Apparent differences in agroclimatic requirements for sweet cherry across climatic settings reveal shortcomings in common phenology models

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Abstract

Temperate fruit trees are widely cultivated across the world's temperate regions. These trees are well-adapted to cold-winter climates through their ability to synchronize their phenology with the seasons. In autumn, they enter a dormant state, which allows them to survive the low winter temperatures and lasts until they resume growth in early spring. We analyzed the agroclimatic requirements (chill accumulation in Chill Portions, CP, and heat accumulation in Growing Degree Hours, GDH) for blooming in three sweet cherry cultivars ('Samba', 'Burlat', and 'Sylvia') grown in distinct climatic settings in Bonn (Germany) and Zaragoza (Spain). We used Partial Least Squares (PLS) regression analysis to relate bloom dates of the three cultivars grown in both locations to local temperatures. In Bonn, the colder location, trees experienced a long period of chill exposure (87-105 CP), which allowed a rapid growth response to warm temperatures (3233-4343 GDH). The flowering dates were mainly driven by conditions during the forcing period. In contrast, in the warmer climate of Zaragoza, chill exposure of the trees was relatively short (48-59 CP). The buds required a long exposure to warm conditions (5444-6988 GDH) to subsequently bloom. In this case, flowering dates were influenced more by exposure to chilling than by conditions during the heat accumulation period. Global warming caused opposite effects on flowering dates depending on location. While in Bonn flowering dates have advanced between 3 and 5 days per decade, bloom dates in Zaragoza did not show such a trend, except for minor flowering delays in 'Sylvia', the late-flowering cultivar. Our results show that the response of the flowering dates to temperature appeared to depend on specific local climatic conditions. Although we applied current methodologies to determine the agroclimatic requirements of these cultivars, our methods were unable to derive consistent estimates of agroclimatic needs across the two locations.

Keywords: Chilling requirements, Dynamic model, Forcing requirements, Growing degree hours model, PLS analysis.

Introduction

In temperate and cold climates, trees enter a dormant state during autumn, which allows them to survive in low winter temperatures and resume growth in early spring, to be active during the climatically favorable seasons of spring and summer (Rohde and Bhalerao, 2007). Temperate deciduous fruit trees are widely distributed across latitudes between 23.5° and 66.5° N/S, especially between 30° and 40°, with only occasional occurrences outside this range. Temperate regions are characterized by a wide temperature range throughout the year, with distinct seasonal changes. Temperate fruit trees have adapted to these annual changes and synchronized their phenology with the seasons. Temperature and photoperiod are the main environmental signals that regulate phenology (Singh et al., 2017). The annual variation of these parameters varies greatly with latitude (Fadón et al., 2020a). How a particular cultivar adapts to the different regions and distinctly responds to the environmental clues remains unclear.

Temperate fruit tree species and cultivars have specific agroclimatic requirements for flowering that must be met to overcome dormancy and resume growth (Richardson et al., 1975). The interaction between these requirements and the local temperature regime determines the timing of spring phenology. Flowering time strongly depends on temperatures during dormancy, in autumn and winter. Dormancy is traditionally divided into two phases, known as endo- and eco- dormancy (Lang et al., 1987). Endodormant trees are not able to grow even under suitable environmental conditions, since a period of exposure to low temperature is necessary to recover the capacity to grow (chilling requirement). After overcoming endodormancy, trees enter the ecodormancy phase. Growth is not immediately resumed, but remains suspended until a specific amount of heat has been accumulated (forcing requirements) (Lang et al., 1987; Rohde and Bhalerao, 2007).

Chilling and forcing requirements appear to be genetically determined (Calle et al., 2020; Castède et al., 2014), constituting an intrinsic trait of each cultivar that plays a key role in the geographical adaptation of temperate fruit trees. The identification of the DORMANCY-ASSOCIATED MADS-BOX genes (DAM) and numerous Quantitative Trait Loci (QTL) associated with phenology are important steps forward in this field (Fan et al., 2010; Jiménez et al., 2010). However, the lack of exhaustive biological or physiological understanding of dormancy prevents accurate delineation of the process (Fadón et al., 2020). This limitation has hampered the development of a process-based model to accurately predict the effect of temperature on dormancy and phenology.

Knowledge of agroclimatic requirements is useful for predicting the suitability of a cultivar in a certain growing region, and it allows anticipating cultivar responses to future climate conditions in a global warming context (Luedeling et al., 2015). However, information on climatic needs is scarce, even for some of the most common cultivars of temperate fruit trees (Fadón et al., 2020b). This scarcity is due, on the one hand, to the cumbersome methodologies involved in determining these climatic needs and, on the other hand, to the low accuracy of models and agroclimatic requirements in approximating the actual behavior of trees (Fernandez et al., 2020; Luedeling and Brown, 2011).

