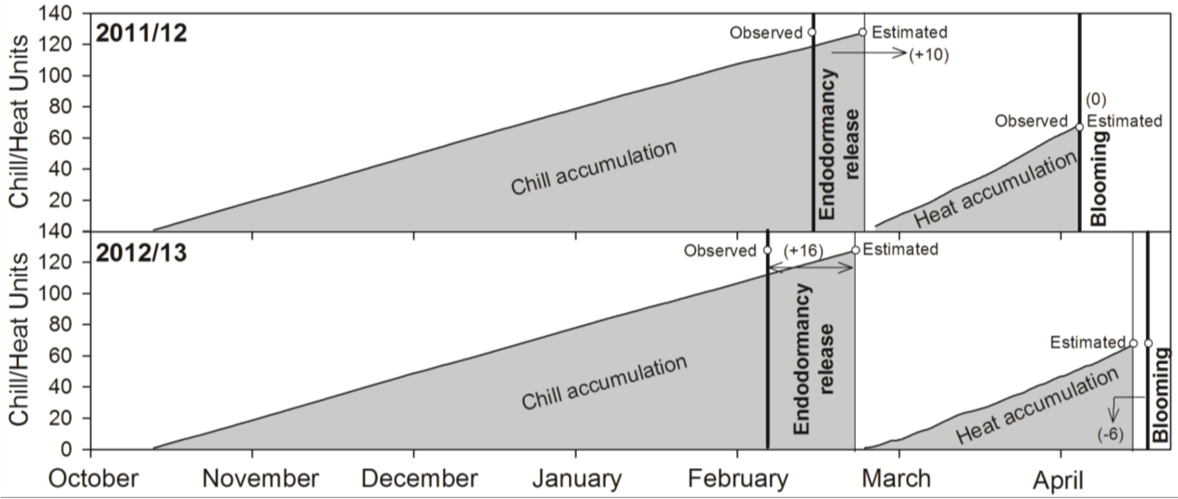

Figure 28. Evolution of chill and heat accumulation in Marsillargues, according to the 'NHGoldenF2' model during 2011/12 and 2012/13.

3.4.4 Evolution of endodormancy release dates and flowering of Golden Delicious using the NHGoldenF1 model

The application of the NHGoldenF1 model to the full historical temperature and phenology series for Bonn, Angers, Forli and Nîmes produced estimated dates of ER, the F1 flowering stage and the duration of these phases for the Golden Delicious cultivar and were also able to visualize their evolution over the historical period (Figure 29).

The estimated dates of ER exhibited small fluctuations at Bonn, Angers, Forli, and Nîmes. Except for Bonn, the remaining locations exhibited significant delays in meeting the chill requirement for ER. According to the Mann-Kendall test, Nîmes exhibited the highest delay of all of the studied locations at 2,20 days per decade (d 10 y^{-1}) between 1975 and 2012. Smaller significant delays were observed at Angers (1,43 d 10 y^{-1}) and Forli (1,00 d 10 y^{-1}) (Figure 29).

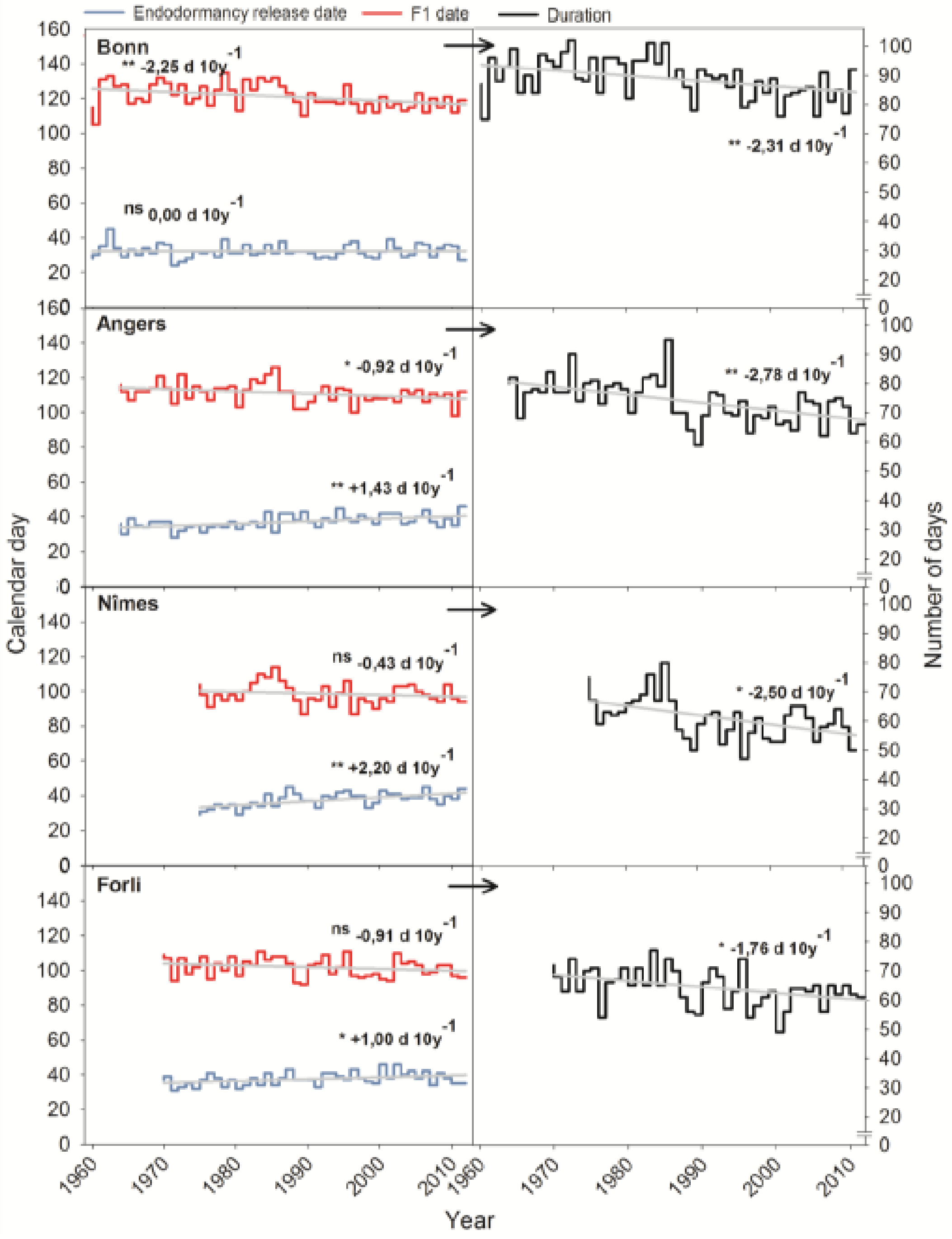

Figure 29. Simulated past time-course changes (day per decade – d 10y^{-1}) of endodormancy release dates, F1 dates (in calendar days from 1st January) (on the left) and duration between these dates (on the right) in Golden Delicious apple tree cultivar at different sites of Western Europe, according NHGoldenF1 model. * and ** significant at 5 and 1%; ^{ns} not significant.

The annual fluctuations were more pronounced for the estimated dates of the F1 flowering stage than for the dates of ER. Significant anticipation trends of approximately 2,25 d 10 y^{-1} at Bonn, starting in 1959, and of 0,92 d 10 y^{-1} at Angers, starting in 1964, were observed. In contrast, no significant differences were observed for the warmest locations of Western European, such as Nîmes and Forli. A significant decreasing trend of the time between the estimated dates of ER and the F1 flowering stage was observed for all of the studied locations, it was more pronounced for Angers and Nîmes, which had decreasing rates of 2,78 and 2,50 d 10 y^{-1}, respectively. The trend of a delay in the meeting of chill requirements for these two locations likely provided an important contribution to the shortening of the transition period between the considered phases. For Bonn, the significant 2,31 d 10 y^{-1} decrease may have been a reflection of the anticipation of flowering dates. The lowest rate of decrease of the transition phase was observed for Forli (1,76 d 10 y^{-1}). The weak delay of ER dates may have contributed to this trend (Figure 29).

Future predictions of phenology between 2015 and 2100 (86 years) revealed situations that were compatible with the RCP temperature scenarios. For the RCP 4.5 intermediate scenario, moderate trends were observed for the dates of ER and flowering (F1 and F2) of Golden Delicious (Figure 30).The future trends of ER dates, estimated using the NHGoldenF1 and NHGoldenF2 models, for Angers were not significant. However, significant predictions of the anticipation of F1 (-0,84 d 10 y^{-1}) and F2 (-0,95 d 10 y^{-1}) flowering stages were observed. In contrast, significant delays in the ER dates of +0,55 and +0,13 d 10 y^{-1} were observed for Nîmes according to the NHGoldenF1 and NHGoldenF2 models, respectively. However, no significant time changes were observed for the dates of the F1 flowering stage, although a -0,53 d 10 y^{-1} significant trend toward anticipation was observed for the F2 stage (Figure 30).

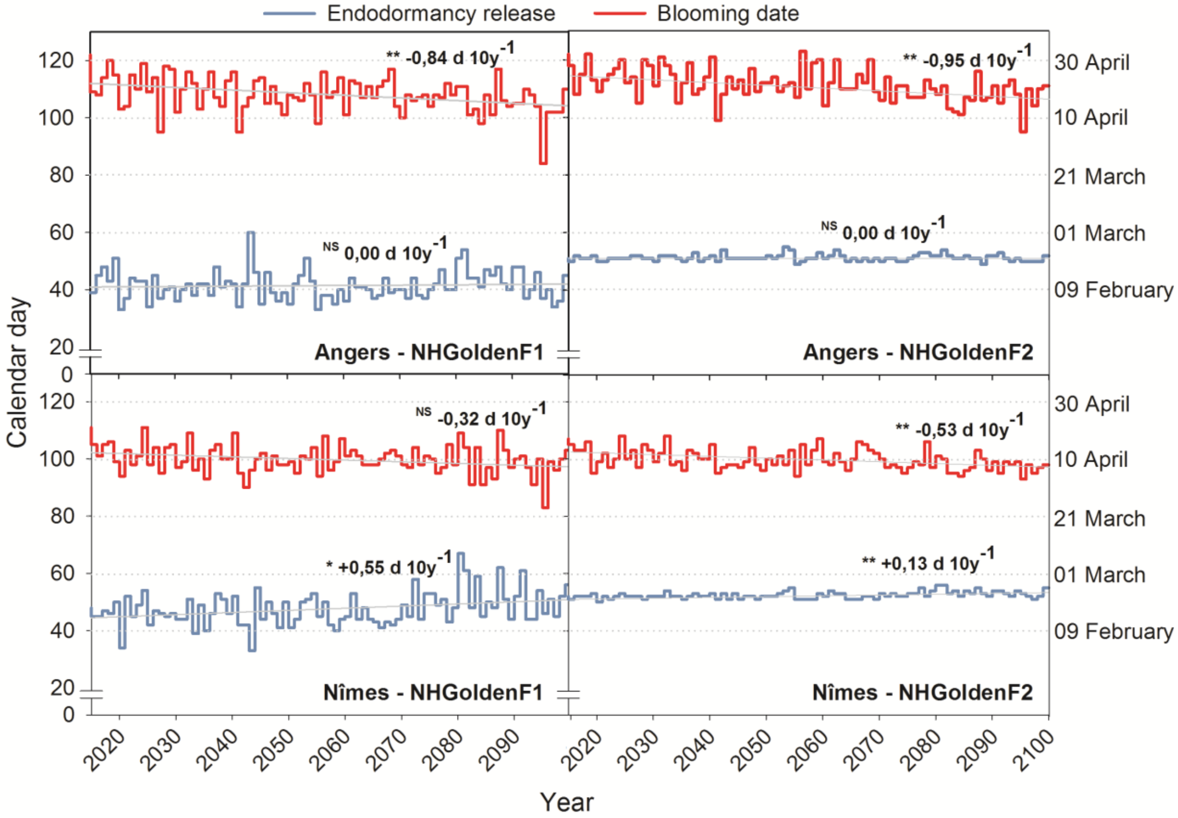

Figure 30. Simulated future time-course changes (day per decade – d 10y^{-1}) of endodormancy release and blooming dates (in calendar days from 1st January) of Golden Delicious apple tree cultivar in Nîmes and Angers, according NHGoldenF1 (on the left) and NHGoldenF2 (on the right) models, considering the intermediate (4.5) "Representative Concentration Pathways – (RCP)" scenario of temperature. * and ** significant at 5 and 1%; ^{ns} not significant.

