Agroclimatic requirements and phenological responses to climate change of local apple cultivars in northwestern Spain

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Abstract:

In a global warming context, analyses of historic temperature records are essential to understand the potential impacts of climate change on spring phenology. To estimate flowering trends over recent decades, we analyzed long-term temperature and phenology records of eleven local apple cultivars in Asturias (northwestern Spain) in a temperate oceanic climate. Our results show that, over a period of 30 years, bloom dates of the local cultivars have experienced relatively minor changes, considering that temperatures increased strongly since 1978, by 0.30°C per decade. An explanation for this weak phenological response to warming may be that these temperature changes only had a small effect on overall chill accumulation, but possibly delayed the onset date of endodormancy, which may have counteracted phenology-advancing effects of warming in spring. At present, chill accumulation in this area is high, at an average of 96 Chill Portions from November to March, which indicates that chill is not currently a limiting factor for the quality of flowering and fruiting in the study area. We used Partial Least Squares (PLS) regression to delineate an effective chilling period between November 12th and February 9th and effective heat accumulation between March 15th and May 4th. While these periods appear plausible, we noticed that this approach was unable to identify well-known differences in chilling requirements among many of the cultivars, with similar chill needs determined for many of them. This observation may be explained by inaccurate expectations about cultivars' climatic needs, by inaccuracy of the chill (and possibly heat) model or, most concerning, by inability of the PLS approach to correctly identify the chilling periods of apple cultivars in this region. Bloom dates were similarly responsive to mean temperature during the chill and the heat accumulation phases, indicating that both processes need to be considered when predicting future phenology.

Keywords: Phenology, Apple, Climate change, PLS regression, Dormancy, Chilling requirements.

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1. Introduction

Apple (*Malus domestica* Borkh.) trees, like most woody perennial species that evolved in temperate or cold climates, spend the winter months in a dormant state that allows them to survive unfavorable conditions and avoid cold damage (Faust et al., 1997; Saure, 1985; Campoy et al., 2012). To break dormancy, trees undergo two distinct phases that ultimately lead to flowering: endodormancy, during which trees must fulfill cultivar-specific chilling requirements and ecodormancy, when heat requirements need to be satisfied (Lang et al., 1987; Egea et al., 2003). Chilling temperatures are important in fruit production, since they are needed for dormancy release, optimal flowering and satisfactory fruit set (Sunley et al., 2006; Campoy et al., 2011). In apple, the dormancy cycle is only regulated by temperature (Heide and Prestrud, 2005) and a sufficient amount of chill and heat is positively correlated with fruit weight, size and firmness (El Yaacoubi et al., 2020). The amount of chill that is required is cultivar-specific, and large variability has been reported among over 8000 apple cultivars and land races across the world. Nevertheless, most commercial cultivars have high to medium chilling requirements (El Yaacoubi et al., 2016; Parkes at al., 2020).

The apple industry plays a relevant economic and social role in Asturias in northwestern Spain, which contributes about 80 per cent of the total cider production in the country. The bulk of the orchards are composed of several local cultivars, which tend to be well adapted to the agro-climatic conditions of the region. Currently, apple production in Asturias relies on cultivars with medium to high chilling requirements (Dapena, 1996; Dapena and Fernández-Ceballos, 2007). So far, consequences of insufficient winter chill accumulation, such as delayed and irregular budburst (Erez, 2000), have rarely been observed in commercial orchards, except for a few occasions in years with particularly mild winters and/or cold early spring.

Global warming may compromise the fulfillment of trees' agroclimatic needs during dormancy (Luedeling et al., 2011; Fernandez et al., 2020a). Mean global air temperature increased by

0.74°C between 1906 and 2005 (IPCC, 2007) and numerous future climate scenarios project major changes in air temperature over the course of the 21st century (IPCC, 2014). Since plant phenology is strongly influenced by air temperature, long-term phenological observations at specific sites that are combined with meteorological data can provide useful information on plant responses to climate change. In recent decades, advances in spring phenological events have been observed for many tree fruit species in many places (Guédon and Legave, 2008; Legave et al., 2008; Luedeling et al., 2011; Darbyshire et al., 2013; El Yaacoubi et al., 2014; Guo et al., 2015; Legave et al., 2015; Yong et al., 2016). These bloom advances are a result of a rise in air temperatures in spring, which has accelerated the fulfillment of heat requirements. However, in some regions, temperature increases in winter appear to have delayed the fulfillment of chilling requirements, sometimes to an extent that could not be compensated by phenology-advancing effects of warming in spring. In such extreme situations, warming during dormancy has been reported to result in delayed bloom dates (Harrington et al., 2010; Campoy et al., 2011; Luedeling et al., 2013a; Legave et al., 2015; Martínez-Lüscher et al., 2017; Bartolini et al., 2019).

Several models have been proposed for quantifying chill and heat accumulation. The most common concept of the dormancy season stipulates that chilling and heat requirements are fulfilled sequentially (Guédon and Legave, 2008; Luedeling et al., 2009; Darbyshire et al., 2013), but some recent studies have proposed more complex concepts that include an overlapping phase of both agroclimatic stimuli (Pope et al., 2014), or the possibility that budbreak can be triggered by various combinations of chill and heat accumulation (Harrington et al., 2010). To measure the accumulation of winter chill in deciduous trees, various models have been developed: the Chilling Hours Model (Hutchins 1932, as cited by Weinberger, 1950), the Utah Model (Richardson et al., 1974) and the Dynamic Model (Fishman et al., 1987a, b). For quantifying heat accumulation, the Growing Degree Hours Model (Anderson et al., 1986) is the most widely used model.

Since buds do not exhibit easily observable changes during dormancy, delineation of the chill and heat accumulation has long remained elusive, especially where no controlled experiments could be undertaken. In recent years, Partial Least Squares (PLS) regression has been used to overcome this limitation (Luedeling and Gassner, 2012). This statistical approach requires long-term temperature and phenology records. For each calendar day of the dormancy season, PLS regression can identify whether high temperatures tend to delay or advance bloom dates. In many climatic settings, this information can then be used to delineate the endormancy phase, when high temperatures should delay budbreak, and the ecodormancy phase, when high temperatures should result in advanced phenology.

The analysis of historic chill accumulation trends is a decisive step towards a better understanding of the impacts of climate change in a particular region. In a context of global warming, chill trend estimations can be very sensitive to the choice of chill model (Luedeling et al., 2009; Fernandez et al., 2020b). In the particular case of Asturias, the potential impacts of climate change on locally available winter chill may include changes in the timing of phenological events, which may have implications for agricultural management. Significant warming during the chilling phase could reduce the number of suitable cultivars for cider production in the region. Another important factor to consider is the possibility of increased frequency of adverse weather events such as late spring frosts, which can be associated with shifts in budbreak dates. While late damaging frosts have traditionally been rare in the study region, advances in spring phenology may lead to earlier appearance of advanced flowering stages, which are more frost-sensitive than fully dormant buds (Westwood, 1999). Anticipating future production risks related to the dormancy season would be facilitated by accurate knowledge of the flowering times of each local cultivar. Reliable characterization of chilling and heat requirements is also important for adapting agricultural practices to possible new constraints, as well as for the design of new orchards and as guidance for future breeding strategies.

To provide information for risk assessment and strategic decisions on the composition of future orchards, we pursued two objectives. First, we analyzed temperature trends in Asturias over the past 41 years to examine how changes in chill and heat accumulation have affected the phenology of apple cultivars in northwestern Spain. Second, we determined the start and end dates of the effective chill and heat accumulation periods to quantify chill and heat requirements of local apple cultivars using Partial Least Squares (PLS) analysis.