Chill and heat accumulation are usually quantified according to temperature models (Richardson et al., 1974). For chill quantification, the Dynamic model has been identified as the most sophisticated and best-adapted model for Mediterranean climate conditions (Fernandez et al., 2020; Luedeling and Brown, 2011). This two-step model relies on the assumption that low temperatures stimulate the production of a chill precursor compound (which is not mapped to a concrete biological phenomenon), which then needs to be converted into a permanent “chill portion” – a quantification of effective chill accumulation, by a process that is most effective at moderately cold temperatures (Erez et al., 1990; Erez and Couvillon, 1987; Fishman et al., 1987). However, this model is based on a hypothetical biological process.

To quantify heat accumulation, the Growing Degree Hours is the most widely used model. This model considers that only temperatures within a certain range (usually between 4º C and 25º C) contribute to plant reactivation and growth after the chill requirements have been fulfilled (Richardson et al., 1975). This model is not specific for the phenology of temperate fruit trees, but also used for phenological stages of annual crops and even insect growth. Chilling and forcing models are usually combined for the prediction of phenology (Anderson et al., 1986). This approach assumes that chilling and forcing are successive phases, although recent studies suggest that chill and heat interact with each other during part or the whole dormancy period (Darbyshire et al., 2016; Harrington et al., 2010; Pope et al., 2014). Even though this combination approach is extensively used, including in many recent studies, it is worth noting that it does not incorporate recent advances in dormancy research.

The chilling and forcing periods, during which chill and heat accumulations are quantified, can be determined experimentally or statistically. The experimental methodology establishes the minimum chill exposure that is needed for buds to recover the capacity to grow. Shoots are cut and transferred to a growth chamber with warm conditions throughout winter. Bud growth is then monitored after a certain time of exposure to the chamber environment (Brown and Kotob, 1957; Fadón and Rodrigo, 2018). This methodology can easily be adapted to different fruit species and regions by adjusting the shoot sampling frequency, parameters of the growth chamber (i.e. temperature and light regimes) and the time shoots spend in the chamber. Evaluation methods have varied across studies, with researchers observing either vegetative or flower buds, recording bud weights or bud phenology, and using different thresholds for the transition from endodormancy to ecodormancy. This variability in experimental designs has limited opportunities to compare results across studies (Fadón et al., 2020b).

The statistical methodology analyzes long-term phenology records by relating the phenological observations to daily temperatures during the previous months. Partial Least Squares (PLS) regression analysis has emerged as a suitable approach to determine the periods when cold or warm temperatures advance flowering dates (Luedeling et al., 2013; Luedeling and Gassner, 2012). The agroclimatic requirements of numerous temperate fruit tree species in several regions have been estimated using this technique, including apricot in China and the UK (Guo et al., 2015b, 2015a; Martínez-Lüscher et al., 2017), apple and almond in Spain (Delgado et al., 2021; Díez-Palet et al., 2019), sweet cherry in Germany and Spain (Fadón et al., 2021b; Luedeling et al., 2013), and pistachio and almond in Tunisia (Benmoussa et al., 2017b, 2017a). However, PLS regression has several prerequisites that limit its generalization to a broad range of species and climatic conditions. A particular constraint is the method's dependence on the availability of long-term phenology records. Long-term records are needed for accurate delineation of the chilling and forcing periods, because the PLS methodology relies on sufficient variation in temperature signals throughout the dormancy phase, the effect of which on bloom timing is then evaluated (Luedeling and Gassner, 2012). However, in some regions, even with long-term data, periods that are commonly assumed to be important for chill contribution appear not to have significant effects on bloom timing in the outputs of PLS analysis (Delgado et al., 2021; Díez-Palet et al., 2019; Fadón et al., 2021b; Guo et al., 2014; Luedeling et al., 2013; Martínez-Lüscher et al., 2017).

We aimed to analyze temperature responses of sweet cherry cultivars in two growing areas with very different climatic conditions. To this end, we used PLS regression analysis to analyze long-term phenology observations of the same three sweet cherry cultivars grown in both locations. We examined the feasibility of delineating the chilling and forcing periods for each cultivar based on the combined data set. However, this analysis was unable to distinguish common chilling and forcing phases of each genotype for these two distinct conditions. Data were finally analyzed separately for each of the locations, revealing very different chilling and forcing periods across the two sites. This analysis provided insights on how the phenology of these cultivars varies across different climate conditions, which has implications for their ability to respond to rising temperatures caused by global warming.