The least optimistic scenario of temperature change (RCP 8.5) predicted more drastic phenological changes for Golden Delicious compared with the RCP 4.5 scenario (Figure 31). For Angers, the NHGoldenF1 model estimated a +1,48 d 10 y^{-1} significant delay of ER dates that implied a cumulative delay of approximately 13 days by the end of this century relative to the current situation; thus, the chill requirement would no longer be met at the beginning of February and would be met at the beginning of March. According to the NHGoldenF2 model, this rate was only +0,24 d 10 y^{-1} ($p>0,01$). Concurrently, the flowering dates exhibited significant trends of advance toward -0,83 and -1,43 d 10 y^{-1}, according to models NHGoldenF1 and NHGoldenF2 the NHGoldenF1 and NHGoldenF2 models. In both cases, the flowering at the end of April would start in the first two weeks of that same month. Delays to the ER dates were more pronounced for Nîmes than for Angers. The NHGoldenF1 model predicted a significant +2,76 d 10 y^{-1} delay to the ER dates, and the NHGoldenF2 model predicted a +0,63 d 10 y^{-1} delay (p>0,01). However, predictions of flowering dates for Nîmes indicated non-significant changes for the F1 flowering stage until the end of the century and a significant anticipation of -0,43 d 10 y^{-1} for the F2 flowering stage (Figure 31).

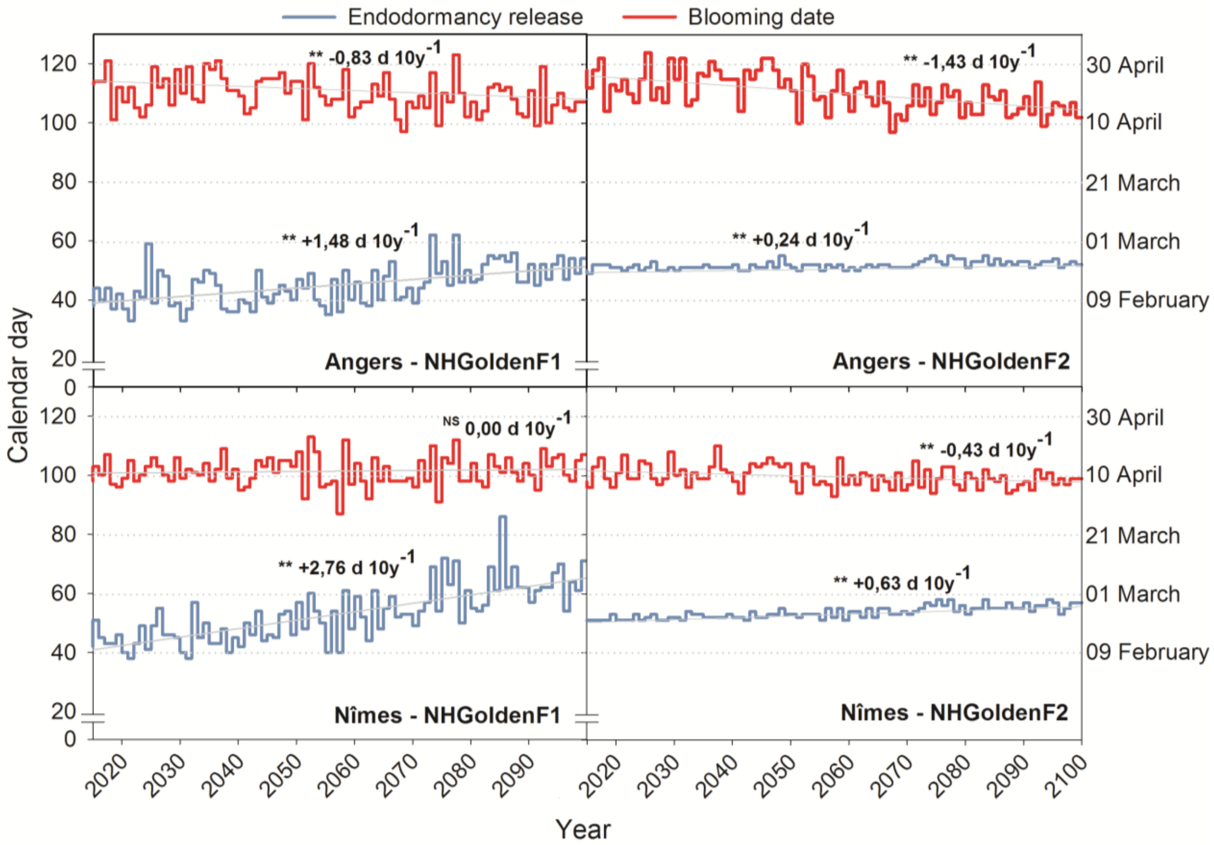

Figure 31. Simulated future time-course changes (day per decade – d 10y^{-1}) of endodormancy release and blooming dates (in calendar days from 1st January) of Golden Delicious apple tree cultivar in Nîmes and Angers, according NHGoldenF1 (on the left) and NHGoldenF2 (on the right) models, considering the high (8.5) "Representative Concentration Pathways – (RCP)" scenario of temperature. * and ** significant at 5 and 1%; ^{ns} not significant.

The predictions of the time between the dates of ER and flowering (F1 and F2) for Golden Delicious were also analyzed for Angers and Nîmes. For both temperature scenarios, significant decreasing trends were observed for this period for Golden Delicious. The estimated decrease in the time between ER and the F1 flowering stage were more pronounced for Nîmes than for Angers, whereas the opposite was observed for the time between ER and the F2 flowering stage (Figure 32).

Therefore, for the first scenario (RCP 4.5), rates of decrease of -1,15 and -0,95 d 10 y^{-1} were observed between ER and the F1 flowering stage. The decreasing rates for the period between ER and the F2 flowering stage were -0,76 and -0,98 d 10 y^{-1} for

Nîmes and Angers, respectively. Similarly, decreasing rates of -2,67 and -2,50 d 10 y^{-1} were predicted using the second scenario (RCP 8.5) for the period between ER and the F1 flowering stage for Nîmes and Angers, respectively. Decreasing rates of -1,11 and -1,67 d 10 y^{-1} were observed for the time between ER and F2 for Nîmes and Angers, respectively (Figure 32).

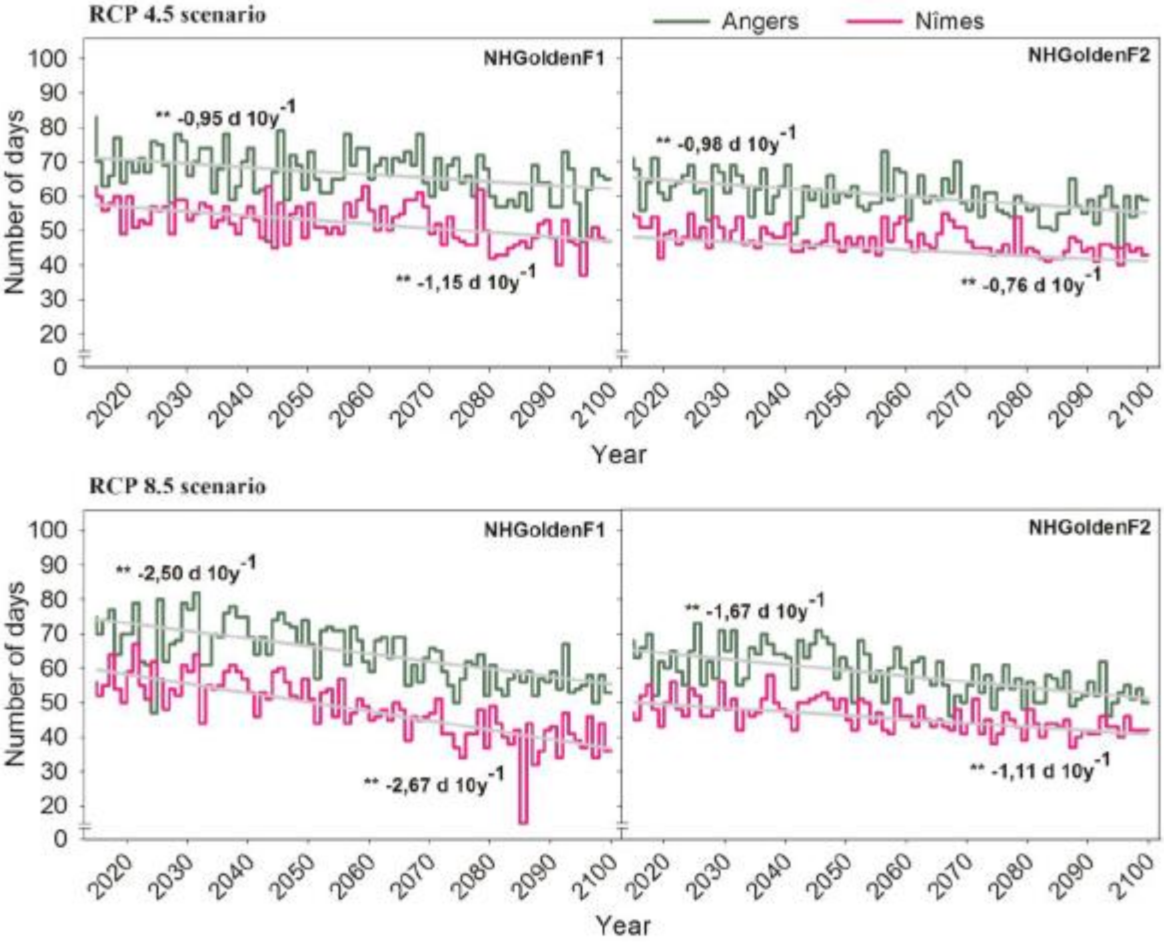

Figure 32. Simulated future time-course changes (day per decade – d $10y^{-1}$) in the duration time between endodormancy release and blooming dates (in calendar days from 1st January) of Golden Delicious apple tree cultivar in Nîmes and Angers, according NHGoldenF1 (on the left) and NHGoldenF2 (on the right) models, considering the intermediate (4.5) and high (8.5) "Representative Concentration Pathways – (RCP)" scenario of temperature. * and ** significant at 5 and 1%; ^{ns} not significant.

3.5. Discussion

3.5.1 Model parameterization and validation

The models NHGoldenF1 and NHGoldenF2 selected for the NH exhibited significant accuracy for locations in Western Europe such as Bonn, Angers, Nîmes, and Forli. The 1,0 and 7,0°C optimum temperatures for the chill accumulation were consistent with most similar studies (Richardson et al., 1974; Shaltout and Unrath, 1983; De Melo-Abreu et al., 2004; Legave et al., 2013). The triangular and parabolic functions considered for the chill sub-models were similar to the functions used in the Utah (Richardson et al., 1974) and North Carolina (Shaltout and Unrath, 1983) models, widely used for temperate fruit-producing regions. Similarly, the exponential and sigmoid functions considered for the heating sub-models were used in the F1Golden1 and F1Golden2 models (Legave et al., 2013) selected for Golden Delicious in Europe. Therefore, the precision of the flowering date estimations, according to the parameters of each model, shows that these models can be used for simultaneous predictions of phenology for locations in Western Europe. However, these models were not validated for Meknès, Morocco, which was possibly because of the smaller amount of temperature and phenology data used for model parameterization at this location compared with that of the Western Europe locations.

Moreover, although the models exhibited excellent precision in the estimation of dates of the F1 and F2 flowering stages for Golden Delicious at Marsillargues, the estimation of ER by the NHGoldenF2 model was relatively different from the observed values for both cycles. This observation assumes that for cold regions, such as Europe, a higher efficiency could be achieved by the simultaneous parameterization of models using dates of flowering and ER. Similarly, the selected models could be validated by the simultaneous application of both data series. This approach has not been previously used for the parameterization of phenological models because of the scarceness of ER dates. Although our pioneer study has attempted to validate the selected models using observed ER dates that were recorded over two years at Marsillargues, this information was not enough to evaluate the model's precision. However, this method of validation can be promising if historical series of the required information are available. The inclusion of this new data in the extensive European phenological database could be performed through annual applications of the Tabuenca test, based on the good results reported in chapter 2. In the near future, these data could be applied to develop more complex parameterizations and model validation, resulting in model fits that are closer to biological reality.

In opposition, the lack of precision of the estimated ER dates by the North Carolina + GDH composite sequential model and models for the NH series reveals a deficiency that is possibly related to the limited data available for parameterization. However, although the sequential models selected in this study exhibited satisfactory accuracy, in cases of variable climate conditions within a region, which is the case of southern Brazil, sequential models may not be the most accurate.

The scarcity of models capable of predicting phenology under subtropical conditions (such as the ones observed at São Joaquim, Caçador, and Pelotas), remains a primary limitation to the management of apple trees in southern Brazil. In the present study, although the selected models exhibited a good fit for predictions of flowering dates for the Fuji, Gala, and Golden Delicious cultivars, the functions were not capable of explaining the variability of the data that were not used for parameterization. This difficulty in selecting models for warmer climates (southern Brazil and Meknès, Morocco) indicates that these climates represent a much more complex challenge than cold regions.