2. Materials and methods

2.1. Study area

The study was carried out at Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA) in Villaviciosa, Asturias, northwestern Spain (43.46°N, 5.43°W, 10 meters above sea level) (Fig. 1). Villaviciosa is located in an area known as “Comarca de la Sidra”, the most important cider apple growing region in Spain. The climate in this region can be defined as temperate and humid oceanic climate. Temperatures are mild in winter, summers are not dry or very hot and the annual rainfall is fairly evenly distributed over the year with an average annual rainfall around 1100 mm.

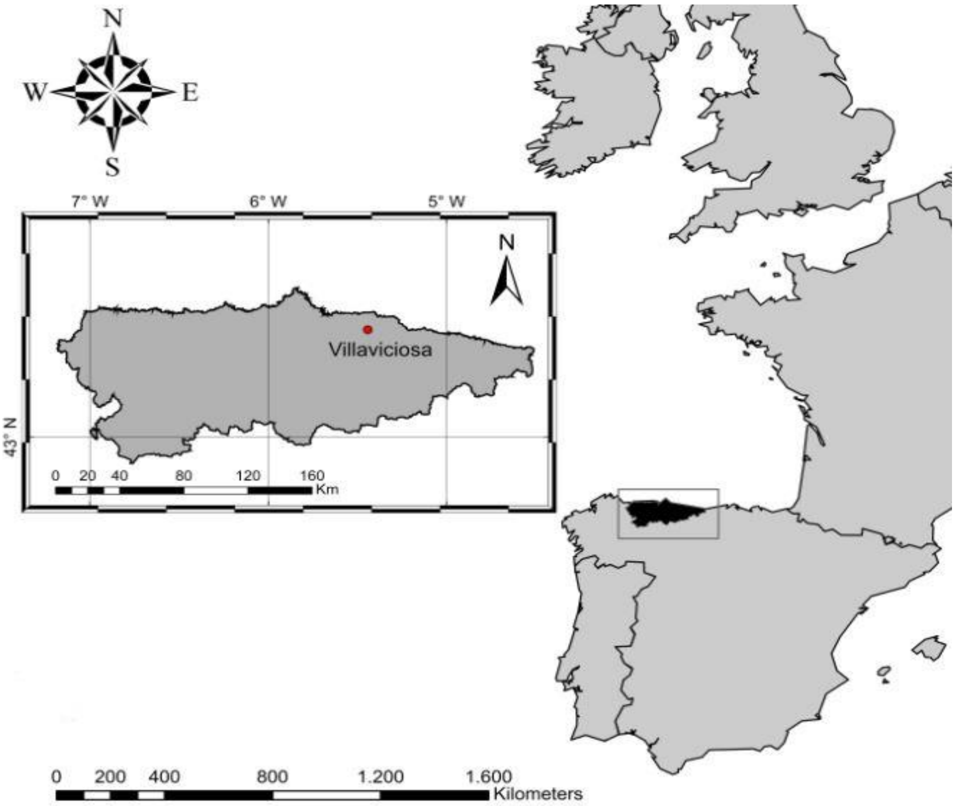

Fig. 1. Map of the study area. The red dot in the inset image shows the location of the weather station used in the study. The larger image shows the location of the Asturias region within Spain.

2.2. Climate data and trends

Daily minimum and maximum temperatures were collected from the weather station of the SERIDA research institute situated just next to the experimental orchards. Sporadic gaps in the meteorological data were filled with information from the nearest weather station (Gijón, 43.54°N; 5.62°W, 30 m a.s.l. and 17 km away). These data were bias-corrected (by -1.37 and +0.35°C for daily minimum and maximum temperatures, respectively) based on an analysis of all days for which both stations had data.

Annual temperature trends were analyzed over a 41-year period (1978-2019), including detailed analysis of temperature variation between October and May, the period that includes all dormancy-related processes for fruit trees in this region. Additionally, the total numbers of frost days (air temperature below 0°C) between November and March and between March and May were identified for each year.

2.3. Phenological observations

Phenology data were collected for eleven Asturian cider apple cultivars. The dataset contains observations for two time periods: 1987-1996 and 2004-2019 (no observations were available in 2017). Trees were monitored twice a week, and flowering dates were recorded when trees reached the F2 stage (full bloom) according to Fleckinger (1945), which corresponds to stage 65 (∼50% of flowers open) of the BBCH code. Phenology was monitored in three experimental orchards located within a radius of less than 1 km, all of which were managed with the same agricultural practices.

The set of local cultivars we investigated varied widely in terms of flowering time. All of them are recognized as local cultivars by a “Protected Designation of Origin” quality label (Dapena and Blázquez, 2009). The cultivars we examined are: ‘Clara‘, ‘Coloradona‘, ‘Perezosa‘, ‘De la Riega‘, ‘Verdialona‘, ‘Blanquina‘, ‘Teorica‘, ‘Xuanina‘, ‘Collaos‘, ‘Perico‘ and ‘Raxao‘.

2.4. Chill and heat accumulation models

Chill and heat models require hourly temperature data. Hourly records were constructed from daily minimum and maximum temperature records based on geographic latitude (Spencer, 1971; Almorox et al., 2005) using procedures proposed by Linvill (1990), which are included in the chillR package (Luedeling, 2019) for the R programming language (R Core Team, 2020).

Daily chill accumulation was calculated according to three chill models. The Chilling Hours Model (Hutchins 1932, as cited by Weinberger 1950) is the simplest model, but it does not perform well in mild and warm areas (Dennis, 2003). The Utah Model (Richardson et al., 1974), which assigns varying chilling efficiencies to several distinct temperature ranges, has also shown problems in mild climates, where it appears to overestimate the chill-negating effect of warm temperatures (Campoy et al., 2011). The Dynamic Model (Fishman et al., 1987a,b), the most recent and most complex of the commonly used models, has been widely acknowledged as the most accurate for mild-winter climates (e.g., Ruiz et al., 2007; Luedeling et al., 2009; Zhang and Taylor, 2011; Campoy et al., 2013; Parkes et al., 2020). In this model, chill is accumulated by a two-step process, in which only the intermediate product formed by the first step can be destroyed by warm conditions. Daily heat accumulation was calculated according to the Growing Degree Hours Model (Anderson et al., 1986), a model that assigns varying heat accumulation efficiencies to temperatures above a base temperature of 4°C, with an optimum temperature of 25°C and a critical temperature of 36°C.

2.5. Identification of the chilling and forcing periods

Chill and heat accumulation were determined by applying Partial Least Squares Regression (PLS) (Luedeling and Gassner, 2012) using full bloom dates observed during the 2004-2019 period. The analysis was implemented using the chillR package (version 0.70.21) (Luedeling, 2019). The onset and the end of the chilling and forcing periods were based on the two major outputs of the analysis: the variable importance in the projection (VIP) statistic, calculated for each independent variable, and the standardized coefficients of the model. As in previous studies, a VIP value of 0.8 was selected as a cut-off for considering coefficients for particular days important (Wold et al., 2001; Luedeling et al., 2013a). A negative coefficient for daily chill or heat accumulation on a particular day of the year indicates that high rates of chill or heat accumulation on that particular date are correlated to an early bloom date. We based the PLS analysis on chill quantified with the Dynamic Model, defining the beginning of the chilling phase as the first date of a pronounced period with consistently negative standardized coefficients and VIP values above 0.8. The onset of the forcing phase was determined using the same criteria, and the median bloom date was established as the end of the heat phase. Cultivar-specific chill and heat requirements were calculated as the mean accumulation of the respective model between the two dates defining the corresponding phase for each year. We estimated uncertainty by computing the standard deviation of chill or heat accumulated during these phases. In order to represent temperature responses of the set of eleven apple cultivars to mean temperatures during the chilling and forcing phases identified by PLS regression, bloom dates were plotted in relation to mean temperatures during both periods using the Kriging interpolation technique (Luedeling et al., 2013a).