Materials and Methods

Plant material, orchard locations and phenology monitoring

We evaluated flowering dates of three sweet cherry cultivars (‘Samba’, ‘Burlat’, and ‘Sylvia’) at two locations, Bonn in Germany and Zaragoza in Spain. Bonn is exposed to westerly Atlantic weather, tempered by the mild buffering climate of the Rhine valley to the east, resulting in an average annual temperature of 9.8°C. August is the hottest month with an average temperature of 17.9°C and January the coldest month at 2.2°C. Annual rainfall is around 600 mm (Blanke and Kunz, 2009). The experimental orchard is located at Campus Klein-Altendorf (50.62° N; 6.99°E; 160 m a.s.l.) (Fig.1), an experimental station of the University of Bonn. The soil is a loess loam (silty loam), with relatively high water retention capacity. Trees of the investigated cultivars were grafted on GiSelA 5 rootstocks and a Tall Spindle Axe (TSA) training system, with fully grown trees reaching a height of about 4 m. The orchard was occasionally irrigated with a sprinkler system.

Zaragoza features an Arid Cold steppe climate, classified as BSk on the Köppen scale (Köppen, 1900; Kottek et al., 2006). The temperature averages 15.6 °C, with July being the warmest month with an average temperature of 25°C and January the coldest month at 7°C (AEMET, 2021). The long-term mean of annual precipitation is about 362 mm. The soil is classified as fine-loamy and calcareous soil. Trees of the three investigated cultivars belong to the cultivar collection at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) (41.72º N; 0.81º W; 220 m a.s.l.) (Fig.1). The trees were grafted on Santa Lucia 64 rootstock (*Prunus mahaleb*) and pruned to form a Spanish Bush. Irrigation is essential, and the system used is traditional blanket (by gravity).

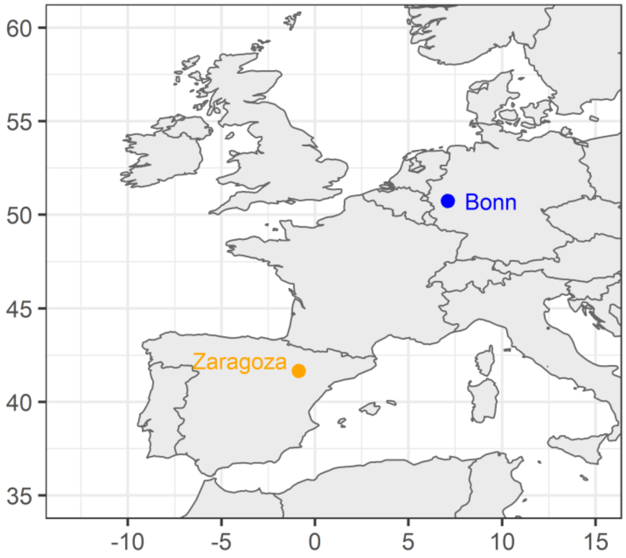

Figure 1. Location of Bonn, Germany and Zaragoza, Spain.

In Bonn, field observations were carried out every two to three days to record three phenological stages according to the BBCH code (Fadón et al., 2015; Meier, 2001). Recorded stages were stage 61 (beginning of flowering), stage 65 (full flowering), and stage 69 (end of flowering), corresponding with stages E, F, and G of the Baggliolini phenology code (Baggiolini, 1952). In Zaragoza, phenology was monitored every two days during spring.

Full bloom was recorded when the F stage of the Baggliolini phenology code (Baggiolini, 1952) was observed as the most frequent stage, corresponding with stage 65 of the BBCH code (Fadón et al., 2015; Meier, 2001). Across the two sites, 38 observations were available for 'Samba', 56 for 'Burlat' and 29 for 'Sylvia' (Table 1).

Table 1. Phenology record availability in Bonn and Zaragoza for the sweet cherry cultivars 'Samba', 'Burlat' and 'Sylvia'.

	Bonn (Klein-Altendorf)		**Zaragoza**	
	Nº years	**Years**	**Nº years**	**Years**
'Samba'	18	2003-2020	20	1993-2001, 2009-2019
'Burlat'	35	1978-1984, 1986-1995, 2002-2019	21	1991, 1993-2001, 2009-2019
'Sylvia'	12	2008-2012, 2014-2020	17	1996-2001, 2009-2019

Chill and heat quantification

Temperature records were obtained from meteorological stations located at the experimental site in Zaragoza (Spain) and at Campus Klein-Altendorf (Germany). Daily minimum and maximum temperatures have been recorded since the 1950s at Campus Klein-Altendorf and since 1990 in Zaragoza.