The factors listed below may be potential agents for overcoming such obstacles. (I) Phenology and temperature variability data obtained from wider observations would allow for the selection of more robust models that are capable of predicting phenology with reasonable precision but remaining flexible, allowing simultaneous predictions of phenology for the majority of the considered locations. (II) In addition to budding and flowering dates, routine methods should be implemented that are capable of recognizing transition limits between endodormancy and ecodormancy phases, such as the Tabuenca test. Such methods have been previously proposed by Chuine et al. (2014), who observed that historical flowering dates were not sufficient for the accurate prediction of flowering dates for a wide region in Europe. These authors also noted the urgent need to identify and record dates of ER for forest and fruit species and to build databases that can be used to produce more accurate phenological models in the future.

Moreover, in-depth modeling (III) that considers new concepts in the interpretation of temperature effects during dormancy may be an interesting alternative for temperate regions. These new concepts would be able to predict phenology more accurately if the model parameters consist of a wider range of effective temperatures and consider the effect of sudden peaks of temperature during winter more strictly, which occurs with the alternating and parallel models. Similarly, flexible models could better fit the effect of delayed low temperatures during spring.

Modeling could also consider the level of complexity (IV) according to the effects of multiple factors, such as photoperiod(White and Nemani, 2003), rainfall, and light (Cardoso, 2011). For southern Brazil, these parameters could provide a significant contribution because the subtropical climate and high latitude favor considerable variations of these variables during dormancy. Studies have revealed increasing rainfall in southern Brazil in recent decades because of the intensification of *El Niño* events (Haylock et al., 2006). In the case of rainfall, the evaporation of water from the bud surface after rainfall results in decreased oxygen concentrations in bud tissues, and anaerobiosis may promote the breaking of dormancy (Petri el al., 2006). Light has a positive effect on dormancy because it can promote temperature variations in plant tissues. Therefore, winters with intense radiation promote the breaking of dormancy, whereas cold and cloudy winters favor dormancy (Petri et al., 2006). However, the inclusion of multiple variables and relatively extended historical data series in the modeling process would demand the use of computing resources with a higher data sorting and combination capacity.

3.5.2 Historical evolution of phenology and its long-term prospects

According to the NHGoldenF1 model, prior changes of ER and the F1 flowering stage dates for Western European locations revealed significant delays for Angers, Nîmes, and Forli, and pronounced advances in dates of the F1 flowering stage for Bonn and Angers. Simultaneously, the variation of days between these events showed a decreasing trend. This result appears to be closely linked to the pronounced temperature increases in winter and especially spring (see chapter 1). A similar study conducted for the same Western European locations revealed a slight delay of estimated ER dates (Legave et al., 2013). In addition, anticipation of flowering dates have been recorded because of increases in spring temperature starting in the 1980s in Western Europe (Chmielewski et al., 2004; Guédon and Legave, 2008; Legave et al., 2013).

The projections obtained for Nîmes and Angers, France confirm the maintenance of the current phenological trends until the end of the current century. The least optimistic scenario (RCP 8.5) exhibited change rates between 2015-2100 that were closer to those historically observed until 2012. Delays in meeting the chill requirement until the end of this century have also been reported by Fujisawa and

Kobayashi (2010) and Legave et al. (2013) for apple trees. The main implication of this observation refers to the time between the meeting of the chill requirement and the flowering dates. Our projections indicate a decrease in that phase of up to 2,67 d 10 y^{-1} d, which is equivalent to a decrease of approximately 23 days until the end of the century because the flowering dates only exhibited slight advance, especially for the scenario RCP 8.5. This scenario may be the equivalent of what occurs currently in subtropical Brazil regions. Another important implication of the delay in chill accumulation is the extension of the flowering period, which can result in higher damage to the floral buds due to late frost. Other possible consequences are pollination problems, desynchronization of pollination-dependent cultivars, and physiological disorders (Legave et al., 2013).

Thus, it is increasingly evident that the effect of chill is required for models to estimate flowering dates. In fact, a comparison of the efficiency of sequential and thermal models clearly indicated that sequential models exhibit the best fit to the variability of phenological data from Western Europe. This result is consistent with the efficiency of the model proposed by Fu et al. (2012). However, it is important to note that if climate changes are more severe than that considered in the RCP scenarios and the simulated phenological changes are underestimated, then sequential models may not exhibit the expected efficiency, which has been observed for the subtropical regions of Brazil. Therefore, the continuity of comparative studies and further development of modeling studies should be requirements of the scientific community.

3.6. Conclusions

For the cold conditions of Western Europe, the NHGoldenF1 and NHGoldenF2 models provided valid estimated dates of the F1 and F2 flowering stages, respectively, in Golden Delicious at Bonn, Conthey (only F2), Angers (only F1), Nîmes, and Forli. These models explained the phenological data variability more efficiently than the sequential ‘North Carolina + GDH’ model and thermal model. This concept of model can be optimized using complementary information, such as historical series of endodormancy release dates. However, the NH models were not valid for the temperate climate conditions of Meknès, Morocco. In the same manner, the selected models for Fuji, Gala and Golden Delicious in Southern Brazil were not valid to predict regionally the annual dates of flowering. These results indicate that the climate conditions of Southern Brazil and Meknès require a more complex approach to select precise phenological models. Thus, we need to consider other model concepts that adjust more effectively the effect of high temperatures during the winter such as parallel, alternating or flexible models and use historical series of endodormancy release dates to validate.

In addition, the NH models were useful for estimating future phenology in Western Europe. They simulated trends toward a delay in endodormancy release dates at Nîmes, principally, and an advance in Golden Delicious flowering dates at Angers, principally, under two temperature scenario (moderate and pessimistic) by the end this century. These simulations also indicated an important reduction of endodormancy-ecodormancy transition. Thus, this case study confirms that the mediterranean region is more vulnerable to climate change, as was hypothesized in the first chapter.

Concluding remarks and perspectives

This study was based on four main strands that converged to a better understanding of the physiological behavior of apple trees grown under contrasting climates: I) spatiotemporal variability of temperature and II) phenology patterns under contrasting climates; III) dormancy in apple cultivars grown under contrasting climates and phenological changes involved in overcoming them; IV) selection and validation of models for annual and future forecasts of flowering dates. The findings generated by these studies can contribute significantly to the making of future decisions regarding the need of adjusting to warming temperatures. The main contributions of this study, the difficulties and perspectives are discussed below.

The analysis of the spatiotemporal variability in temperature patterns (I) and phenology (II) under subtropical (Brazil) and temperate (Western Europe and Morocco) climates revealed significant regional differences. While in Western Europe and Morocco advancing of flowering dates was mainly associated with warming temperatures in the spring, in Brazil they were associated with winter temperatures, although these parameters have not shown significant changes in recent years.

In the Northern hemisphere, especially in Western Europe, studies of this scope have been conducted since the end of 80s, when the warming became more intense, and the consequences on plant development intensified, as was also observed in this study. The similarities between the projected temperatures for Nîmes, France in 2040, and the current temperatures at São Joaquim, Brazil, according to the A1B scenario, instigates the broadening climate studies and their implications on the physiology of apple trees, focusing the development of solutions for the adaptation of this culture to warming. Currently the subtropical region of Brazil has a low vulnerability to future warming, as revealed in this study, however, this does not rule out the need for monitoring temperature patterns over future years, given that studies have projected an increase in the frequency of the *El Niño* and *La Niña* phenomena.

The phenological responses in relation to environmental factors can be better understood if studied regionally. In this study, the regionalized focus and the comparison between different zones of apple production was innovative in relation to the global approach presented in the literature in recent years. The integration of research and educational institutions, data accessibility, the recording of phenological and temperature data, and the long term data series contributed massively to the study of regional variability. On the other hand, the study was restricted in Brazil and Morocco because the limited number of local records, interruptions to the series, short data sets, and the lack of information unification in a database record. Another difficulty still observed in Brazil, was especially related to flowering quality. Although the flowering stage BBCH 61 and 65 have been registered, the amount of floral organs analysed is usually low in trees not sprayed with products to overcoming dormancy. Therefore, the same stage of flowering in the subtropical region of Brazil and the temperate region of Western Europe was probably evaluated using different amounts of flowers. This can be used to reflect on the methodologies used to assess the phenology in the field.

Because of these current difficulties, future measures in Brazil and Morocco should consider the need to establish a network for observing phenological events. These procedures could comprise of a greater number of observation sites, standardization of the evaluation events, and a unified data system. This could provide phenological data that may be useful for future phenology studies and validation of regional phenological models. Although for Brazil the threat of immediate warming is not a worry, the implementation of these measures could contribute to a better understanding of the physiology of apple trees grown under a subtropical climate. This particular climatic situation causes physiological changes, requiring a different management approach and increasing costs. For this reason, and due to recent changes in phenology, European countries, such as France, have already established measures to implement a network for the observation of phenology. The structure of this network may help the understanding of the relationship between phenology and environmental factors. This could also contribute to a better understanding of the factors controlling flowering period, which was not clear in our study.

On the other hand, the full view of kinetic dormancy in vegetative buds of apple trees highlighted for the first time the differences and similarities between cultivars within the same region and between contrasting climates. This study allowed the observation of marked differences in the transitional period between endo- and ecodormancy, and launched the hypotheses regarding the physiological variables involved in this transition. Although the activities related to the remobilization of carbohydrates, water status and vascularization in buds were highlighted during this transition, the replication of this study could confirm in more detail the impact of each event on the physiology of trees during dormancy. Furthermore, other factors could be demonstrated.

Factors involved in the dormancy mechanism, the changes that occur, and the extension of phases is the key points for defining the best concept of phenological model and their parameters. Thus, according to the efficiency in defining the extension of the transition period between endo- and ecodormancy, even in contrasting climates, the Tabuenca’s test can be used routinely in apple trees. Its application may provide real dates of endodormancy release in floral buds and contribute to the development and validation of future phenological models.

In relation to the parameterization aspects and the selection of phenological models, our study presented an unconventional method of the mixing of data series from the same region. This methodology contributed to the selection of robust sequential models, valid for a wide region in Western Europe, and more efficient than classical sequential ‘North Carolina + GDH’ and thermal models. The models selected for Western Europe (NHGoldenF1 and NHGoldenF2) presented parameters consistent with those typically reported in the literature. Currently, under the climatic conditions of Western Europe, the concept of sequential models has the ability to predict the date of flowering. However, the quick progression of temperatures toward levels close to those recorded currently in the subtropical region of Brazil, can cause these models to lose their accuracy. This is a reasonable question considering that the current temperature level in Southern Brazil did not allow validation of regional phenological models.

The lack of valid models for subtropical conditions corroborates the evidence of low interference of temperature on phenology, reported in the first chapter. The development of phenological models for apple tree in the subtropical region of Brazil has been in demand for a long time. Despite the efforts made in this study the demand persists, however, the results highlighted are important for guiding future modelling studies. This situation suggests the expanding of studies including other modelling concepts (not sequential concept), that consider more effectively the occurrence of high temperatures and large fluctuation during winter. It also encourage us to consider the action of multiple factors on dormancy, and the development of tools with a greater capacity for screening and combining data to obtain robust models.

Another significant contribution of this study was the phenology projections for Golden Delicious in Western Europe. The projections indicated that the Mediterranean region as more vulnerable to the effects of warming. When considering the RCP scenarios intense increase in temperature, the NHGoldenF1 model showed considerable elongation of the chill accumulation period from 2040, and a drastic elongation from 2070. Surprisingly, important changes in temperature will also be recorded in 2040 and 2070, respectively, at Nîmes, considering the A1B scenario in chapter 1. These changes are comparable with the current temperature levels of São Joaquim and Meknès, respectively. Despite these results obtained and considering a pessimistic future scenario of warming, the implementation of adaptation measures from today seems to be unavoidable in the Mediterranean region of Europe, given that current advances in flowering dates have caused many problems with crop management. Thus, the models selected in this study for the Northern hemisphere, and the consistency of their phenological projection with future temperature changes at Nîmes, may be useful for the prediction of annual flowering dates and to evaluate the most suitable locations for the cultivation of apple in the coming years.

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APPENDICES

Abstracts and papers submitted

1 - MALAGI, G.; LEGAVE, J. A comparison between two forcing tests for determining the end of apple bud endodormancy. In: **XXII Congresso Brasileiro de Fruticultura**, 2012, Bento Gonçalves. Anais do XXII Congresso Brasileiro de Fruticultura, 2012. v. 1. p. 2652-2655, 2012 (abstract);

2 – MALAGI, G.; EL YAACOUBI, A.; BONHOMME, M.; LEITE, G. B.; LEGAVE, J. M. Contrasted warming patterns in both hemispheres result in divergent blooming trends for apple. **14th EMS Annual Meeting & 10th European Conference on Applied Climatology** (ECAC). Prague, Czech Republic, 2014 (abstract);

3 – EL YAACOUBI, A.; MALAGI, G.; OUKABLI, A.; HAFIDI, M.; LEGAVE, J. M. Global warming impact on floral phenology of fruit trees species in Mediterranean region. **Scientia Horticulturae**. 180:243-253, 2014 (original article);

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A COMPARISON BETWEEN TWO FORCING TESTS FOR DETERMINING THE END OF APPLE BUD ENDODORMANCY

GUSTAVO MALAGI[1]; JEAN MICHEL LEGAVE[2]

INTRODUCTION

Recently, predictive studies about the impacts of climate change on fruit tree production have stimulated renewed interest for breeding tools to improve climatic adaptation. In this context, phenological changes in fruit trees, as advances of blooming dates, were linked to temperature increases particularly in apple (GRAB and CRAPARO, 2011; GUÉDON and LEGAVE, 2008). That triggered needs to improve the breeding methodology to select apple varieties with appropriate chilling requirements to break the endodormancy in the warming context. In past and recent literature, the methods involved for the estimation of chilling requirement were numerous and hardly compared. Therefore, inconstant chilling estimates currently obtained for a same variety might be partly due to the use of different or inappropriate forcing tests.