2.6. Statistical analysis

Trends in air temperature series, historic chill levels and flowering records were determined using the non-parametric Mann–Kendall test (Mann, 1945; Kendall, 1975). Kendall's Tau coefficient (τ) and a critical p value of 0.05 were used to detect significant time series trends from 1978 to 2019. Flowering records from the studied cultivars were analyzed by hierarchical cluster analysis using the average linkage (between groups) method. PLS regression analysis was performed using version 0.70.21 of the chillR package (Luedeling, 2019). Chill and heat accumulation dynamics over the past decades were studied using a running mean function (Luedeling and Gassner, 2012; Luedeling et al., 2013a). All analyses were run in the R programming environment (R Development Core and Team, 2020; version 3.6.3).

3. Results

3.1. Temperature trend and variability

Annual and seasonal temperature trends were analyzed using meteorological data collected in Villaviciosa during the period 1978–2019. This location has a mean daily temperature of 13.35°C, with mean daily minimum and maximum temperatures of 8.62°C and 18.34°C, respectively (Table A1 in the supplementary materials). Over the past 41 years, the average daily temperature increased significantly ($\tau = 0.48$, $p < 0.001$) by 1.21°C, at a rate of 0.30°C per decade. The warmest year was 2014 (14.3°C) and the coldest mean annual temperature was recorded in 1980 (12.4°C). From October to May, the period which involves the physiological processes of relevance for the flowering time of fruit trees in the study region, mean daily minimum, mean and maximum temperatures were 6.35°C, 11.14°C and 16.39°C, respectively (Fig. 2). Warming trends during this period were similar to trends for the whole year, with the mean daily temperature registering an increase by 1.16°C (+0.29°C/decade). Temperature rise was more pronounced for the minimum temperature (+1.25°C in total, at +0.31°C/decade; $p < 0.001$) than for the maximum temperature (+0.56°C in total, at +0.14°C/decade, $p = 0.12$). The strongest positive trend was found for January (+0.41°C/decade, $p = 0.013$), the month with the coldest mean temperature (8.24°C).

Flowering of apple trees is generally observed in Asturias in April and May. In terms of air temperature, the spring phase commonly occurs from February to April at mid-latitudes of the Northern Hemisphere (Chmielewski and Rötzer, 2001). In this spring phase, we found an increase in mean temperature by 0.29°C/decade, with the strongest warming trend observed in April (+0.43°C/decade).

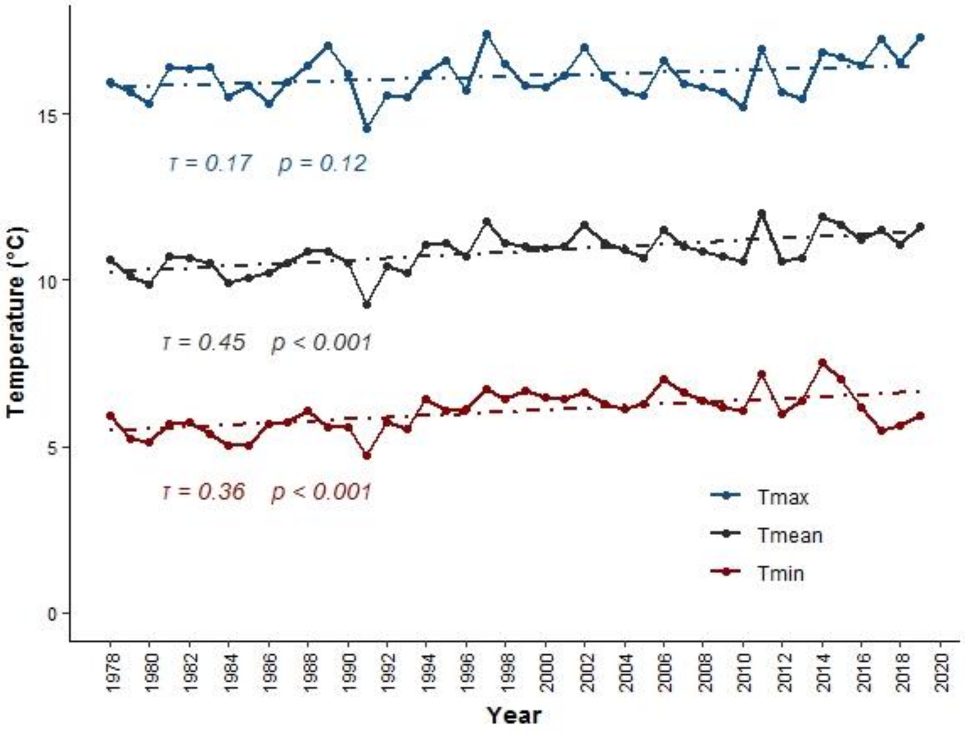

Fig. 2. Trends of annual means of daily minimum, mean and maximum temperatures recorded at Villaviciosa during the phenological season of fruit tree species (October-May) from 1978 to 2019. Kendall's rank correlation coefficient (τ), as well as the probability of the observed results occurring in the absence of a temporal trend (p) are indicated for each time series.

Annual mean temperature anomalies, compared to the mean of the entire record we analyzed, showed a clear warming signature, with years that were cooler than the long-term mean being a rare occurrence after 1996 (and then just slightly cooler than the mean; Fig. 3).

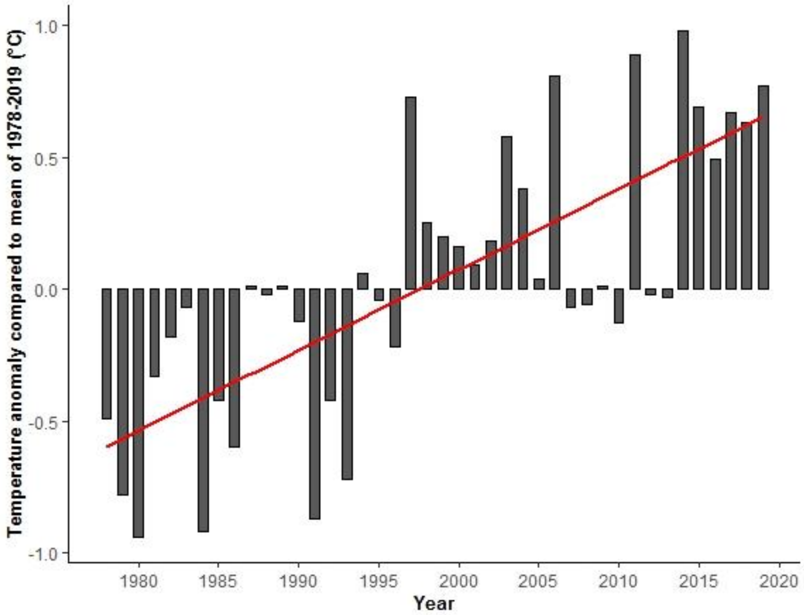

Fig. 3. Anomalies in mean annual air temperature in Villaviciosa, compared to the 1978-2019 average. The red line represents the result of linear regression analysis of the annual anomalies over time. Anomaly stands for the temperature deviation in degrees Celsius relative to the standard period.

The long-term temperature record indicated a significant decrease in the annual number of frost days between November and March during the 1978-2019 period, showing a decline by 2.5 days per decade since 1978 ($\tau = -0.24$ and $p = 0.03$). Few frost days were identified between March and May (average of 0.9 days per year). Approximately 60% of the years did not experience any frost events between March and May. In particular, during the flowering months of most cultivars, frost risk has historically been low, with no frost events detected in May, and only two days in April, over the entire study period.