We used the recorded temperature data to estimate chill and heat accumulation during the respective periods of investigation at both locations. Chill accumulation was computed in Chill Portions (CP), according to the Dynamic Model (Erez et al., 1990; Erez and Couvillon, 1987; Fishman et al., 1987). The Dynamic Model assumes that chill accumulation results from a two-step process, which is mediated by a thermally labile precursor (not yet identified). In the first step, low temperatures stimulate the production of an intermediate product that can be destroyed by warm temperatures. In the second step, this intermediate product is converted into a permanent "chill portion" when a certain quantity has accumulated (for equations, see Luedeling et al., 2021).

Heat accumulation was quantified using the Growing Degree Hours (GDH) Model, a linear model with a base temperature of 4.5ºC and an upper limit of 25ºC. One GDH was defined as one hour at a temperature 1ºC above the base temperature (calculated by subtracting 4.5ºC from each hourly temperature between 4.5ºC and 25ºC), and all temperatures above 25 ºC were assumed equal to 25ºC (Richardson et al., 1974; Richardson et al., 1975; Anderson et al., 1986) (for equations, see Luedeling et al., 2021).

Chill accumulation was calculated over the chilling period defined by Partial Least Squares (PLS) regression (described below) across all years of observation. In a similar manner, heat requirements were quantified between the start of forcing and the flowering date. The final chill and heat requirements were calculated as the mean values across all years for each cultivar, with the standard deviations across all years providing an estimate of uncertainty.

Determination of chilling and forcing periods

Chilling and forcing periods were determined by applying Partial Least Squares (PLS) regression (Luedeling et al., 2013; Luedeling and Gassner, 2012). For this procedure to be successful, the PLS analysis requires some variation in the signal variables (i.e., chill and heat), which can be related to variation in bloom dates. We assessed this variation by relating the temperatures at the study sites to the temperature responses of chill and heat models. First, we produced a plot illustrating the response of the Dynamic Model and the GDH Model to a particular daily temperatures pattern, defined by daily minimum and maximum temperatures. Daily temperature variation patterns are latitude-specific since they are influenced by sunrise and sunset times. We selected an intermediate latitude (46º N) between Bonn and Zaragoza so that the differences in the temperature variation patterns were minimal. We then plotted the daily minimum and maximum temperatures grouped by month during the last 20 years (2000-2020) for both locations over the model response plots.

PLS regression analysis handles highly autocorrelated data better than most other regression approaches and can be used in situations where the number of independent variables substantially exceeds the number of dependent variables. In this case, we used PLS regression analysis to relate sweet cherry bloom dates (one observation per year) to a much larger number of observations of daily accumulated CP and GDH over the previous 8 months of each year. We ran all analyses with 11-day running means of daily CP or GDH to facilitate interpretation of the results.

To delineate the chilling and forcing periods, we examined the PLS regression outputs, considering both the model coefficients and the variable-importance-in-the-projection (VIP) scores. Model coefficients indicate the strength and direction of the influence of temperature on particular dates on bloom dates, whereas VIP values signal for each variable whether it makes an important contribution to the PLS prediction model. We considered all variables with VIP scores greater than 0.8 as important. To identify the chilling period, we examined PLS outputs for extended periods where model coefficients assigned to daily chill accumulation were negative and variables exceeded the VIP threshold. Such outputs mean that early bloom dates are associated with high chill accumulation during the respective periods – a property that is characteristic of the endodormancy phase. For delineating the forcing phase, we used an analogous procedure, except that we focused on negative model coefficients for heat rather than chill accumulation. High heat accumulation during the identified periods is associated with early bloom dates, a pattern that is characteristic of the ecodormancy period.

All analyses were performed using the chillR v.0.70.24 package (Luedeling, 2019) in the R v.4.0.1 programming environment.

Flowering response to temperature variation during the chilling and forcing periods

We characterized the relationship between flowering dates and temperature variation during the chilling and forcing periods for each cultivar and study site. To do so, we plotted the flowering dates as a function of the mean temperature during the chilling and forcing periods identified by the PLS procedure. We used the Kriging technique to interpolate a continuous surface of flowering dates (Guo et al., 2015a; Martínez-Lüscher et al., 2017). Kriging is a Gaussian regression method commonly used in spatial statistics to estimate values at locations where no data are available (Oliver and Webster, 1990). This illustration facilitated the interpretation of responses of flowering dates to multiple climatic drivers. We also demonstrated the direction and magnitude of the effects of chilling and heat accumulation on flowering dates (Guo et al., 2015a).