The forcing method using one-bud cuttings (POUGET, 1963) allow to avoid correlative inhibitions between buds and to study the changes in their dormancy depth. This method was currently used in literature for determining the dormancy depth of vegetative buds in fruit tree species (CARVALHO et al., 2004; HERTER et al., 2001). Also, the comparison of time evolution of flower primordia weight without forcing (in orchard) and after forcing (in experimental warming conditions) allows assessments of the end of endodormancy for floral buds during the rest period, according to Tabuenca (1967). Reliable results have been obtained by this method in apricot (LEGAVE et al., 2010) and plum (TABUENCA, 1967). Thus, the aim of this work was to compare preliminary results obtained by these two forcing tests for three apple varieties, with the objective to define how the end of endodormancy can be correctly estimated.

MATERIAL AND METHODS

The experiment was developed from orchards of the experimental station CEHM located near Montpellier in France, using adult trees of ‘Mondial Gala’, ‘Golden Delicious’ and ‘Granny Smith’ apple varieties, grafted on M9 rootstock. For the one-bud cutting test, long annual shoots bearing

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[1] Doctoral student, Federal University of Pelotas-BR/Montpellier SupAgro-FR, e-mail: malagi@agronomo.eng.br
[2] Researcher, UMR AGAP, INRA, Montpellier, FR, e-mail: legave@supagro.inra.fr

lateral vegetative buds were regularly collected in orchard from August 2011 to March 2012 to produce cuttings in laboratory that were placed inside a forcing room (25ºC±1; 16 hours of light) with water supplies. For each sampling date (80 cuttings), observations were made three times by week during 62 days to highlight the date when the ‘green tip’ stage was emergent for each bud in forcing room. At the end of the observation period, the percentage of budburst and the average time for budburst (ATB value) were calculated for each cutting sample. The time-course changes of these variables were graphically elaborated. Regarding the Tabuenca’s test, short shoots bearing terminal flower buds were sampled regularly from November 2011 to April 2012. At each sampling date, the shoots were separated in two sets: the first one was used to estimate the fresh and dry weights in orchard condition of 30 floral primordia randomly extracted under binocular within flower buds (weighting of 6x5 primordia, dehydration at 90ºC); the second set was placed inside the forcing room, the shoots being stored in containers with water supplies. One week later, 30 primordia were also extracted from forcing buds and weighted. The, time-course changes in fresh and dry weights of flower primordia were graphically elaborated before and after forcing.

RESULTS

The results obtained through the forcing of vegetative buds highlighted three periods (Figure 1 A, D, G). This was particularly clear for Golden Delicious (Figure 1 D): a first period from the end of August to mid-November showed high percentages of budburst and relatively low ATB values that corresponded to the paradormancy phase according to LANG et al. (1987). Then, a marked decrease of budburst percentage and high ATB values were observed from the end of November to the beginning of January: this expressed a physiological change toward the endodormancy phase (a peak was reached round mid-December). From mid-January, a marked and rapid increase of budburst percentage was inversely observed associated with a clear decrease of ATB values: this expressed another physiological change toward the end of endodormancy (dormancy break). Then, from the end of January to mid-March, the budburst percentage reached values round 60% while the ATB values were more and more decreasing: this expressed a final phase of ecodormancy. Similar results were obtained for Granny Smith, although the peak of endodormancy was reached a little later for Granny Smith than for Golden Delicious (Figure 1 A and D). Lower budburst percentages and higher ATB were recorded for Mondial Gala during the paradormancy phase, followed by a little earlier decrease toward very low budburst percentages (endodormancy), compared to the two other varieties (Figure 1 A, D, G).

Regarding the results obtained by Tabuenca’s test, the end of endodormancy for Golden Delicious was observed in the second part of January, since the first significant increase of fresh weight after forcing was observed for the sampling date of January 24 (Figure 1 E). This was in agreement with the time of endodormancy end highlighted for vegetative buds, as previously described. The end of endodormancy for Granny Smith appeared in the beginning of January (significant weight increase in January 10; Figure 1 B), i.e. earlier than for Golden Delicious. In the case of Mondial Gala, the end of endodormancy inversely appeared later than for the two other varieties (significant weight increase in February 14; Figure 1 H). When the dry weight of floral primordia was considered, significant increases were observed clearly later for the three varieties (Figure 1 C, F, I) than considering the fresh weight.

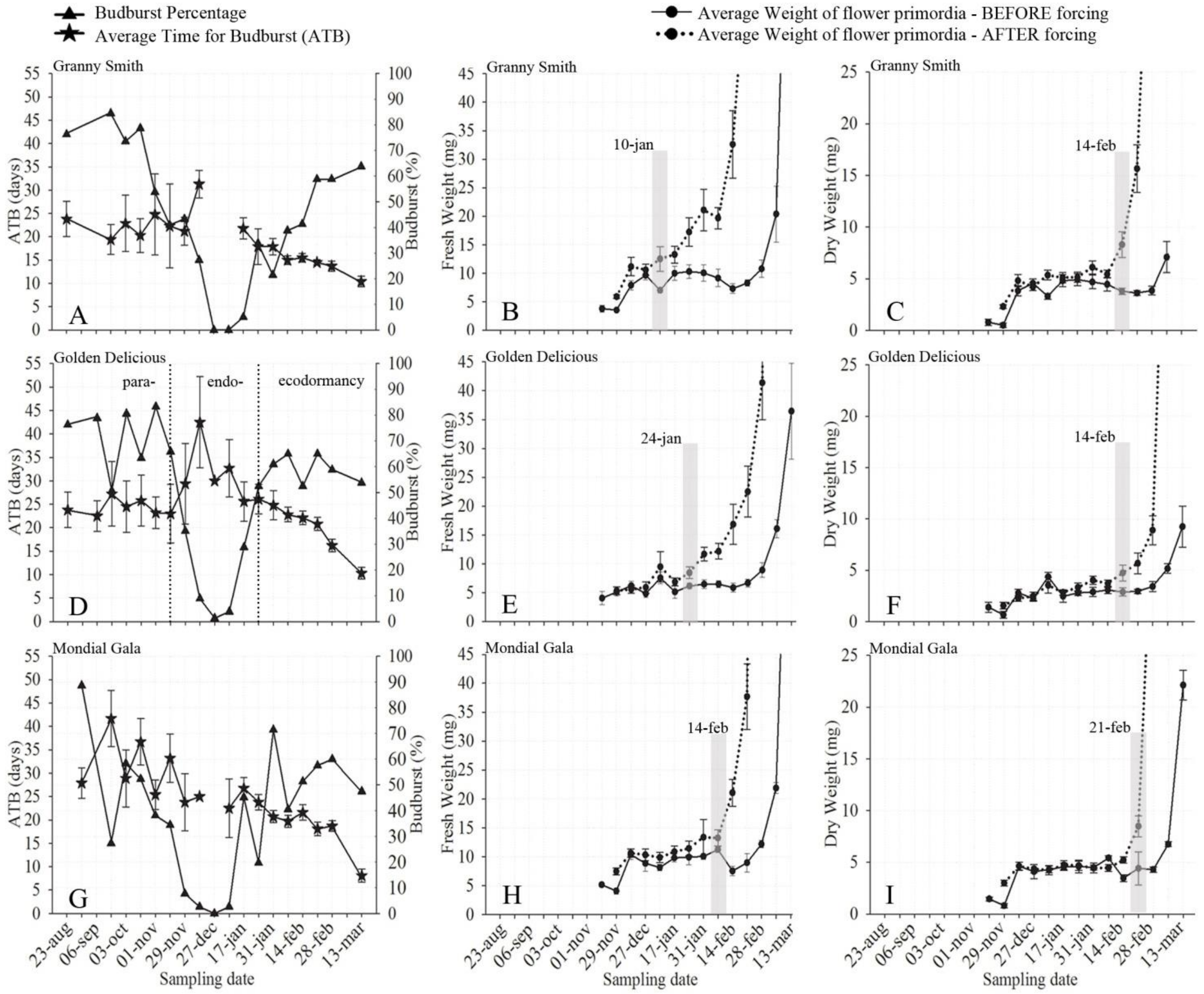

Figure 1. Time-course changes in dormancy depth observed in southern France from August 2011 to March 2012 for three apple varieties. Changes in vegetative buds after forcing: Average Time for Budburst (days) and Percentage of Budburst (A, D and G). Changes in floral buds before and after forcing: Average Fresh Weight (B, E and H) and Average Dry Weight (C, F and I). Vertical bars represent confidence intervals (P<0.05).

DISCUSSION AND CONCLUSION

Finally, the results obtained by the one-bud cutting test and the Tabuenca's test, considering changes in fresh weight, suggested similar times (dates) of the end of endodormancy for vegetative and floral apple buds, and this for the three varieties studied. Therefore, in a first approach, we may propose to estimate the annual date of satisfaction of chilling requirement by using these tests (only one or the two). Moreover, the two tests could highlight varietal differences in time-course changes of bud dormancy. Particularly, the Tabuenca's test efficiently showed differences in the ending date of endodormancy (chilling satisfaction) when changes in fresh weight of floral primordia were considered. As vascular connections between the buds and shoots could not have been functional before the overcoming of ecodormancy (ALONI, 1987), significant increases of primordia dry weight would have been recorded later than the end of endodormancy.

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Contrasted warming patterns in both hemispheres result in divergent blooming trends for apple.

Gustavo Malagi (1,6), Adnane El Yaacoubi (2), Marc Bonhomme (3,7), Gabriel Berenhauser Leite (4), and Jean Michel Legave (5)

(1) Federal University -UFPel, Pelotas, Brazil (malagi@agronomo.eng.b), (2) University Moulay Ismaïl, Faculty of Science, Department of Biology, Meknès, Morocco, (3) INRA UMR 547 PIAF, Clermont-Ferrand, France (Marc.bonhomme@clermont.inra.fr), (4) Center of Agricultural Research and Extension of Santa Catarina (EPAGRI), Caçador, Brazil (gabriel@epagri.sc.gov.br), (5) INRA UMR AGAP, Montpellier, France (legave@supagro.inra.fr), (6) INRA SupAgro, Montpellier, France , (7) Clermont Université Université Blaise Pascal UMR 547 PIAF, Clermont-Ferrand, France

This study aimed to compare the difference of temperature and apple phenology patterns and their relationships, in contrasting climates of Southern (SH) and Northern (NH) Hemispheres, using long historical series of data. Relatively high divergences of temperature and blooming patterns were found. The most important changes were observed in the NH sites, experiencing a significant warming of temperatures (especially maximum temperature) in whole year and mainly during early-spring. The oceanic and Mediterranean sites of Europe were also warmed during winter. Similarly, important increase of heat during early-spring was found in all sites and a strong decrease of chill during winter was highlighted in the mild sites of Europe. We outlined that blooming dates toward early for some apple cultivars with a higher advance in the continental regions (cold). In these colder regions, we found a strong relationship between blooming dates and heat accumulation during early-spring (February and March). In the mild sites, advance is lower and moderate relationship with heat accumulation in the mild sites can be linked to chill decrease during winter, leading us to consider this zone as more vulnerable to climate changes. By contrast, in the SH sites, only the continental site (Caçador) outlined a little warming and small advance of the blooming dates. Significant correlations were found between blooming dates and accumulated chilling doses, supporting the idea that blooming dates are mainly controlled by winter temperatures, especially June one's. We concluded that blooming dates and blooming duration are distinctly determined according climate pattern. The important warming of European sites led us to predict that apple crop in this region can be soon (around 2050) under climatic conditions observed today in mild climate regions like Southern Brazil, and, consequently, submitted to the same cropping difficulties.