3.2. Flowering trends and phenological changes in local apple cultivars

The average flowering date of apple trees in Villaviciosa spanned a period of 30 days, ranging between April 21st (in 2011) and May 21st (in 2004). The least variation was found in the earliest flowering cultivar ‘Clara’ (23 days) and the widest spread in the late flowering cultivar ‘Raxao’ (36 days) (Fig. A1 in the supplementary materials). The cluster analysis revealed five distinct groups according to the flowering time (Fig. A2 in the supplementary materials). Based on this analysis, the local apple cultivars can be grouped into five flowering groups: an early-flowering group formed by ‘Clara’ and ‘Coloradona’, an intermediate-flowering group (‘Perezosa‘), an intermediate/late-flowering group (‘Verdialona‘, ‘De la Riega‘ and ‘Blanquina‘), a late-flowering group (‘Xuanina‘, ‘Collaos‘ and ‘Teorica‘) and a very late-flowering group (‘Perico‘ and ‘Raxao‘). The recent warming did not lead to pronounced variation in flowering dates over the study period (Fig. 4). Mean flowering dates across all cultivars in the study region showed a slight advancing trend (-0.13 days/year), which was not statistically significant ($\tau = -0.12$, $p = 0.44$). The predominant trend among these cultivars is a moderate advancement of flowering (Fig. 4). The changes in temperature led to slight delays in flowering in the three cultivars with the earliest bloom dates (i.e. ‘Clara’, ‘Coloradona’ and ‘Perezosa’) and slight bloom advances in the other study cultivars. It should be noted that for all cultivars except ‘Xuanina’, the possibility that there was no trend in the dataset could not be statistically excluded ($p > 0.05$).

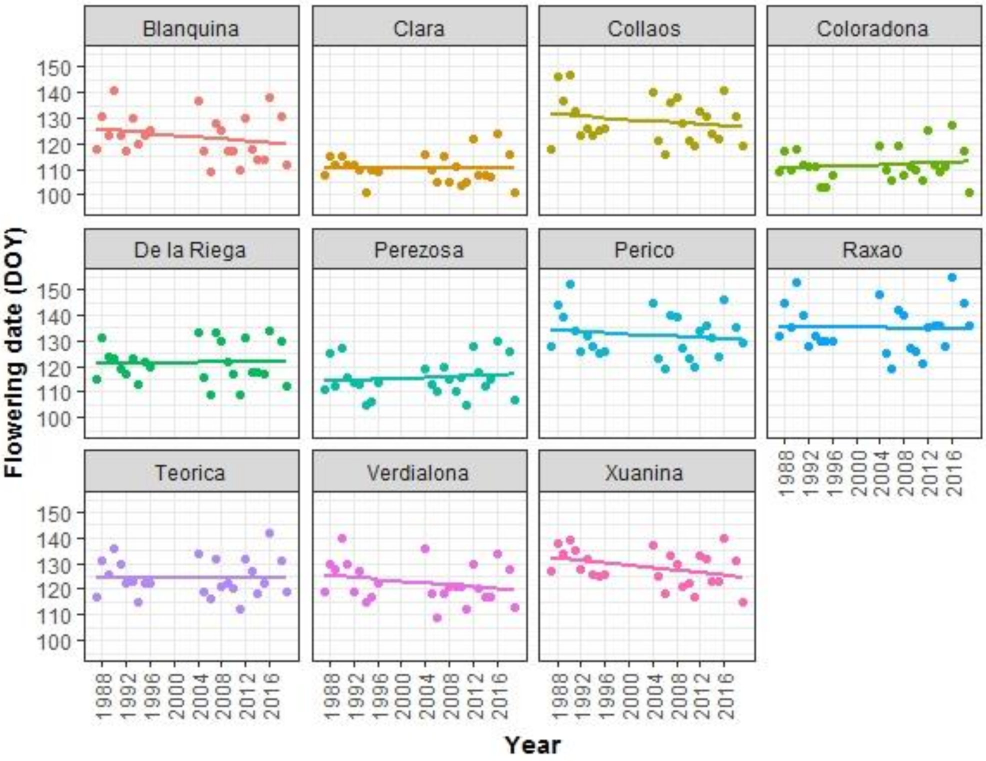

Fig. 4. Flowering date (DOY = day of the year) of 11 apple cultivars in Asturias, Spain, between 1987-1996 and 2004-2019 (with 2017 missing), with trends visualized by linear regression.

3.3. Historic chill and heat trends

We calculated chill accumulation from November to the end of March for each winter season and evaluated the results for trends. We chose November 1st as the start date, since this time corresponds roughly to the accumulation of the first Chill Portions.

Even though all the models indicated a decline in winter chill over the past 41 years, change trends differed across the three models (Fig. 5). The Chilling Hours (CH) model showed the most severe decline (-5.7 CH/year; $\tau = -0.24$; $p = 0.03$) followed by the Utah model (-1.91 CU/year; $\tau = -0.1$; $p = 0.36$). The Dynamic model, which measures chill in Chill Portions (CP), indicated only negligible changes in winter chill levels (-0.04 CP/year; $\tau = -0.03$; $p = 0.45$). Across all winter months, January accounted for the greatest chill accumulation (21.8 CP), followed closely by December (20.6 CP). The coldest winter on record was the 1990/1991 season, with a mean temperature of 7.64°C. This cold winter registered the highest total winter chill according to the Chilling Hours Model. Interestingly, neither the Dynamic Model nor the Utah Model were in agreement with this assessment, and they also did not identify the warmest winter as the one with the lowest chill accumulation.

While overall chill accumulation remained relatively stable, we observed a gradual trend towards later onset of endodormancy. In consequence, chill accumulated during the first fifteen days of November, as quantified by the Dynamic Model, decreased by 0.51 CP/decade ($\tau = -0.31$; $p = 0.006$). For the whole month of November, chill accumulation experienced a similar rate of decline (-1.13 CP/decade; $\tau = -0.28$; $p = 0.009$). A particularly large decline was observed since the 2000s, and chill accumulation in November only exceeded 5 CP in four years since the beginning of the current century.

We calculated heat accumulation following the Growing Degree Hours (GDH) Model for the months of February, March and April. The average flowering date for the set of local cultivars was May 4th. Experimental work performed by forcing buds in a temperature-controlled environment suggests that some of the cultivars completed their endodormancy phase in February (Delgado et al., in preparation). Heat accumulation significantly increased by an average of 52.5 GDH per year ($\tau = 0.27$; $p = 0.01$). This increase was stronger between 1980 and 2009, with a rate of 63.3 GDH per year, than during the following decade, when heat accumulation appeared fairly stable. It is important to highlight that heat increases in April (27.8 GDH/year; $\tau = 0.27$; $p = 0.01$) contributed most strongly to the overall rise in heat accumulation.