Results

Flowering periods of the sweet cherry cultivars 'Burlat', 'Samba', and 'Sylvia' in Bonn (Germany) and Zaragoza (Spain)

The sweet cherry cultivars 'Burlat', 'Samba', and 'Sylvia' are successfully cultivated in the North Rhine region (Bonn, Germany) and in the Ebro valley (Zaragoza, Spain). Over the study periods, flowering dates in Bonn occurred between March 22nd and May 1st, while in Zaragoza, the flowering time ranged from March 12th to April 10th, occurring approximately 10-20 days earlier than in Bonn (Fig. 2A). In both locations, the earliest-flowering cultivar was 'Samba' and the latest was 'Sylvia'.

The order of flowering times across cultivars was consistent in both locations, but bloom occurred earlier in Zaragoza where mean temperatures were about 5ºC higher than in Bonn (Fig. 2B). The temperature differences led to location-specific patterns of daily accumulation of agroclimatic metrics. Chill accumulation started about one month later in Zaragoza than in Bonn, reaching its maximum rate of about 0.75 CP per day during the coldest months (December, January, and February). It is worth noting that despite its warmer climate, chill accumulation in Zaragoza reached higher and more stable levels than in Bonn (Fig. 2C). Spring heat started to accumulate about one month earlier in Zaragoza than in Bonn (Fig. 2D).

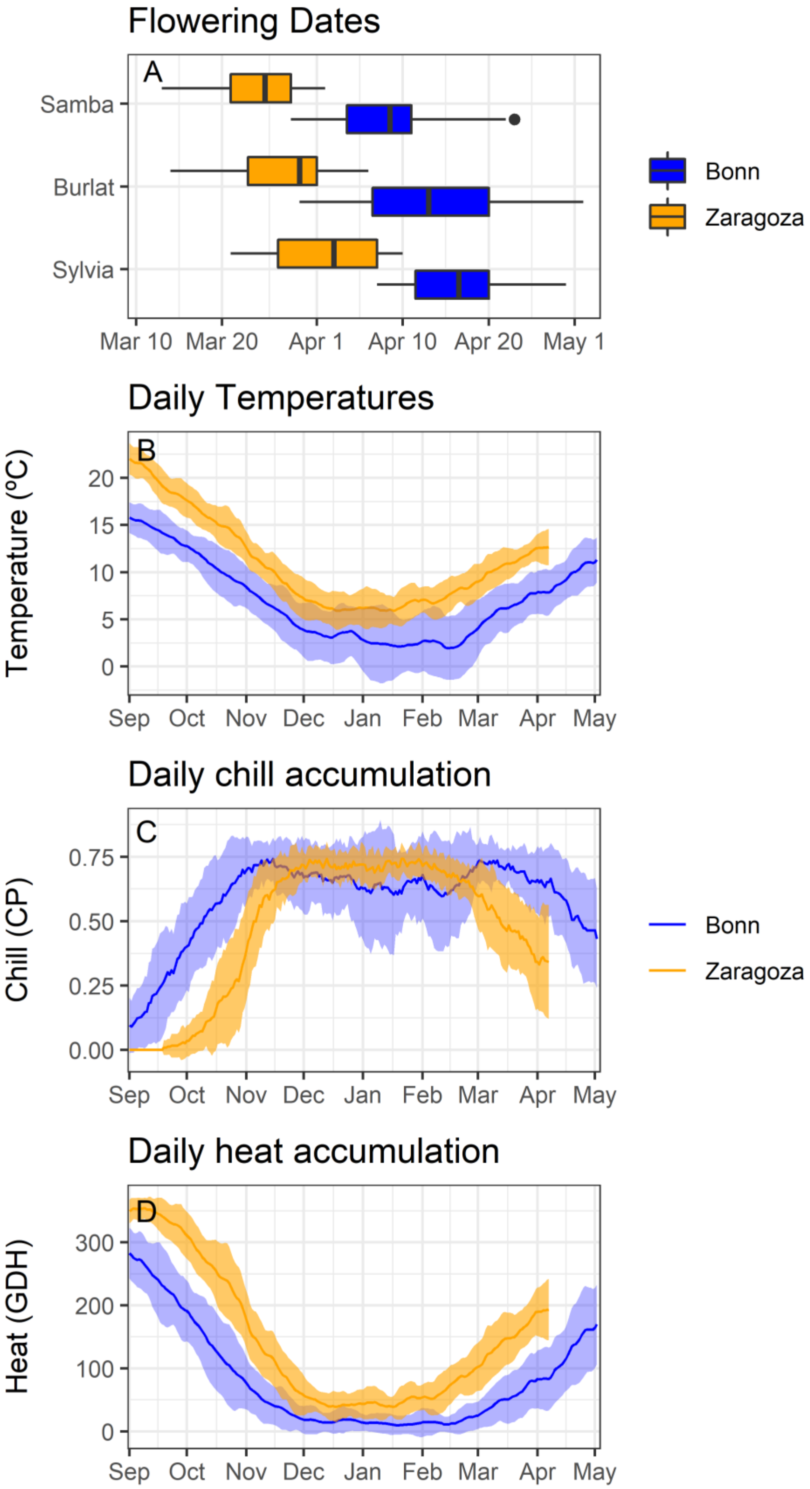

Figure 2. Flowering dates of three sweet cherry cultivars (A) and climatic conditions at Bonn and Zaragoza, including temperatures (B), chill accumulation quantified as Chill Portions - CP (C) and heat accumulation quantified as Growing Degree Hours - GDH (D). In B, C and D, lines indicate means and shaded areas signify standard deviations.