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Global warming impact on floral phenology of fruit trees species in Mediterranean region

Adnane El Yaacoubi[a,c], Gustavo Malagi[b], Ahmed Oukabli[c,*], Majida Hafidi[a], Jean-Michel Legave[d]

[a] *Moulay Ismaïl University, Faculty of Science, Department of Biology, BP 11 201 Zitoune, Meknès 50000, Morocco*
[b] *Federal University of Pelotas, 96010-900 Pelotas, RS, Brazil*
[c] *INRA, Plant Breeding and Genetic Resources, Regional Agricultural Research Center of Meknès, Box 578, Meknès, Morocco*
[d] *INRA – UMR 1334 AGAP, F-34398 Montpellier, France*

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ABSTRACT

This study was carried out in three geographically contrasting countries of the Mediterranean region. It aims to understand the impact of climate change, particularly the temperature increases, on phenological stage of three taxonomically different species (early and late-spring-flowering species). Three species, namely olive (*Olea europaea*), apple (*Malus domestica* Borkh.) and almond (*Prunus dulcis* (Mill.)) were investigated to highlight the phenological behavior of one species at different locations and different species at one location. Climatic and phenological data were collected from Morocco, France and Italy over the last 40 years. Analysis of data on temperature showed a strong warming in the northern locations (coldest areas), particularly in Nîmes, compared to the southern ones (warmer locations) during the period October to May. The marked spring warming in all locations since the end of 1980s in France (the beginning of 1990s in Italy and Morocco) resulted in blooming earliness, with regional patterns in terms of impact. The late-spring-flowering species (olive and apple) showed a remarkable sensitivity to continuous warming in different areas. No flowering earliness was observed in early-spring-flowering species (almond), due to the stability of mean temperature during February. Thus, a strong control of mean temperature during the forcing period on flowering earliness of apple was found in all areas. Physiological processes (dormancy and dormancy release) of trees during the dormant and growth period explain, in part, the regional differences observed in flowering dates among sites and species. Overall, the pronounced warming in the southern France reflects a relative trend toward aridity of climate at this site, and consequently some vulnerability of fruit trees. As result, the process of flowering in a high latitude locations (northern areas) in the future can be represented by that in a low latitude locations at present (southern areas), particularly for apple.

1. Introduction

Mediterranean fruit tree production is facing major changes that have environmental and socio-economic consequences. Climatic changes related to temperature warming have been reported worldwide. Thus, the period 1995–2006 has been ranked among the 12 warmest years since 1850 (IPCC, 2007). Other phenomena associated with climate change such as rainfall variability and higher drought frequency are become increasingly intense.

Most meteorological stations in Europe, East Asia, and Alaska recorded significant increase in annual maximum and minimum temperature, specifically during winter and spring. However, summer warming remains not significant (Schwartz et al., 2006). In the Mediterranean, a temperature increase was pointed over a long period throughout Sicily, with higher increases at the end of the past century (Viola et al., 2013). In Spain, Martínez et al. (2010) reported increases in annual minimum and maximum temperatures during spring and summer, and only for maximum temperatures in winter. In France, a mapping of temperature trends has been established by Moisselin et al. (2002). They showed a significant increase in minimum temperatures and moderate increase in maximum ones. Minimum temperatures presented an

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* Corresponding author at: INRA Centre Régional de la Recherche Agronomique, BP 578, Meknès, Morocco. Tel.: +212 5 35 30 02 66; fax: +212 5 35 30 02 44.

E-mail addresses: adnan_el_yaacoubi@hotmail.com (A. El Yaacoubi), malagi@agronomo.eng.br (G. Malagi), oukabli2001@yahoo.fr (A. Oukabli), hafidimaj@yahoo.fr (M. Hafidi), legave@supagro.inra.fr (J.-M. Legave).

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east-west gradient, whereas maximum temperatures exhibited a north-south gradient. Temperature trends studied in Maghreb countries showed increases in Algeria, Egypt, Libya and Tunisia. Rainfall remained, however, unchanged in Egypt, Tunisia and Libya (Schilling et al., 2012). In Morocco, Benassi (2008) showed an important increase in average temperature since 1961, particularly important in April-May-June and September-October-November. This increase is most rapid during April to June period. McSweeney et al. (2010a,b) confirmed this trend and reported increases reaching 1.4–5.6 °C in the 2090s. Rainfall outlined a noticeable decrease since the 1970s in different places in Morocco (Schilling et al., 2012). Many other studies showed temperature increase across the globe (Bradley and Bertrand, 2008; Rebetez and Reinhard, 2007; Codero et al., 2011; Marengo and Camargo, 2008).

The Mediterranean is differentiated by its horticultural crop production including olive (*Olea europaea*) and almond (*Prunus dulcis* (Mill.)). Fruit tree crops are vulnerable to climate change: it is hardly suited in changing climatic conditions and needs more equipment and investments. The influence of climatic factors on trees phenology has implications on their production behavior. Oukabli and Mahhou (2007) observed irregularities in annual yield associated with flowering date changes, bud fall, maturity earliness, resulting into additional water needs (Legave, 2009). Current studies have shown that phenological stages are closely related to temperature characteristics of the cropping site during months preceding the phenological evolution (Lopez and Dejong, 2007; Galán et al., 2001; Osborne et al., 2000). Indeed, temperature increase is linearly correlated with earliness of flowering dates. The rate of flowering earliness varies from one species to another, although recent studies have shown that this trend is not always linear (Pope et al., 2013). In the northern hemisphere, Legave et al. (2008) underlined advances in apple (*Malus domestica* Borkh.) flowering dates during the 1980–2011 period in France and other European countries (Legave et al., 2013). In the southern hemisphere, Grab and Craparo (2011) confirmed flowering advances in apple through an advanced full bloom around 1.6 days/decade over the period 1973–2009. In Cordoba, an increase in mean temperature of 1 °C during March–April–May induced an advance in olive full blooming of 7.6 days (Orlandi et al., 2009) with a projected flowering advance of 6.2 days/°C by the end of the twenty first century in western Mediterranean (Osborne et al., 2000). Concerning almond species, advanced blooming dates after warm periods of dormancy were already highlighted in Spain (Alonso et al., 2011). Early blooming cultivars have shown higher variation in blooming dates because of a slower heat completion and more variable temperatures during February. As a consequence, late blooming cultivars would show more stable blooming dates because their higher heat requirements are quickly satisfied by the higher temperatures during March (Alonso et al., 2011). Flowering advances were similarly reported in many other fruit tree species (Lu et al., 2006; Črepinšek et al., 2012; Miller-Rushing et al., 2007; Abu-Asab Mones et al., 2001).

Most studies covering the impact of climate change on the phenology of fruit trees often focused on one species in a given site. The present study assesses relationships of climate and phenological trends based on data from contrasting Mediterranean countries and aims to understand how fruit-trees are responding to climate change in these areas. In terms of floral phenology, three different-determinism species (early and late-spring-flowering species) are investigated: olive without consistent chilling requirements and highly dependent on heat requirements (Galán et al., 2005; Alba and de la Guardia, 1998; Fornaciari et al., 1998), apple have instead the highest chilling requirements (Hauagge and Cummins, 1991), with problems of adaptation in Morocco (Oukabli et al., 2003) and almond with moderate chilling requirements (Egea et al., 2003) and therefore well adapted to different climatic conditions (Alonso et al., 2005). In North Africa, namely Morocco, the few studies available on this topic are limited to annual assessments of phenological stages such as dormancy and flowering. Conclusions derived from the current study will contribute to suggest new effective ways to manage and adapt this fruit crop to observed climate change phenomenon.

2. Materials and methods

2.1. Sites and climate data

Data on temperatures recorded for each location are presented in Table 1. Three climate-contrasting Mediterranean areas are chosen in this study: Morocco (Meknès and Tassaout), France (Nîmes and Montpellier) and Italy (Forli). The investigated sites are located on both banks of the Mediterranean and are distant in terms of latitude and longitude to cover a large geographic comparison (Table 1). The European sites are lowlands of around 34–64 m, while uplands are represented in North Africa (400–500 m). Nîmes and Montpellier are coastal areas and are influenced by Mediterranean air masses. Forli is close to the Adriatic coast of 30 km, with sub-continental climatic condition, evolving gradually to Mediterranean climate toward the coastal part of the region. Northern Mediterranean areas (Nimes, Forli) and southern ones extend between 44°N and 32°N respectively. During the period 1973–2012, the annual average of mean temperature was around 14 °C. In Northern Africa, Meknès and Tassaout have a semi-arid climate, with higher temperatures by comparison to European sites. The average annual mean temperature is around 17–18 °C over the period 1973–2012. The European sites are suitable for all fruit trees species investigated while the Moroccan locations, particularly in Tassaout, are not appropriate for apple. Olive and almond are mostly cultivated in Meknès while olive is predominant in Tassaout. Daily maximum and minimum temperatures data obtained throughout 40–47 years depending on the site were collected from governmental meteorological stations where trees are planted. All temperature data were manually verified and were considered reasonably accurate for further analysis (checked to ensure that no daily minimum temperature value was larger than the corresponding daily maximum temperature value). The mean temperatures were calculated using maximum and minimum temperatures.

2.2. Phenological data

Five locations were selected to represent major trees-growing areas in the Mediterranean: Two in North Africa (Meknès and Tassaout) and three in the Europe (Nîmes, Montpellier and Forli). In each of the five locations, phenological stages were observed from 1970 until the year 2012. The oldest chronological series date back to 1970 for apple in Forli. They extended from 1974 to 2012 and from 1984 to 2012 in Nîmes and Meknès respectively. For almond, the longest series were collected in Meknès during 1977–2012 for both Ferragnès and Marcona cultivars. The observation period was, However, short in Nîmes where it extended only from 1988 to 2000, with one missing data in 1997. The longest series for olive were collected in Montpellier (1973–2012) for the maximum emission of local olive pollen. In Meknès, the observation period extended from 1993 to 2012, with one missing data in 1999 while in Tassaout it extended from 1986 to 2012 with a gap from 1992 to 1996. Inputs were reliable and verified using field observation data which were conducted by qualified observers on adult trees. This latter are not influenced by any artificial products to break dormancy, and growing in long-term orchards according to standard horticultural practices receiving the annual pruning, fertilization and plant protection. Collection of these data was achieved through an international research network allowing comparable data across the

Table 1
Phenological and temperature data collected from different sites of Mediterranean countries and their geographic localization.

Cropping region recording sites/origin data	Latitude/longitude	Altitude (m)	Climatic area/climatic specificity	Phenological data recorded			Temperature
				Species/cultivars	Stage(s)	Period	Period recorded
Northen Italy			Mediterranean upper basin				
Forli (CRA)	44°13′N/12°2′E	34	Mediterranean climate	**Apple** Golden Delicious	61, 65	1970–2012	1966–2012
Southern France							
Nîmes, Balandran (Ctifl)	43°44′N/4°30′E	52	Mediterranean climate	**Apple** Golden Delicious	61, 65, 67	1974–2013	1966–2012
Nîmes, Balandran (INRA Fr.)				**Almond** Ferragnès	61	1988–2000	
Montpellier (SupAgro)	43°36′N/3°52′E	64	Mediterranean climate	**Olive** Local cultivars	65	1973–2013	
Northern Morocco			Mediterranean lower basin				
Meknès (INRA Mo.)	33°55′N/5°13′W	499	Semi-arid climate	**Apple** Golden Delicious	61, 65, 67	1984–2013	1972–2012
				Almond Ferragnès	61, 65, 67	1977–2013	
				Marcona	61, 65, 67	1977–2013	
				Olive Picholine Marocaine	61, 65, 68	1993–2012	
Southern Morocco							
Tassaout (INRA Mo.)	32°8′N/7°16′W	416	Semi-arid climate	**Olive** Picholine Marocaine	61, 65, 68	1997–2012	1972–2012

Apple and almond: Meier et al., 1994. F1 (stage 61): 10% of flowers open; F2 (stage 65): at least 50% of flowers open, first petals falling; H (stage 67): majority of petals fallen.
Olive: Sanz-Cortés et al., 2002. F1 (stage 61): 10% of flowers open; F2 (stage 65): at least 50% of flowers open, first petals falling; H (stage 68): majority of petals fallen or faded.

Mediterranean areas. For each species and cultivar, flowering data in each site were collected as described in Table 1. For each species, flowering data concern F1 (stage 61) when 10% of flowers open, F2 (stage 65) when at least 50% of flowers open, first petals falling and H (stage 67) when the majority of petals have fallen according to the description outlined in international BBCH code (Bayer, Basf, Ciba-Geigy, Hoechst). By definition, the maximum emission of pollen recorded for olive in Montpellier corresponds to full bloom stage (Orlandi et al., 2009). Linear trends of blooming dates are compared in order to show the spatiotemporal differences among earlier and late species, and therefore differences in terms of chilling and heat requirements on one hand, and native and introduced species on the other hand.

2.3. *Statistical analysis*

Due to the non-normal distribution observed on some variables of temperature data, mainly in Tassaout for May and in Nîmes for January, Mann–Kendall test was used for monthly and seasonal temperatures during autumn and winter periods (October, November, December, January and February) and spring (March, April and May) for each site to highlight the spatiotemporal temperature changes. Statistical analyses were implemented on maximum, minimum and mean temperatures. All phenological time-series, except for Ferragnès cultivar in Meknès, showed a normal distribution. Flowering dates were transcribed to day of year (DOY). In this study, we were interested only in full bloom dates (F2) corresponding to the date 65, which are available for all species. Because of non-linear regression observed on phenological data of some species/cultivars, a descriptive analysis was used to demonstrate trends of full blooming dates using annual dates.