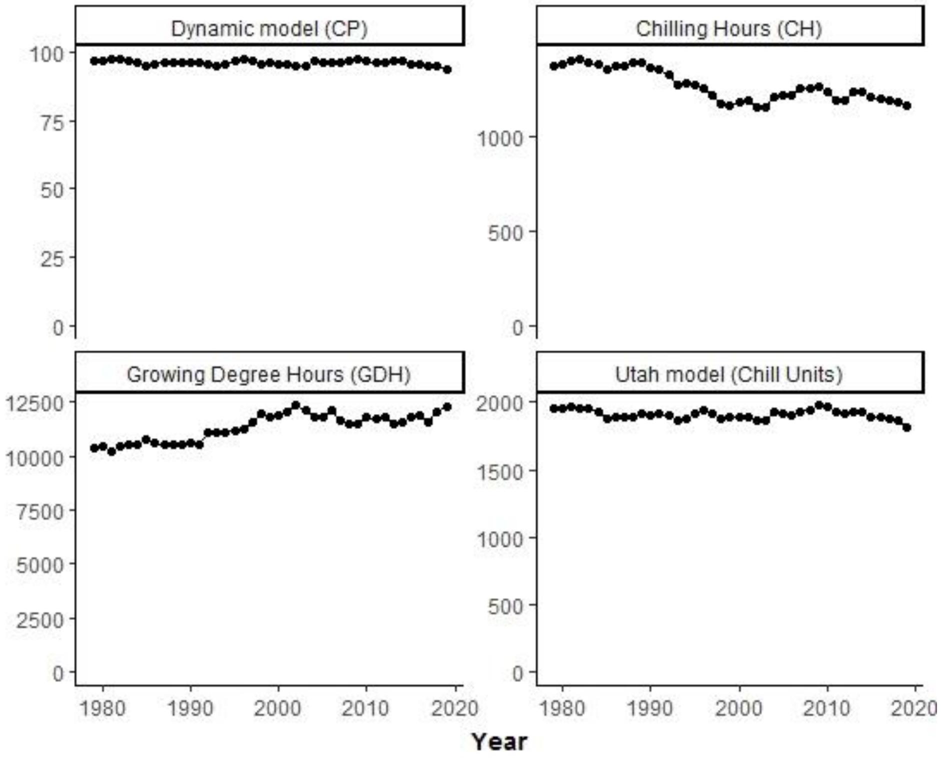

Fig. 5. Chill accumulation, calculated in Chill Portions (CP), Chilling Hours (CH) and Chill Units (CU), during the dormant season (November 1st – March 31st), and heat accumulation calculated in Growing Degree Hours (GDH) from February 1st to April 30th, in Asturias, Spain, between 1978 and 2019.

3.4. Chilling and forcing periods for apple cultivars in the study region

Chilling and forcing periods were delineated by PLS regression. While we conducted this analysis for all three chill models, we only report results for the Dynamic Model, which has been found to be more appropriate than the other models in mild winter regions (Luedeling, 2012; Luedeling and Brown, 2011; Guo et al., 2015). Using phenological observations from 2004 to 2019, effective chilling and forcing periods for apple trees in the SERIDA institute occurred from November 12th to February 9th (88 days) and from March 15th to May 4th (50 days), respectively (Fig. 6). Chilling and forcing phases were delineated very clearly, with the large majority of days during the chilling phase and every single day during the forcing phase showing negative model coefficients and a VIP score above 0.8. These two criteria indicate significant effects of the rates of chill and heat accumulation, respectively, on flowering dates, but variation in VIP scores and model coefficients during both periods imply that not every date of these periods has an equally strong effect. The greatest responsiveness to chill was found between mid-December and mid-January, and the greatest responsiveness to heat occurred during the first half of April.

The delineation of chilling and warming phases differed slightly between the models selected to obtain the outputs. We did not find evidence of a significant period of overlap between chilling and forcing periods in this region, where winters are moderately cold and springs are mild. According to the delineation of the chilling period obtained by applying the Dynamic Model, the average chill accumulation during the identified period was 809 CH, 1128 CU or 59.5 CP, respectively. For all three models, the year with the lowest chill accumulation was 2015/2016, yet the highest-chill year varied according to the model. The average heat accumulation was 8647 GDH. Interannual variability was greater for heat accumulation (17%) than for chill (7% with the Dynamic Model).

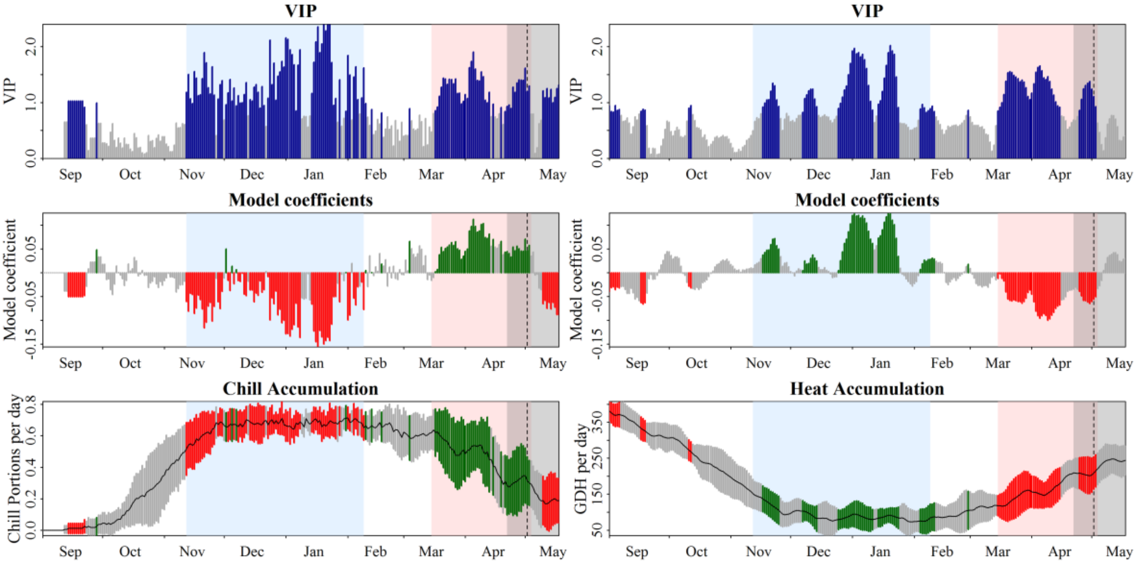

Fig. 6. Results of the Partial Least Squares (PLS) regression analysis for bloom of local apple cultivars in Villaviciosa, northwestern Spain, between 2004-2019, using the Dynamic Model and the GDH Model for chill and heat accumulation, respectively. Blue bars in the top row indicate that VIP scores are above 0.8. Red bars mark negative model coefficients, which represent an important correlation between flowering and daily chill and heat accumulation. GDH stands for

Growing Degree Hours; CP for Chill Portions; VIP for Variable Importance in the Projection. Blue shading indicates the chilling phase, red shading represents the forcing phase, grey shading shows the range of bloom dates, with the dashed lines marking median flowering dates.

For the purpose of distinguishing the effects of air temperature during chilling and forcing periods identified by the PLS procedure, flowering records of the study cultivars were plotted in relation to mean temperatures during both periods (Fig. 7). Based on the graphical presentation, we observed approximately diagonal contour lines, which means that the local apple cultivars showed similar sensitivity to temperatures during both periods. Hence, advances in spring phenology in the whole set of local apple cultivars may arise from cooler temperatures during the chilling period and/or warmer temperatures during the forcing period.

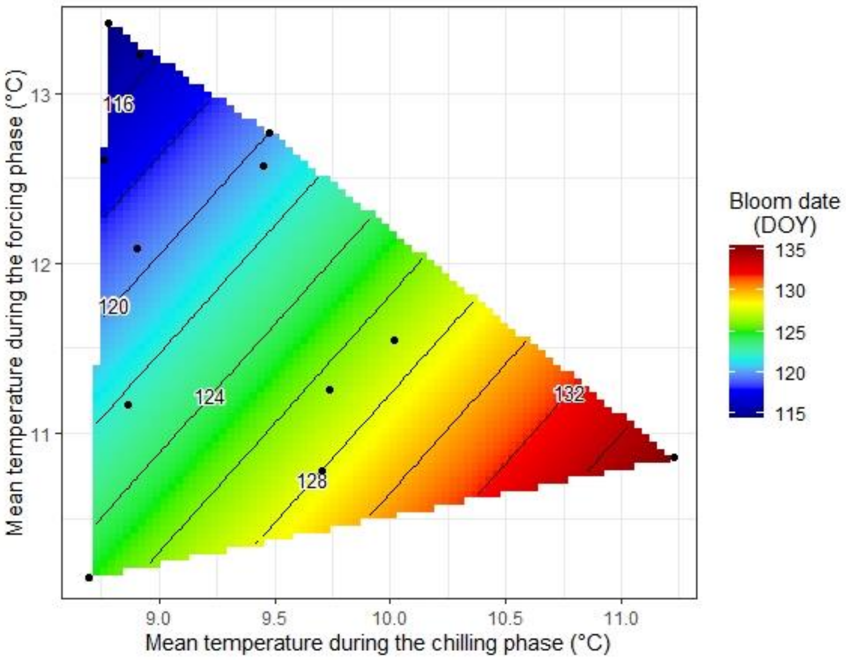

Fig. 7. Response of the average bloom date of eleven apple cultivars to mean temperatures during the chilling and forcing periods delineated by PLS regression (November 12th – February 9th and March 15th – May 4th, respectively). Black dots represent observed apple flowering dates and colors and contour lines indicate the timing of flowering dates expressed in Julian dates (days of the year; DOY). The color spectrum represents a gradient between early and late flowering dates.