Chilling and forcing periods and agroclimatic requirements

We used PLS regression analysis with daily chill and heat accumulation to delineate the chilling and forcing phases. We first assessed the variation in the signal variables (i.e., chill and heat), which is a prerequisite for the PLS analysis to be sensitive to these metrics (Fig. 3). The months that appeared to have daily minimum and maximum temperatures that resulted in a gradient of chill accumulation were October, November, February, and March in Zaragoza, and October, January, and February in Bonn. We expected PLS to produce useful results for these months, because recorded temperature regimes included both temperature conditions that are effective and conditions that are not effective for chill accumulation (too warm or too cold). With this meaningful variation we expected PLS to produce useful results.

In contrast, months that strongly contributed to chill accumulation, i.e. December and January in Zaragoza, and November and December in Bonn, presented temperature values within a temperature range that was constantly highly effective for chill accumulation. A consistently high daily accumulation resulted in very little variation that could be exploited by PLS analysis. However, considering both locations together, a wider range of daily chill accumulation was covered, which suggested a more accurate delineation of the chilling phase. For daily heat accumulation, there was wide variation in October, November, February and March, both in Zaragoza and in Bonn.

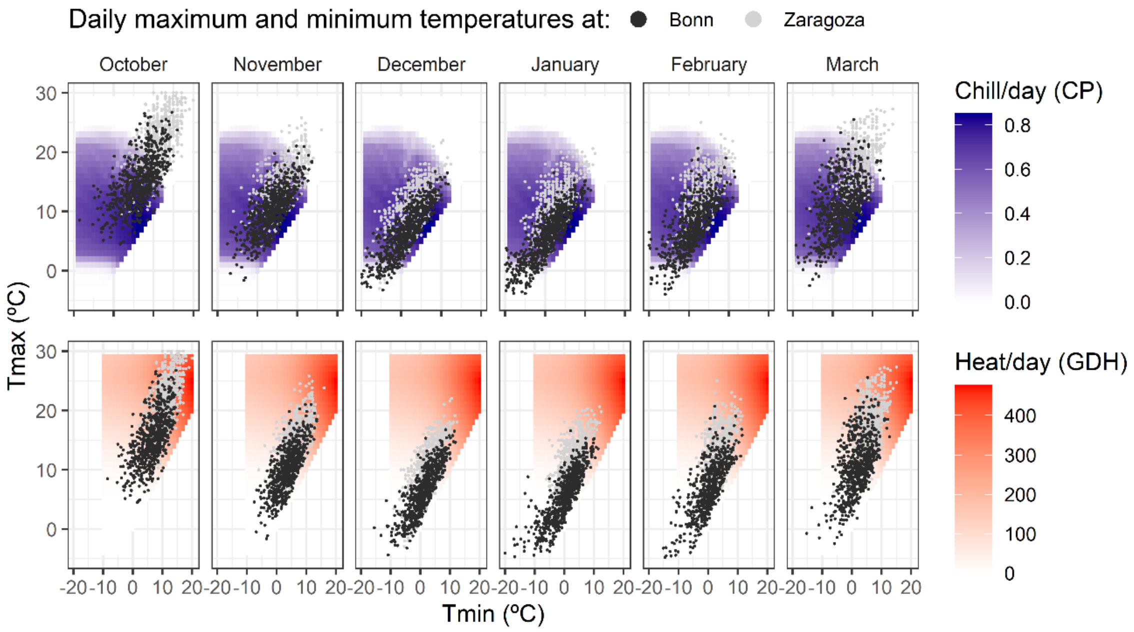

Figure 3. Daily maximum and minimum temperatures grouped by month during the last 20 years (2000-2020) for Zaragoza (light grey) and Bonn (dark gray) in relation to daily chill (CP) and heat (GDH) accumulation (indicated by blue and red gradients, respectively), according to daily minimum and maximum temperatures simulated for a location between Zaragoza and Bonn (at 46º N).