Moreover, in order to analyze the change in flowering dates in relation to temperature changes, mean temperatures were assessed to determine precisely the chilling period which play an important role in breaking bud endodormancy (Lang et al., 1987) and the forcing period which is essential to active flower bud growing. To do this, we determined the corresponding periods of these two phases for each species/cultivars using partial least squares (PLS). This latter is a recent statistical approach used for modeling relations between phenology and temperature: a dependent variable (response), which is full blooming in our case, and a set of predictor variables block, which are 11 days-running mean daily temperatures from October to May over the studied period for each species/cultivar. In this study we focused our analysis on months corresponding to chilling and forcing period which are involved in the flowering process during the existence of floral buds. Partial Least Squares is commonly used in many fields, such as business and management research (Hulland, 1999), bioinformatics (Nguyen and Rocke, 2002) and chemometrics research (Nilsson et al., 1997). Recently, this method was effectively used to highlight the correlation between daily temperatures and phenology response for walnut (Luedeling and Gassner, 2012), chesnut (Guo et al., 2013) and cherry (Luedeling et al., 2012). Variable importance in the projection (VIP: commonly set to 0.8) and model coefficients are the two major outputs of PLS analysis. All data are analyzed following the method described by Guo et al. (2013). After identification of the major phases (chilling and forcing periods) related to flowering dates, Spearman's test was done to show the relationship between mean temperature during chilling and heat periods and full bloom, the slope value was determined using Sen's test. To investigate which period (chilling or forcing period) affects flowering dates the most, a three dimensional analysis was executed to interpolate response of full blooming as function of mean temperature during chilling and forcing periods, using Kriging technique. We choose a Gaussian model to interpret relationships between phenological dates (variance) and distance.

Table 2
Temperature data collected characteristics in the four studied sites in the Mediterranean region during 40 years (1973–2012).

Annual period	Daily temperature	Mean daily temperature (°C)				Temperature slope (°C/decade)			
		Tassaout	Meknès	Nîmes	Forli	Tassaout	Meknès	Nîmes	Forli
October	Average	20.0	18.9	15.2	15.5	0.53*	0.70**	0.72***	0.30
	Maximum	26.9	24.7	20.1	18.8	0.31	0.70*	0.65**	0.31
	Minimum	13.1	13.2	10.4	12.2	0.82**	0.60*	0.75***	0.25
November	Average	15.2	14.5	10.2	9.3	0.07	0.06	0.38**	0.48**
	Maximum	21.9	19.8	14.5	11.9	–0.14	–0.08	0.30	0.49*
	Minimum	8.6	9.1	5.9	6.6	0.24	0.21	0.53**	0.43*
December	Average	11.9	11.4	7.2	5.0	0.26	0.16	0.19	0.19
	Maximum	18.5	16.4	11.2	7.5	0.19	0.02	0.19	0.19
	Minimum	5.2	6.4	3.2	2.5	0.39	0.17	0.18	0.19
January	Average	10.8	10.3	6.7	3.9	0.02	0.08	0.21	0.15
	Maximum	17.7	15.6	10.9	6.4	0.10	0.18	0.26	0.21
	Minimum	4.0	5.1	2.5	1.3	0.10	0.05	0.15	0.11
February	Average	12.5	11.5	7.8	5.8	–0.09	0.08	0.12	0.10
	Maximum	19.1	16.8	12.5	8.9	–0.14	0.27	0.22	0.29
	Minimum	5.8	6.2	3.1	2.7	–0.02	–0.22	–0.06	–0.15
March	Average	15.0	13.5	10.6	10.0	0.50*	0.56**	0.49*	0.32
	Maximum	22.2	19.3	15.8	13.7	0.51	0.79*	0.63**	0.51
	Minimum	7.8	7.7	5.4	6.4	0.42*	0.35	0.29	0.12
April	Average	16.7	14.9	13.0	13.6	0.64*	0.54*	0.67***	0.42**
	Maximum	23.6	20.8	18.4	17.5	0.69	0.77*	0.75***	0.49**
	Minimum	9.8	9.0	7.6	9.7	0.43*	0.29	0.69***	0.36*
May	Average	19.7	18.2	17.1	18.6	0.77**	0.82**	0.69***	0.47*
	Maximum	27.0	24.6	22.8	22.9	0.82*	1.11**	0.80***	0.64**
	Minimum	12.3	11.7	11.3	14.4	0.66**	0.44**	0.56***	0.29
October–May	Average	15.3	14.2	11.0	10.3	0.31**	0.35***	0.44***	0.33**
	Maximum	22.1	19.8	15.8	13.5	0.28	0.47***	0.49***	0.43***
	Minimum	8.4	8.6	6.2	7.0	0.39**	0.22*	0.40***	0.24*

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

3. Results and discussion

3.1. Spatiotemporal temperatures trends

Trends in monthly and seasonal temperatures (maximum, minimum and average) collected during the period 1973–2012 in different sites are presented in Table 2. Focusing on the period from October to May (period affecting the physiological process of floral buds of most fruit trees in the Mediterranean), averages mean daily temperature in Tassaout and Meknès were 15.3 °C and 14.2 °C respectively. The hottest temperatures were recorded during 2010 and 2002 for both Moroccan sites, whereas the hottest temperatures registered in Nîmes were in 1988, 2001 and 2007, with an average of mean daily temperature of 11 °C. In Forli, it was 10.3 °C, with warmer temperatures in 2007, 2001 and 1994. Forli and Nîmes showed a difference of monthly regime evolution explained by coldest average temperature in Forli during November, December, January and February, compared to Nîmes where average temperature during April and May becomes colder than Forli. However, in Moroccan sites, Tassaout was warmer than Meknès in all months (Table 2).

In terms of spatial temperature change, a significant increase of temperature (minimum, maximum and average) was observed during October–May period at all sites, except for maximum temperature recorded in Tassaout. During this period, temperature increase was most rapid for minimum temperature in Tassaout, while it was more pronounced in Meknès, Nîmes and Forli for maximum temperature. In Nîmes, average temperature showed an increase of 0.44 °C/decade as a highest value, compared to other sites that showed relatively the same increase: 0.31 °C/decade in Tassaout, 0.35 °C/decade in Meknès and 0.33 °C/decade in Forli (Table 2). In this context, the amount of winter chilling available in Nîmes might be strongly affected in the near future as reported by Luedeling et al. (2009) in California, where Baldocchi and Wong (2008) highlighted a noticeable decrease of annual winter chilling accumulation.

In term of temporal temperature changes, a significant temperature increase was observed mainly during spring months in all sites (Table 2), especially for maximum temperature and in autumn namely for average temperature. Spring warming was relatively similar between the north of Morocco and the south of France. The annual variation of average temperature during March/April showed an accelerated temperature increase since 1988 in Nîmes and ten years later in Tassaout, Meknès and Forli (Fig. 1). This change reflects a relative trend toward greater climate vulnerability following a gradient from the south (Northern Morocco) to the north of the Mediterranean (south of France). This warming more pronounced in Nîmes (compared to Forli and Meknès) is explained firstly by warming in fall temperature (October and November) which was less marked in Meknès and spring temperature warming which was more pronounced in Nimes than in Forli. In fact, no significant increase of temperature was observed during the winter season in all sites.

3.2. Spatial trends of full bloom

Our goal in the present study was to compare the flowering responses of different species and cultivars in contrasting environments in relation to temperature increase. For this purpose, we compared the responses of long-term phenological trends of apple, olive and almond within three contrasting Mediterranean countries (Morocco, France and Italy) in the beginning and the

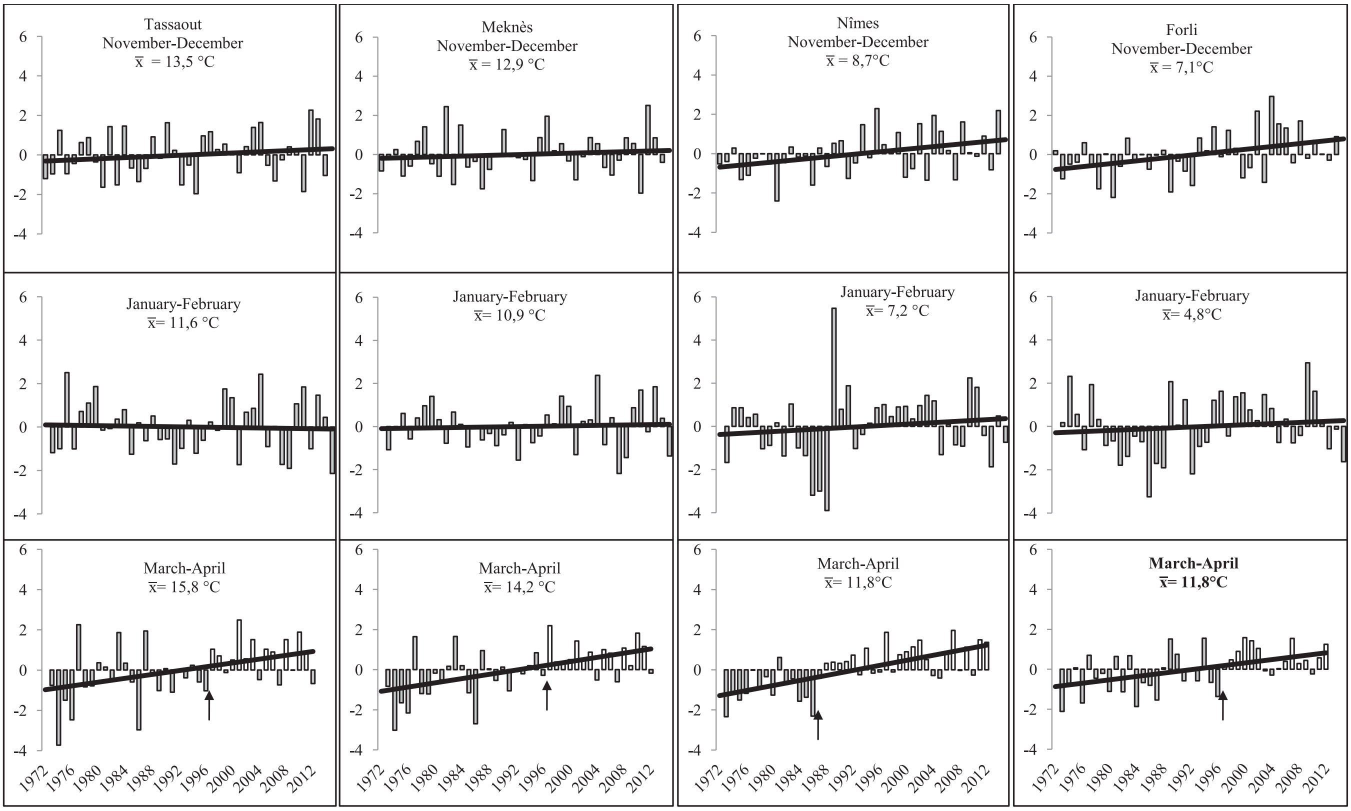

Fig. 1. Annual average air temperature variation during chilling and heat period in four Mediterranean sites from 1973 to 2012.

end of growing season. Indeed, it is widely reported that spring phenophases are occuring earlier, and strongly influenced by species, altitude/latitude and extent of temperature increases, particularly mean temperature during the months preceding flowering date (Chmielewski and Rötzer, 2001; Lu et al., 2006; Fujisawa and Kobayashi, 2010; Miller-Rushing et al., 2007; Legave et al., 2008; Benaouda and Balaghi, 2009). Differences among locations induced some differences in terms of phenological sensitivity to air temperature among early and late-spring-flowering species.

Flowering responses of two almond cultivars (Ferragnès and Marcona) in European and North Africa sites showed similar trend. In southern area, the average date of full bloom was in March 3rd for Ferragnès and February 19th for Marcona during the period 1977–2012. The average date of first bloom for Ferragnès in Nîmes was Februrary 28th during the period 1988–2000. No trend toward flowering earliness of almond, particularly for Ferragnès, was observed in Meknès as well as in Nîmes (which are geographically and climatically different). This result showed no effect of elevation on almond flowering earliness and could be explained by: (i) stability of mean temperature during February (Table 2) which corresponds to the decisive period for satisfaction of heat requirement of the majority of almond cultivars and (ii) the strong correlation between full blooming dates and mean temperature during chilling period (Table 3). The same results were reported by Alonso et al. (2011) for some late blooming cultivars of almond in Spain. In fact, Ferragnès is a highly-chill-demanding species as compared to Marcona (Alonso et al., 2005; Egea et al., 2003). However, in Marcona, a miniature flowering advance of 2 days was observed in Meknès during 1977–2012. A slight change in full blooming began since the year 2000 for Marcona.