3.5. Chill and heat requirements of local apple cultivars

Chilling and forcing periods were delineated for each cultivar (Fig. 8), and the specific requirements were calculated according to chill and heat accumulation during the effective periods (Table 2). The cultivars ‘Perezosa’, ‘Verdialona’, ‘Xuanina’, ‘Clara’ and ‘Coloradona’ showed some periods with high VIP values and negative coefficients before the dates chosen as the beginning of the chilling phase (Fig. 8). These days at the beginning of October were discarded for biological reasons, since trees were still bearing fruit at that time.

The length of the chilling period ranged from 89 days (‘Collaos’ and ‘Verdialona’) to 108 days (‘Perico’ and ‘Raxao’). The end of the chilling period was quite similar for all of the cultivars ranging between February 5th and February 10th, with the exception of ‘Perico’ and ‘Raxao’, which finished their chilling phase on February 28th. The onset of the forcing phase was similar for most of the cultivars and only differed significantly for ‘Clara’, ‘Coloradona’ and ‘Verdialona’. The length of the forcing phase varied more strongly than the chilling phase, but this variation was largely driven by variation in flowering dates rather than by the onset of ecodormancy.

For most cultivars, the forcing period started after the end of the chilling period. The only cultivars for which we detected a small overlap between the chilling and forcing phases were ‘Clara’ (9 days) and ‘Verdialona’ (6 days). For all other cultivars, the gap between chill and heat accumulation phases among the different cultivars lasted between 7 and 20% of the period between the first day of the chilling phase and the last day of the forcing phase in each cultivar.

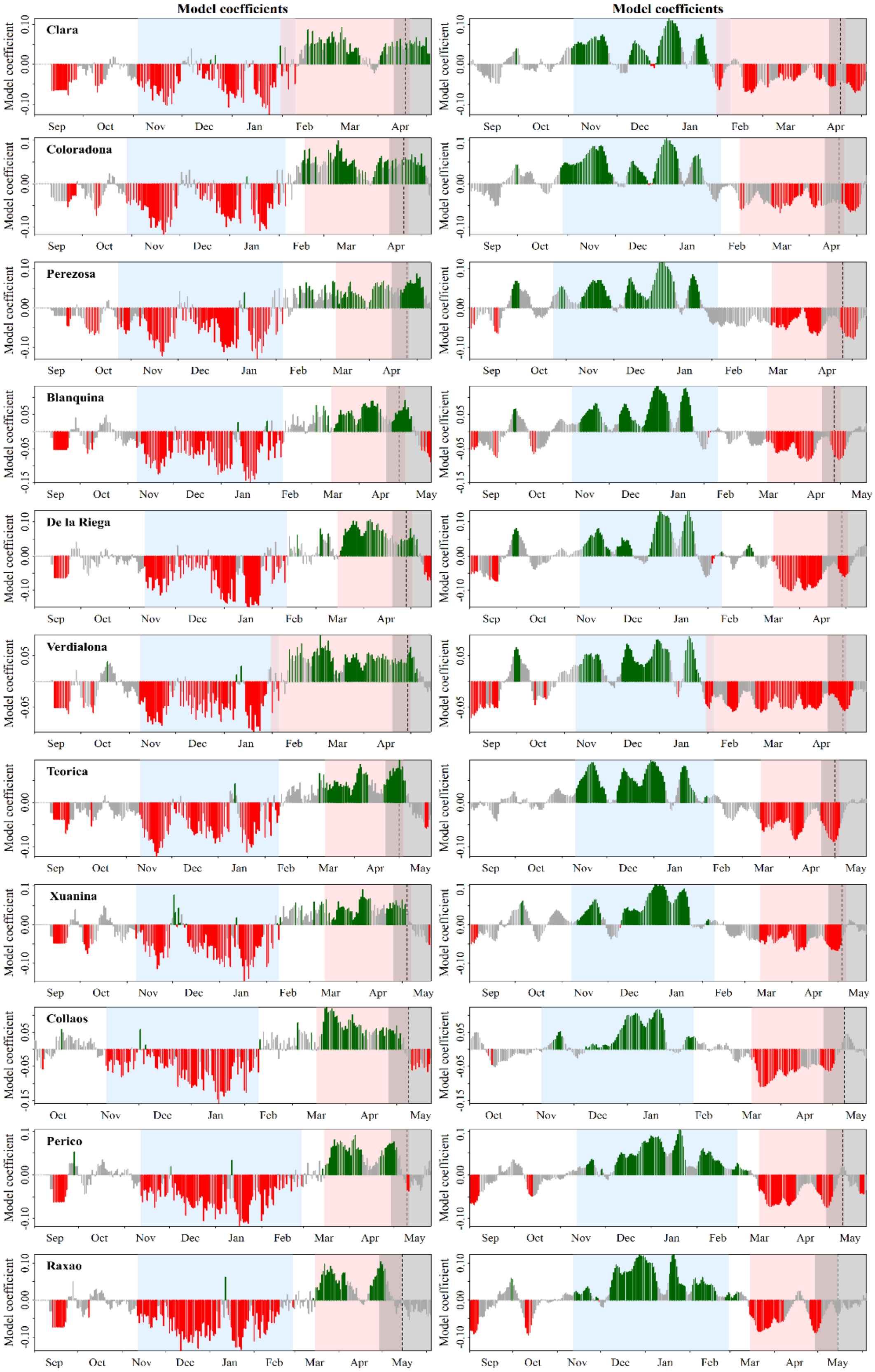

Fig. 8. Model coefficients of Partial Least Squares (PLS) regression between daily chill and heat accumulation and flowering dates of eleven local apple cultivars in Villaviciosa, northwestern

Spain between 2004-2019, using the Dynamic Model and the GDH Model for chill and heat accumulation, respectively. See caption of Fig. 6 for more details.

Table 2. Bloom dates, chilling and forcing periods of 11 local apple cultivars in Villaviciosa (northwestern Spain) between 2004 and 2019. The range of bloom dates represents the difference (in days) between the earliest and the latest full bloom date for each cultivar in our dataset.