Considering our analysis of chill and heat model sensitivity in both locations, we ran PLS analyses for each cultivar combining the data from both sites. However, the outcomes appeared to show a continuous forcing period throughout the winter that did not allow distinguishing between chilling and forcing phases. This effect can probably be explained by the substantial temperature differences between the locations, with temperatures always being higher and bloom dates always earlier in Zaragoza than in Bonn. Across the whole dataset, this situation likely led to a strong association between warm conditions in any season and early bloom dates, manifesting in negative model coefficients for almost all days during the dormancy season. Since a joint analysis of the two datasets did not produce useful results, we analyzed the data from the two locations separately to determine the chilling and forcing periods of the three cultivars.

PLS regression analysis resulted in much longer chilling periods in Bonn than in Zaragoza, with average durations of 148 and 106 days, respectively (Fig. 4). In Zaragoza, the typical chilling period ranged from October 2nd to January 22nd for ‘Burlat’ and ‘Samba’. The cultivar ‘Sylvia’ had a shorter chilling phase, from October 6th to January 7th (Fig. 4A). Chill accumulations during these periods were 59 ± 5 CP for ‘Burlat’ and ‘Samba’, and 48 ± 4 CP for ‘Sylvia’. The typical forcing periods started on February 2nd – 3rd and ended at the flowering time for each year (Fig. 4B). Heat accumulation was 5945 ± 570 GDH for ‘Burlat’, 5444 ± 544 GDH for ‘Samba’, and 6988 ± 659 GDH for ‘Sylvia’ (Table 2). The typical forcing period did not follow directly after the chilling period but was preceded by a transition phase, during which model coefficients were neither clearly negative nor clearly positive, and they were not considered important by the VIP analysis. This transition period lasted 12 days for ‘Burlat’ and ‘Samba’, and 26 days for ‘Sylvia’. In Bonn, typical chilling periods ranged from Oct 23rd to Feb 24th for ‘Burlat’, from Oct 2nd to Mar 10th for ‘Samba’, and from Oct 4th to Mar 5th for ‘Sylvia’ (Fig. 4A). Chill accumulation was 87 ± 6 CP for ‘Burlat’, 105 ± 6 CP for ‘Samba’, and 101 ± 6 CP for ‘Sylvia’ (Table 2). Typical forcing periods in Bonn started between February 7th and 15th and lasted until flowering time (Fig. 4B). Heat accumulation was 3273 ± 548 GDH for ‘Burlat’, 3233 ± 546 GDH for ‘Samba’, and 4343 ± 435 GDH for Sylvia’ (Table 2). On average, the chilling and forcing phases overlapped by around 22 days.