The average date of full blooming for Golden Delicious (GD) in Meknès, Nîmes and Forli was April 17th (April 4th for F1), April 10th (April 8th for F1) and April 15th (April 12 th) during 1986–2012, 1976–2012 and 1970–2012 periods respectively. During these periods, the rate of flowering earliness was intermediate in Meknès (5 days) low in Nîmes (4 days) and greater in Forli (6 days), in accordance with differences in latitude. In Europe, flowering dates of GD advanced similarly by 4 days both in Nîmes and Froli during the common period 1976–2012. A remarkable earliness of flowering dates “break” started from 1988–1989 in Nîmes and Forli without apparent gradual transition over the studied period. These phenological changes toward greater precocity confirmed the results obtained by Legave et al. (2008). This “break” was however, delayed six years later (1994) in Meknès (Fig. 2). Overall, the flowering earliness can be considered as an indicator of global warming which impacted apple; a species highly demanding of chill and heat requirements. A remarkable change in flowering date since the end of 1980 was reported by Legave et al. (2008) who observed that flowering dates showed a shift to earlier dates in Nîmes and Forli. Moreover, although the increase in mean temperature (during spring) was more rapid in Nîmes than in Forli, the latter showed a great flowering earliness than in Nîmes. This observation could be explained by the high value (2.07 days) of year-to-year variations (standard deviation) in mean temperature during February in Nîmes (1.94 days in Forli), which represents an important part in the forcing period revealed by PLS regression. However, this month was not included in this period in Meknès.

The average date of full bloom for Picholine Marocaine (PM) in Meknès and Tassaout was May 9th and April 27th during 1993–2012 and 1986–2012 respectively. The average date for maximum pollen emission, which corresponds to full bloom stage in Montpellier, was June 02nd. Therefore, flowering date was earlier in Meknès in comparison to those of Montpellier which was later. In

Table 3
Spearman's correlation between full bloom of apple, almond and olive and mean temperature during the relevant phases obtained by PLS regression over studied periods.

Sub-periods	GD			Ferragnès	Marocna
	Meknès	Nîmes	Forli	Meknès	Meknès
Chilling period	0.25	0.21	0.12	0.41*	0.29
Forcing period	−0.48*	−0.77***	−0.73***	0.27	−0.22

	Olive		
	Meknès	Tassaout	Montpellier
First period	0.55*	0.30	–
Second period	−0.35	−0.34	−0.81***

* $P < 0.05$.
*** $P < 0.001$.

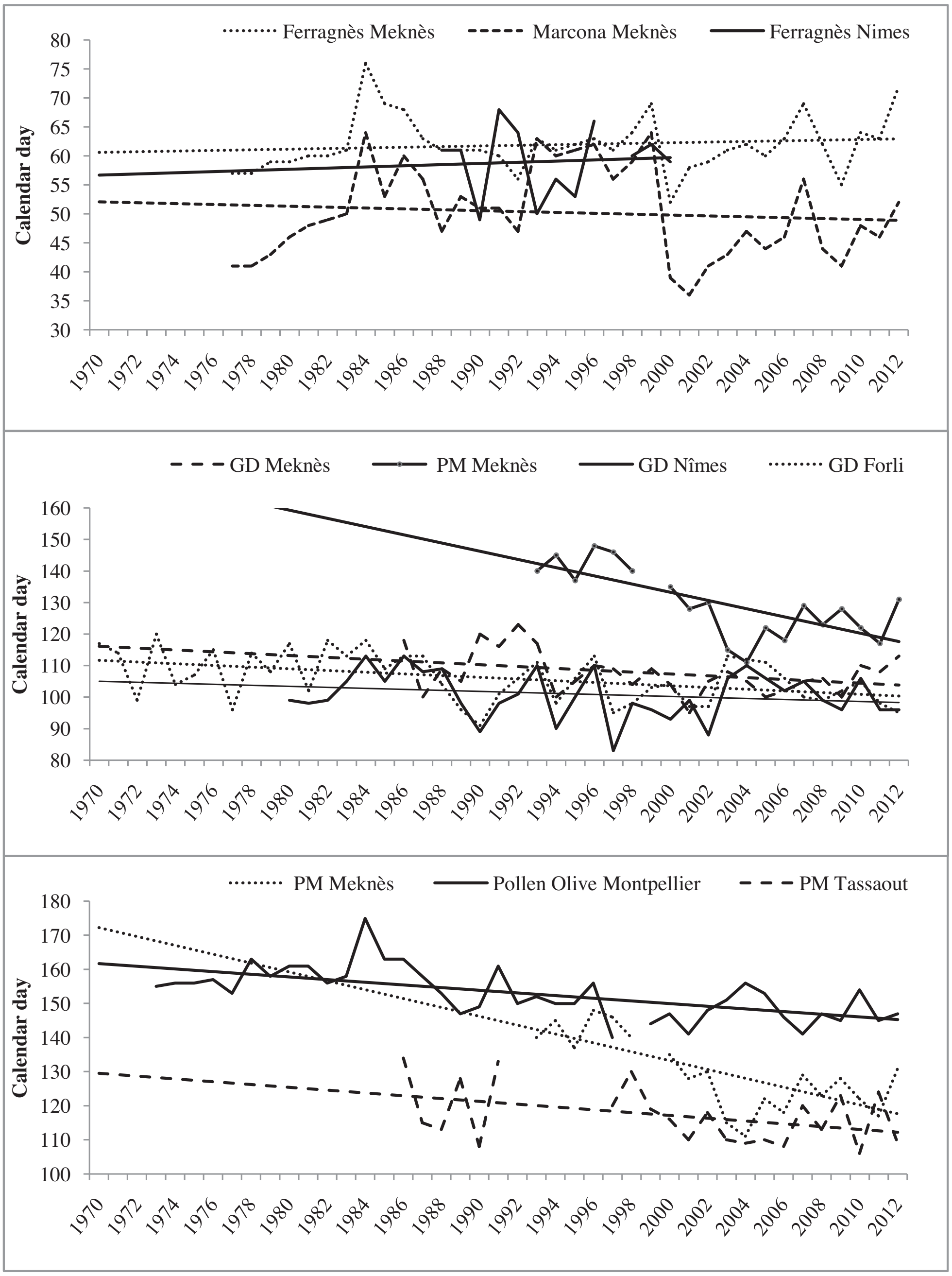

Fig. 2. Evolution and linear regression of observed annual dates of full blooming stages (expressed in calendar days from January 1st) for apple, almond and olive in Mediterranean region during different periods.

Meknès, full bloom was advanced by 17 days during 1993–2012, 7 days in southern Morocco during 1986–2012 and 10 days in the southern France during 1973–2012. Flowering earliness of olive was more pronounced in Tassaout (7 days) than in Montpellier (2 days) throughout the period 1986–2012. A remarkable earliness of flowering date was observed in Meknès and Tassaout since the beginning of 2000 (Fig. 2). Similar trend was observed in Montpellier, starting in 1988 (the same year observed for apple). Flowering times of olive and apple trees tend to approach the course of time in Meknès. In 2004 for example, full blooming of Picholine Marocaine was only six days later (April 20th) than those of GD (April 16th) (Fig. 2).

Generally for late-flowering-species (apple and olive), flowering earliness was strongly apparent. The olive tree, as an indicator of global warming (Osborne et al., 2000) and highly demanding of heat requirement in spring, showed a remarkable flowering earliness compared to apple. Earliness in flowering dates appear to be greater for olive both in Morocco and in France, showing a strong sensitivity to climate change, and moderate in apple trees. However, they are stables for almond cultivars as Mediterranean and early-flowering species (Ferragnès and Marcona).

3.3. *Major periods influencing flowering dates*

Despite the annual variations of length of chilling and forcing periods, due to endodormancy induction and its release, PLS regression pointed out the date dividing the two relevant periods for each cultivar in each location. The two periods showed a spatial variation in blooming (Fig. 3). Positive correlation in standardized model coefficients means that increase in mean temperature delayed the date of full blooming. Negative correlation indicates instead that earlier full blooming is related to a rise in mean temperature. In general, PLS regression showed consistent correlation during the second phase during which correlation is negative, and a good fit of the model to the data has been found, based on low root mean square error (RMSE).

The response of two varieties of almond to mean temperature showed some differences: For Ferragnès, PLS regression performed on data collected during the period 1986–2012 in Meknès, showed onset of the first period which started from October 1st to January 18th, with significant correlation during October, November and first ten-days of January. The second phase began from January 19th and lasted to March 16th (RMSE = 2.82 days). In Nîmes, however, we were not able to determine the two relevant phases, as relationship between phenology and mean temperature was not obvious during the period extending from 1988 to 2000. Two periods delaying phenology were found in Nîmes (3rd to October 20th and December 22nd to January 6th) (Fig. 3). Periods from November 14th to December 18th and from January 18th to February 15th seemed to induce earlier phenology (RMSE = 0.17 days). For Marcona, the phase which seemed to delay full blooming was identified from October 12th to November 30th. The second period started from December 1st to March 16th: it was interrupted by discontinued negative correlation, mainly during the third week of January and the first fortnight of March. This date (December 1st dividing the two relevant periods for Marcona) matched closely the date of breaking dormancy (December 2nd) obtained experimentally by Alonso et al. (2005) for Marcona but not for Ferragnès (December 3rd) in Aragon, Spain. In Meknès, Ferragnès showed a long chilling period (110 days) and short forcing period (58 days). However, Marocona showed a short chilling period (50 days) and a long forcing (107 day). Our results confirm the conclusion of many authors whose reported that prolonged exposure to chilling temperature, particularly when dormancy is overcome, reduced the heat requirements for flowering even when the temperature of exposure is below that of the heat accumulation threshold (Couvillon and Hendershott, 1974; Couvillon and Erez, 1985; Citadin et al., 2001). This could partially explain, on the other hand, why no significant correlation was observed between flowering dates and mean temperature during forcing period in the case of Ferragnès. In addition, high heat requirements (107 days) of Marcona have been partly attributed to a residual effect of dormancy (Spiegel-Roy and Alston, 1979). This result confirms the effect of species-specific differences on the flowering dates reported by Price and Waser (1998).

For apple (GD), PLS regression showed two different periods during which full blooming was significantly correlated to mean temperature during the period 1986–2012. In Forli, the first period started from November 16th to January 17th and showed mostly a positive correlation (essentially during November and the end of December). A negative correlation was identified throughout the second period extending from January 18th to April 2nd, with a low RMSE of 1.34 days. In Nîmes, the first phase began from November 8th to February 4th while the second phase took place between February 5th and April 3rd, with an RMSE of 3.78 days. In Meknès, the periods extending from October 9th to February 26th and from February 27th to April 29th represent the two relevant periods for apple (Figure 3), with an RMSE value of 4.15 days. Indeed, the second period (forcing period) is more extensive in Forli (76 days) and relatively the same in Nîmes (59 days) and Meknès (62 days). However, the chill period (the first period) was longer in Meknès (mild climate) and intermediate in Nîmes. This gradient indicates the transition of Nimes from short chill period (similar to those of Forli) toward longer periods like in Morocco. Legave et al. (2013) highlighted also a remarkable trend toward shorter simulated durations of forcing period and delayed of chilling period. The long chill period in Morocco is explained by a lack of chill during this period, which plays an important role in delaying flowering dates of apple. The slight increase in days during the forcing period observed in Forli could be due to the shorter exposure of GD to cold temperature during post-dormant phase such as previously mentioned for almond. Generally, physiological process in GD during the dormant and growth period (Heide, 1993) may explain, in part, the regional differences observed in flowering dates.

For olive (1997–2012), two relevant periods were identified in Meknès: the first lasted from January 2nd to March 28th, the second period extented from March 29th to May 31st, with an RMSE of 5.75 days. In Tassaout, phenology stages were delayed during the period between December 27th and March 8th. However, analysis of data collected during the period between March 9th and May 31st exhibited a negative relationship with earlier full blooming in olive (Figure 3). The RMSE of this model is 3.86 days. Globally, in Morocco, PLS regression showed relatively the same correlation. In Montpellier, local cultivars of olive showed one consistent period in which pollen emission correlated negatively with mean temperature. It extended from February 5th to May 23rd, with an RMSE of 1.36 days. In low latitude locations, determinism of flowering dates in Picholine Marocaine is difficult to understand. Partial Least Squares regression showed two relevant periods inside the one forcing period if we consider olive as a species not influenced by chilling period. This suggests that flowering earliness of olive is impacted by minimum or maximum temperature more than mean temperature. In addition, PLS regression using minimum temperature showed negative correlation between full blooming and temperature during the period which lasted from March 9th to May 27th in Meknès ($r = -0.51$, $P = 0.03$) over 1993–2012 period. However in southern Morocco, the average temperature from March 21st to May 31st, revealed by PLS regression using maximum temperature, correlated strongly with flowering earliness of olive ($r = -0.64$, $P = 0.001$). This result confirms that olive phenology is being influenced by changes in climate.