Cultivars	**Bloom date**				**Chilling period**			**Forcing period**		
	First	**Last**	**Median**	**Range (Days)**	**Start**	**End**	**No. of days**	**Start**	**End**	**No. of days**
‘Clara’	11-Apr	4-May	21-Apr	23	4-Nov	9-Feb	97	31-Jan	21-Apr	80
‘Coloradona’	11-Apr	7-May	23-Apr	26	29-Oct	5-Feb	99	17-Feb	23-Apr	65
‘Perezosa’	15-Apr	10-May	26-Apr	25	24-Oct	5-Feb	104	11-Mar	26-Apr	46
‘Verdialona’	19-Apr	16-May	1-May	27	8-Nov	5-Feb	89	31-Jan	1-May	90
‘Blanquina’	19-Apr	18-May	1-May	29	7-Nov	10-Feb	95	14-Mar	1-May	48
‘De la Riega’	19-Apr	14-May	2-May	25	11-Nov	10-Feb	91	15-Mar	2-May	48
‘Teorica’	22-Apr	26-May	4-May	34	4-Nov	10-Feb	92	13-Mar	4-May	52
‘Xuanina’	25-Apr	20-May	7-May	25	7-Nov	9-Feb	94	11-Mar	7-May	57
‘Collaos’	26-Apr	20-May	8-May	24	12-Nov	9-Feb	89	15-Mar	8-May	54
‘Perico’	29-Apr	26-May	11-May	27	12-Nov	28-Feb	108	15-Mar	11-May	57
‘Raxao’	29-Apr	4-Jun	15-May	36	12-Nov	28-Feb	108	15-Mar	15-May	61

Chilling requirements (CR) for endodormancy release did not vary significantly among most of the cultivars (Table 3). ‘Verdialona’ had the lowest CR (59.4 CP), whereas ‘Perico’ and ‘Raxao’ had the highest CR (72.7 CP). Heat requirements (HR) ranged between 7,326 GDH (for ‘Perezosa’) and 11,917 GDH (for ‘Verdialona’), indicating that HR were slightly more variable among cultivars than CR.

Table 3. Cultivar-specific CR and HR (± standard deviation) for local apple cultivars in Asturias, Spain. Chill and heat requirements were estimated with the Chilling Hours (CH), Utah (CU), Dynamic (CP) and GDH models (GDH). Chilling-Forcing overlap (%) is the percentage of time of the total period between the beginning of chilling phase and the end of forcing phase with an overlap between phases.

Cultivars	Chill requirements			Heat requirements (GDH)	Chilling-Forcing overlap (%)
	CP	CU	CH		
'Clara'	63.9 ± 5.2	1,296 ± 149	842 ± 123	9,921 ± 1,554	5%
'Coloradona'	63.5 ± 5.5	1,263 ± 157	815 ± 120	8,909 ± 1,406	-
'Perezosa'	64.4 ± 6.2	1,267 ± 173	826 ± 126	7,326 ± 1,182	-
'Verdialona'	59.4 ± 4.7	1,213 ± 132	789 ± 111	11,917 ± 1,799	3%
'Blanquina'	63.3 ± 5.0	1,292 ± 142	847 ± 122	7,940 ± 1,369	-
'De la Riega'	61.3 ± 4.5	1,260 ± 135	830 ± 116	8,022 ± 1,419	-
'Teorica'	61.7 ± 4.5	1,266 ± 135	833 ± 117	8,646 ± 1,479	-
'Xuanina'	62.6 ± 5.1	1,278 ± 143	836 ± 120	9,570 ± 1,547	-
'Collaos'	60.3 ± 4.5	1,240 ± 135	818 ± 113	9,585 ± 1,495	-
'Perico'	72.7 ± 5.1	1,495 ± 167	1,005 ± 148	10,156 ± 1,594	-
'Raxao'	72.7 ± 5.1	1,495 ± 167	1,005 ± 148	11,111 ± 1,657	-

4. Discussion

4.1. Temperature response of bloom dates and chill and heat accumulation

The observed temperature changes have important consequences for apple cultivation, because temperature is the primary driver of phenological development (Walther et al., 2002; Chmielewski et al., 2004). Temperatures at SERIDA (Villaviciosa) have been increasing at a faster rate (+0.30°C) than the mean global land surface temperature, which has only risen by approximately 0.18°C per decade since 1981 (NOAA 2019, Global Climate Summary). Our analysis also revealed that the pace of temperature increase was faster for minimum temperatures than for maximum temperatures, confirming earlier reports that have indicated greater sensitivity to climate change for the lowest than for the highest temperatures of the day (Luedeling et al., 2009). The average temperature during the phenological season in Asturias showed a positive trend of +0.29°C/decade since 1978. Nevertheless, the resulting increase by 1.16°C for temperatures between November and March during this 41-year period has not led to a statistically significant reduction in winter chill accumulation. Even though winters in Villaviciosa have not been particularly cold during recent decades (mean temperature of 8.5°C between 1978 and 2019), chill accumulation was high, at an average of 96 CP per year. This observation indicates that current winter conditions at this location are favorable for chill accumulation (according to the Dynamic Model) and have apparently been cool enough for recent warming to have no negative impacts on this agroclimatic metric.

A major driver of the timing of phenological events in temperate regions of the Northern Hemisphere is variability in mean air temperature from February to April (Chmielewski and Rötzer, 2001). In Asturias, air temperature during these three months increased by approximately 1.16°C over the past four decades, with the most remarkable warming, by 1.71°C, occurring in April. Temperature during this month, which immediately precedes full bloom in most of the local cultivars, has been identified as the strongest driver of flowering time (Lu et al., 2006). Significantly warmer mean temperatures in the month responsible for almost half of the heat accumulation have thus led to a faster fulfillment of HR, which particularly impacted late-flowering cultivars.

Declining winter chill has been reported for several regions (Baldocchi and Wong, 2008; Luedeling et al., 2009; Atkinson et al., 2013), and the relationship between winter chill and spring events in the context of global warming has been widely studied (e.g., Luedeling et al., 2011; Campoy et al., 2011; Luedeling, 2012; Bartolini et al., 2019). For apple trees, previous reports have reported advances in spring events over the past few decades in France (Legave et al., 2008), Germany (Chmielewski et al., 2004), Japan (Fujisawa and Kobayashi, 2010), northern Italy (Eccel et al., 2009), Australia (Darbyshire et al., 2013), China (Yong et al., 2016) and Belgium (Drepper et al., 2020). Only a few authors found a delay in flowering dates in mild winter areas, resulting from a delayed onset of the dormant season, which subsequently delayed winter events and the fulfillment of CR and HR (Legave et al., 2013; Legave et al., 2015; Guo et al., 2019). It is important to note that the impacts of global warming vary across countries and regions. In central Italy, for instance, Bartolini et al. (2019) found a significant reduction in chill unit accumulation, with a loss of a third of the initial accumulation over approximately the same forty-year period as considered in our study.

In Asturias, the lack of a clear shift in mean bloom dates over the past 30 years likely resulted from a later onset of chill accumulation, a longer time to accumulate enough chill to meet cultivar-specific chilling requirements and a shorter duration of the forcing phase. A delay in the accumulation of the first Chill Portions due to a significant temperature increase in November may have contributed to later fulfilment of cultivar-specific CR, especially in high-chill cultivars. In this context, and assuming a sequential transition between phases, the ecodormancy phase may have been shortened in recent years, but local cultivars may have reached their heat needs faster than before as a result of the marked warming trend observed in April. The occurrence of the highest year-to-year variation in the latest-blooming cultivar may have resulted from the combination of high chill and heat requirements. Cultivars with high CR and HR values (as appears to be the case for 'Raxao') are particularly sensitive to year-to-year variation in climatic conditions and may thus exhibit a wide range of bloom dates. On the other hand, winter chill is not a limiting factor in early-blooming cultivars. This may explain the low variation in flowering dates found in 'Clara', which is largely a result of different heat levels during the ecodormacy phase.

The lack of a strong phenological response to warming also indicates that local apple cultivars in Asturias are well adapted to the particular climatic setting of this region and resilient to some variation and change in winter temperatures. Overall, a tendency towards advancing flowering dates was found for most of the study cultivars, whereas early-blooming cultivars tended towards later bloom dates. This is in agreement with previous reports that early-blooming species and cultivars are particularly prone to showing delayed flowering in response to warming (Doi et al., 2008).

Our results of only minor phenology changes in response to warming have potential agronomic implications. Hazardous spring frost events, which may increase in frequency due to flowering advances, do not seem to present a major risk in Asturias, since the number of frost days in April is currently small and appears to be decreasing further. Temperature increases in spring may have favorable effects on apple cultivation, in particular during flowering, when temperature may positively affect pollen quality, pollen viability and pollinator activity.

4.2. Temperature response phases and chill and heat requirements

Through the selection of a reasonably long series of flowering records, PLS regression allowed clear identification of the days of the year when the accumulation of chill or heat had a significant impact on flowering dates. Luedeling et al. (2013b) stated that delineation of the phases is clearer in areas where freezing temperatures are rare, an observation that is confirmed by our analysis, in which all cultivars showed almost uninterrupted periods of negative model coefficients. Several authors have shown that in a cool winter location, where chilling requirements are easily satisfied and variability in chill accumulation does not greatly influence flowering dates, the PLS approach can be a useful tool to determine the chilling and forcing periods (Guo et al., 2014; Darbyshire et al., 2017). Using this statistical approach, other authors have reported an overlap between the chilling and forcing periods in *Prunus* spp. (Guo et al., 2015; Benmoussa et al., 2017a; Martínez-Lüscher et al., 2017) and apple (Guo et al., 2019). Under the fairly mild winters of northwestern Spain, we did not find an overlap between phases for the set of eleven apple cultivars. For the period between February 9th and March 15th, we did not identify a consistent pattern of negative coefficients. We suspect that the PLS procedure is unable to clearly assign these days to one of the phases, as they can be part of the endormancy phase in some years and be associated with the ecodormancy phase in others. It also seems possible that, in some years, chilling requirements have already been fulfilled at this time for the bulk of the local cultivars, but temperatures during this early part of the forcing period are too cool to have a significant impact on spring phenology. On the other hand, the beginning and the end of the chilling period fell on the exact same days for several of the cultivars, resulting in similar or even identical estimates of their chilling requirements. The studied cultivars were selected in the study region, and to date, they are only cultivated in northern Spain, so that no estimates of their chill requirements are available from other locations. The calculated chill requirements between 59 and 73 CP are consistent with the designation of these local cultivars as medium-high chill cultivars (Dapena, 1996). Given the wide range of flowering dates across the eleven apple cultivars, with a maximum difference in mean bloom dates of 30 days, we are somewhat surprised by the relatively low variation in estimated chilling requirements across the eleven cultivars. Such similar estimates across multiple cultivars, also determined with the PLS approach, have been reported previously for almonds and pistachios in Tunisia (Benmoussa et al., 2017a, b). It is of course possible that cultivars selected in the same region have similar agroclimatic needs. On the other hand, the delineation of chilling and forcing phases may also be responsive to typical local temperature dynamics, with temperatures usually rising or dropping at certain times, which may produce PLS coefficient patterns that are not directly related to tree physiology. To resolve this question, comparisons of statistically and experimentally derived cultivar-specific chilling requirements should be undertaken.

In general, the delineated chilling and forcing periods, as well as the estimated chill and heat requirements, appear plausible, especially when based on the Dynamic Model, which has been found superior to alternative models, particularly in warm environments (Luedeling and Brown, 2011). We note, however, that we used the Dynamic Model with predefined parameters that were initially obtained from experimental work with peach (Fishman et al., 1987a,b). These parameters should ideally be calibrated for apple, or even for each cultivar, as recently suggested for apricot by Egea et al. (in press).

Compared to the chilling period, the heat accumulation phase varied strongly in length across the 11 cultivars, lasting between 46 and 90 days. Assuming that the climatic requirements we derived are accurate, this finding indicates that variation in bloom dates among these locally selected cultivars derives primarily from genetic differences in heat requirements, while chill needs are relatively similar.

Several studies have presented CR estimates for apple cultivars, with most of them based on laboratory experiments, where shoots were forced in a growth chamber (e.g., Hauagge and

Cummins, 1991; Guak and Neilsen, 2013; El Yaacoubi et al., 2016; Parkes at al., 2020). Only a few analyses so far have estimated CR and HR using the PLS procedures (Darbyshire et al., 2017; Diez-Palet et al., 2019; El Yaacoubi et al., 2020). Comparisons between experimentally and statistically derived CR estimated in mild winter regions showed considerable differences, with PLS regression consistently reporting lower requirements for the same cultivar and geographical location. For example, in southern Australia, the cultivar-specific chilling requirements for the cultivar 'Cripps Pink' varied from 52 CP according to the PLS regression analysis (Darbyshire et al., 2017) to 73 CP using a forced bud method (Parkes et al., 2020). Similar variation was found for the cultivar 'Gala' in northern Morocco where El Yaacoubi et al. (2016) reported 61 CP in a controlled environment experiment, contrasting with 44 CP found by applying the PLS approach (El Yaacoubi et al., 2020).

Of particular interest for the present analysis is a study by Diez-Palet et al. (2019), who evaluated apple cultivars in Girona (northeastern Spain). Similar to our results, they reported the presence of gaps between chilling and forcing phases, as well as only small differences in CR among apple cultivars (Diez-Palet et al., 2019). The beginning of the chilling period at our study site on November 12th seems to be in accordance with previous studies on apple in the Mediterranean climates of southern France (El Yaacoubi et al., 2014) and northeastern Spain (Diez-Palet et al., 2019). Studies from colder regions, in contrast, have reported earlier onset dates, as well as later end dates for the chilling phase. Guo et al. (2019) identified a chilling period between September 24th and February 19th in northwestern China, and the chilling phase was found to last until the last week of February in Belgium (Drepper et al., 2020). Diez-Palet et al. (2019) identified a similar onset of the chilling phase in dessert apple cultivars, although the length of the period was shorter than in our study, possibly because the specific chilling requirements of dessert apple cultivars are lower.

The bloom timing responses to temperature vary considerably across regions (Menzel and Sparks, 2006). In warm regions, bloom dates have been reported not to be very responsive to temperatures during the forcing phase (Benmousa et al., 2017b). In colder locations, in contrast, it is conditions during the chilling phase temperatures that barely affect bloom dates (Guo et al., 2015; Martínez-Lüscher et al., 2017). The tree responses to temperature during the delineated phases obtained by applying the PLS procedure indicate that for apple cultivars grown in a typical oceanic climate location, where temperatures are mild all year round, bloom dates are controlled in equal measure by temperatures during both the chilling and the forcing phases. In such locations, both processes need to be equally considered for developing climate-resilient apple cultivars.

5. Conclusions

Our results represent an advance in assessing the possible influence of climate change on apple phenology in mild humid climates such as that of northwestern Spain. Under local climate conditions, winter chill accumulation did not show a significant decrease despite temperature increases by 0.30°C per decade since 1978. Our results indicate that local apple cultivars have shown a high degree of phenotypic plasticity to respond to gradual changes in the environmental conditions. However, their resilience to warming winters appears to vary across cultivars. The early-blooming cultivars showed a slight tendency towards a flowering delay, whereas slight advances in flowering dates were generally observed in intermediate/late and late-blooming cultivars. This information suggests that the local apple breeding program should prioritize the use of locally maintained germplasm in the process of obtaining environmentally adapted new cultivars. Finally, our results confirm that the amount of winter chill available in coastal areas in northern Spain has not been significantly affected by recent warming. An average chill accumulation of 96 CP implies that a transition to cultivars with lower chilling requirements and/or geographical shifts to other suitable cultivation areas is not currently an urgent priority.

Author´s contributions

AD and ED designed the study. ED collected phenology and meteorological data. AD performed the analysis with input from EL, JAE and ED. AD wrote the manuscript and all authors contributed to interpretation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at.

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