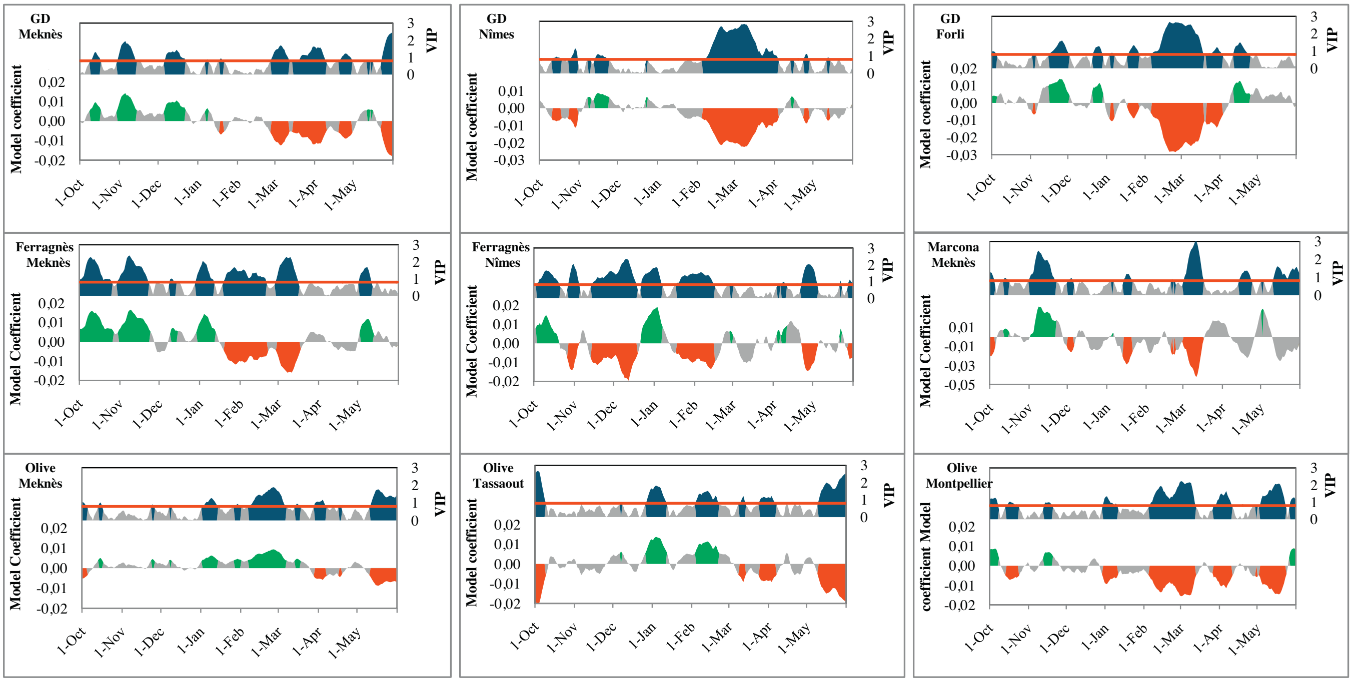

Fig. 3. Auto-correlation between full blooming (except Ferragnès in Nîmes which correspond to first bloom) dates (dependent variable) of fruit trees in Mediterranean region and 11-day running means of daily mean temperatures from the 1st October to the end of May, using partial least squares (PLS). PLS regression was conducted on temperature and phenological data of Golden Delecious (GD) in Meknès, Nîmes and Forli during 1986–2012; Ferragnès and Marcona in Meknès during 1986–2012; Ferragnès in Nîmes during 1988–2012 and olive (Picholine Marocaine in Morocco and local cultivars in France) during 1997–2012. Blue plots in the top of each panel indicate VIP values greater than 0.8 chosen as the threshold for variable importance and indicated by red line. In the below panels, red color indicates negative and considerable correlation, green color indicates positive and considerable relationships between full bloom and mean temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.4. Relationship between temperature and full blooming dates

For GD, earliness of blooming dates was significantly related to mean temperatures during the second period (forcing period) revealed by PLS in Meknès ($r = -0.48$), Nîmes ($r = -0.77$) and Forli ($r = -0.73$) (Table 3). However, no significant relationship was observed between the first period (chilling period) and blooming dates. It explains the important role of this period in satisfaction of heat requirements for floral growing. For Ferragnès in Meknès, the flowering earliness was strongly linked to chilling period ($r = -0.41$). In contrast, no correlation was observed between the chilling/forcing period and flowering dates in Marcona. For olive, the first relevant period seemed to play an important role in delaying the flowering dates in Meknès ($r = 0.55$). Conversely, no relation between the two periods and flowering dates was shown in Tassaout. In the coldest area (Montpellier), the flowering earliness of olive was highly related to an increase in mean temperature during the single long forcing period revealed by PLS regression during 1997–2012 with very highly significant correlation of −0.81 (Table 3). The earliness of flowering dates observed on olive in southern Spain (Cordoba) for the same phenological stage (maximum emission of pollen) was about 7.5 days/decade during the 1982–2011 period (García-Mozo et al., 2014).

3.5. Crucial pattern of apple

Given that determinism of blooming dates in apple trees is often related to temperature during chilling and forcing periods, the three dimensional analysis showed that early full blooming is strongly related to high mean temperature during the forcing period. Spring temperature seemed to be essential in the full bloom in Meknès (Fig. 4A). In this latter, early full bloom dates occurred when mean temperature during forcing period exceed 15 °C (four spots in blue colors). The cold mean temperature during the chilling period probably accelerates occurrences of full blooming (spot at the left, Fig. 4A). Such conclusion cannot, however, be considered final because of lack of sufficient number of observations. In Forli, early full blooming is related both to high mean temperature during the forcing period and cold mean temperature during the chilling period (three spot in the left corner, Fig. 4B) when mean temperature is above 9 °C during forcing period. This is explained by the short chill period in Forli and fast satisfaction of chill during this period. In Nîmes, early full blooming occurred usually during high spring temperature (Five spot in the top, Fig. 4C), when temperature was above 11 °C. Differences in the threshold of mean temperature (revealed by Kriging method) to start the forcing period after breaking dormancy confirms that the physiological process in GD during the dormant and growth period explain the regional differences observed in flowering dates previously mentioned. It was 15 °C, 11 °C and 9 °C respectively in Meknès, Nîmes and Forli. This would mean that the phenological process in high latitude areas (northern areas) in the future can be represented by that in low latitude areas at present (southern areas). These changes in apple behavior are translated by mean temperature increase pointed out in the northern areas, particularly in Nîmes, during October–May period (0.44 °C/decade). Similar trend was also observed in warmer climates of the south.

Overall, the results have clearly proven a remarkable sensitivity of late-spring-flowering Mediterranean species (apple and olive) to global warming than early-spring-flowering ones (almond). This is due to an increase in mean temperature during the months of late spring (March, April and May). Apple, as a temperate species is an intermediate species between olive and almond trees in terms of sensitivity to global warming. The results derived from this study

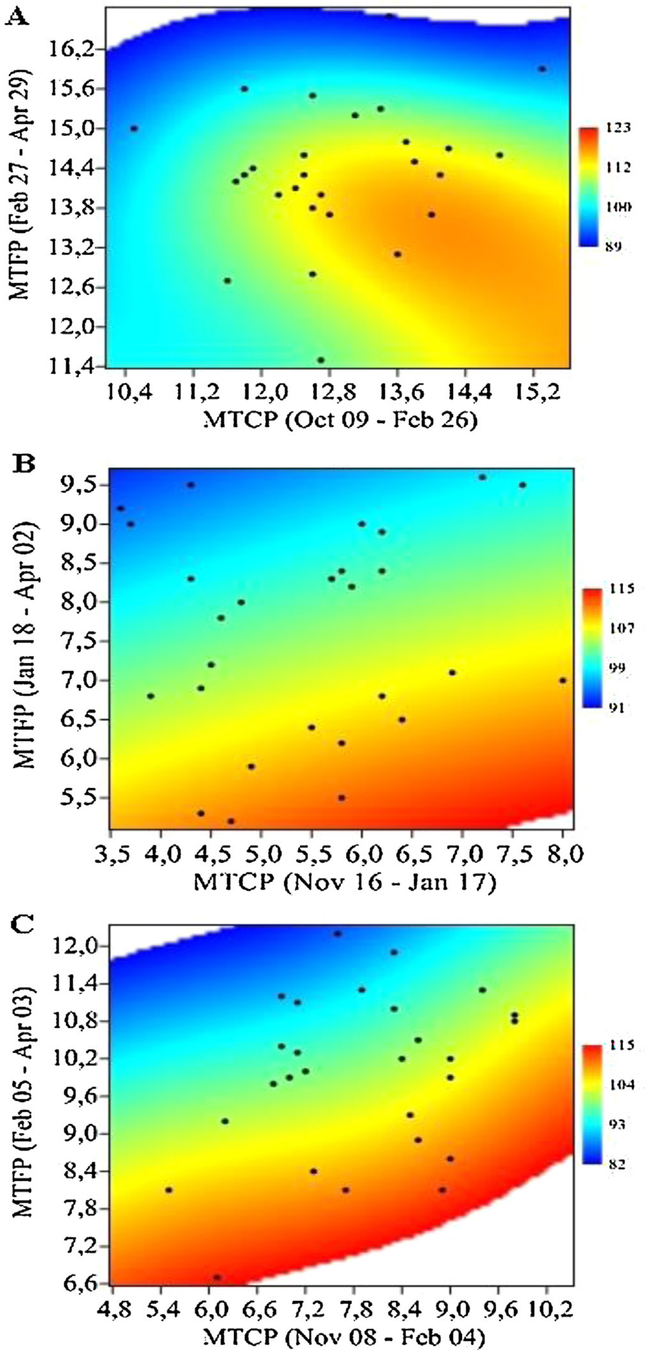

Fig. 4. Full bloom dates of Golden Delicious in Meknès (A), Forli (B) and Nîmes (C), as a function of mean temperatures during the chilling and forcing periods. Colors indicate variation in bloom dates (earlier in blue and late in red color); black points signify full bloom times (day of the year) between 1963 and 2008. MTCP: mean temperature during chilling period; MTFP: mean temperature during forcing period. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

showed some genetic variability of phenological responses to climate change in the Mediterranean region. Indeed, a comparison of flowering data among almond, apple and olive trees in Meknès confirmed the contrasts between these species in terms of flowering dates variability. Here we have also shown that each one species was responding very differently to warming temperature in contrasting areas due to difference in physiological process of the tree, revealed by PLS regression during chilling and forcing period. Results obtained with this new statistical approach are in line with the commonly used sequential chill-heat model; in which forcing period has no effect until all chilling requirements have been met. For almond, which is characterized by a low chilling requirement and an early flowering date (mid-February and early March), no temporal trend in flowering earliness has been revealed. This explains its ability for adaptation to different climatic conditions. However, apple presents some problems for adaptation in the southern areas because it is limited by climatic conditions, and presents some vulnerability in northern areas. Chilling requirement could not be achieved in the southern locations in the future, and phenological studies in the warmer areas of the current apple crops would be necessary. In olive, a pronounced trend toward earlier flowering pattern emerged from this study, although some return to stable flowering dates were observed since the beginning of 2000s. In fact, previous research has pointed out that water deficit can delay olive flowering phenology (Oterosa et al., 2013). In addition, limited availability of water in soil, particularly during flowering period, may slow growing of flowers during bud development, which prompt lengthening this stage (Galen et al., 1999).

This study focused on showing the effects of temperature increase on flowering advance of fruit trees. This phenomenon seems to be more complex given that other factors, not included in this work, may be involved in this process such as photoperiod, rainfall (Grab and Craparo, 2011) or genes control of flowering (Primack, 1980; Ishii, 1990; Baliuckas et al., 2005). In all cases, these changes have severe agronomic consequences such as fall buds, maturity advances and additional needs of water (Legave, 2009), which alter production in the fields (Oukabli and Mahhou, 2007). In addition, early and late-flowering individuals within a population may have less opportunity for cross-pollination (case of almond) and as result, irregularities in annual yield associated to flowering date changes. Longer annual flowering durations, associated with heterogeneity of bud flowering times in the same tree, widely observed for apple in the southern locations, is also a symptom of lack of chill due to global warming. These perceptible climate changes disrupt slowly cropping conditions in production areas and result in geographical shifts of some vulnerable species such as apple. Replacing them by introducing new fruit crops and/or varieties with low chilling and heat requirements could be a way of adaptation to climatic constraints.

4. Conclusion

This study provided evidence of global warming in three contrasting environments from the Mediterranean region, showing spatial and temporal variations between the northern and the southern Mediterranean. A high temperature increase was observed during spring compared to that of autumn, particularly in the southern France, which revealed vulnerability of crop production due to climate change. In response to this global warming, fruit tree species showed contrasting behavior in terms of flowering dates, resulting in differences in physiological process. This explains, in part, the regional differences observed in flowering dates among sites and species. A high sensitivity of flowering earliness was marked for olive both in the north and the south of the Mediterranean. Apple is an intermediate species, while almond did not show any change in flowering dates. Flowering earliness seems to be more affected by mean temperature during forcing period than by chilling period, especially for apple. The stability of flowering dates in almond was explained by the absence of temperature changes during February, which is a critical period in accumulation of amounts of heat.

Here, we concluded that climatic conditions and therefore phenological process of trees, in high latitude areas in the future can be represented by that in low latitude areas at present, particularly for apple, as a consequence to global warming.

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