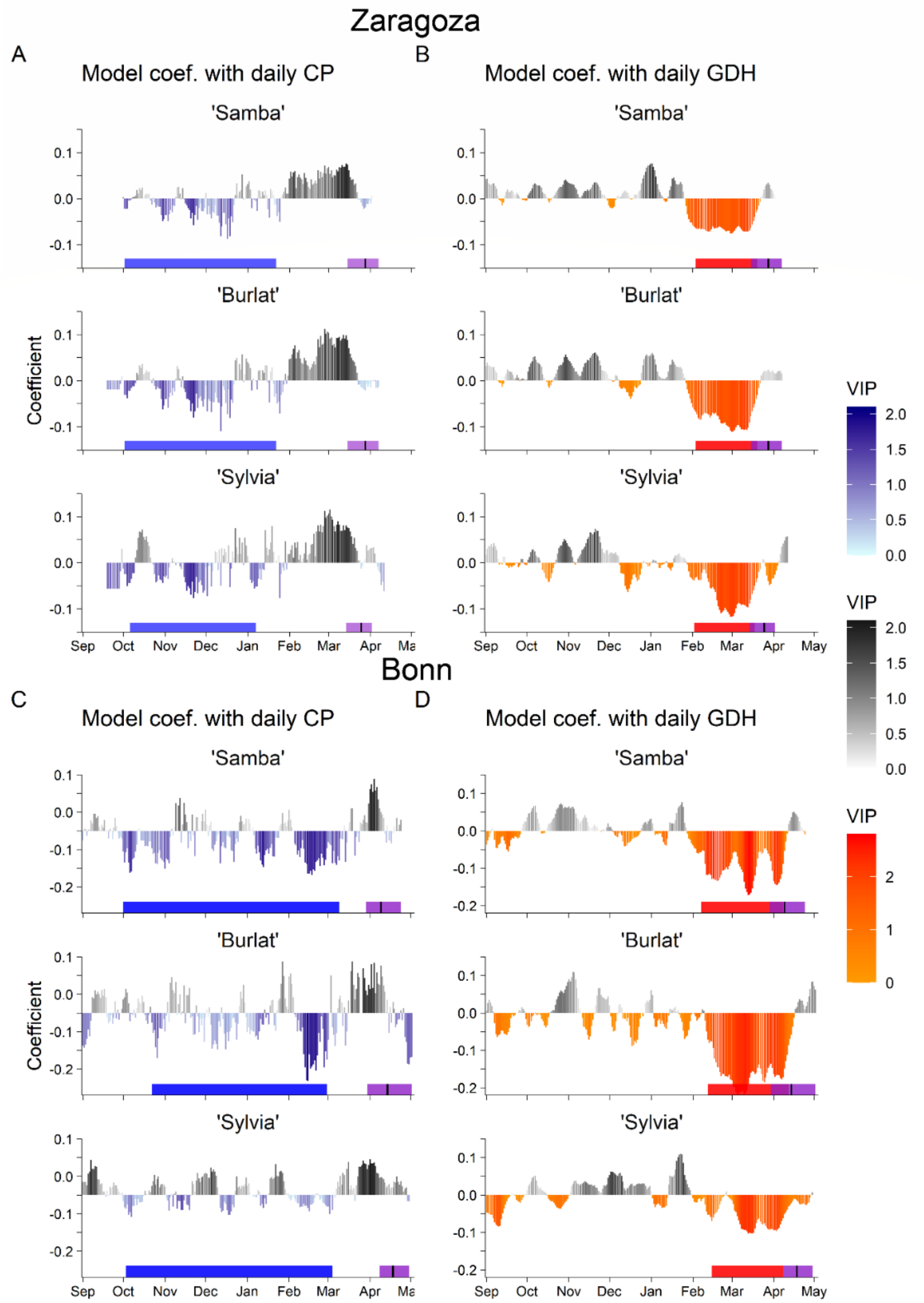

Figure 4. Partial Least Squares regression model of daily accumulation of winter chill (according to the Dynamic Model), daily accumulation of heat (according to the GDH Model), and flowering dates of three sweet cherry cultivars grown in Zaragoza, Spain **(A and B)** and Bonn, Germany **(C and D)**. Bar colors indicate the Variable Importance in the Projection (VIP) (gray scale for positive coefficients, and blue and red scales for negative coefficients for chill and heat, respectively). Rectangles at the bottom of each plot indicate the delineated chilling period (blue), the forcing period (red) and the range of observed flowering dates (purple), for which the median is indicated by the dark purple line.

Table 2. Chilling and forcing periods and temperature requirements of three sweet cherry cultivars grown in Zaragoza and Bonn, derived by PLS regression analysis.

	Zaragoza			
	Chilling		**Forcing**	
Cultivar	**Period**	**Accumulation**	**Period**	**Accumulation**
Samba	Oct 3 – Jan 22	59 ± 5	Feb 3 – flowering	5444 ± 544
Burlat	Oct 3 – Jan 22	59 ± 5	Feb 3 – flowering	5945 ± 570
Sylvia	Oct 7 – Jan 7	48 ± 4	Feb 2 – flowering	6988 ± 659
	Bonn			
	Chilling		**Forcing**	
Cultivar	**Period**	**Accumulation**	**Period**	**Accumulation**
Samba	Oct 2 – Mar 10	105 ± 6	Feb 7 – flowering	3233 ± 546
Burlat	Oct 23 – Feb 24	87 ± 6	Feb 12 – flowering	3273 ± 548
Sylvia	Oct 4 – Mar 5	101 ± 6	Feb 15 – flowering	4343 ± 435

Flowering response to chill and heat accumulation

Once the chilling and forcing periods had been identified, we analyzed temporal trends of chill and heat accumulation, as well as flowering dates (Fig. 5). Chill accumulated during the typical chill period in Zaragoza was about 56 ± 5 CP, tending to decrease by 1.3 CP per decade (Fig. 5A). In Bonn, 100 ± 8 CP were accumulated during the chilling period (Fig. 5B), almost twice the chill amount recorded in Zaragoza, showing no clear trend over time. During the respective forcing period, about 6404 ± 1459 GDH were accumulated in Zaragoza (Fig. 5C), while less heat was accumulated in Bonn (2908 ± 1255 GDH; Fig. 4D). Heat accumulation in the two locations showed opposite trends, which affected phenology. While heat accumulation decreased by 200 GDH per decade in Zaragoza, Bonn showed an increase of 180 GDH per decade (1960-2020). In Zaragoza, the cultivars 'Burlat' and 'Samba' did not show any change in the flowering dates, but bloom of the late flowering cultivar 'Sylvia' has been delayed by 2 days per decade (Fig. 5E). In Bonn, flowering dates have advanced about 3 - 5 days per decade for all three cultivars (Fig. 5F).

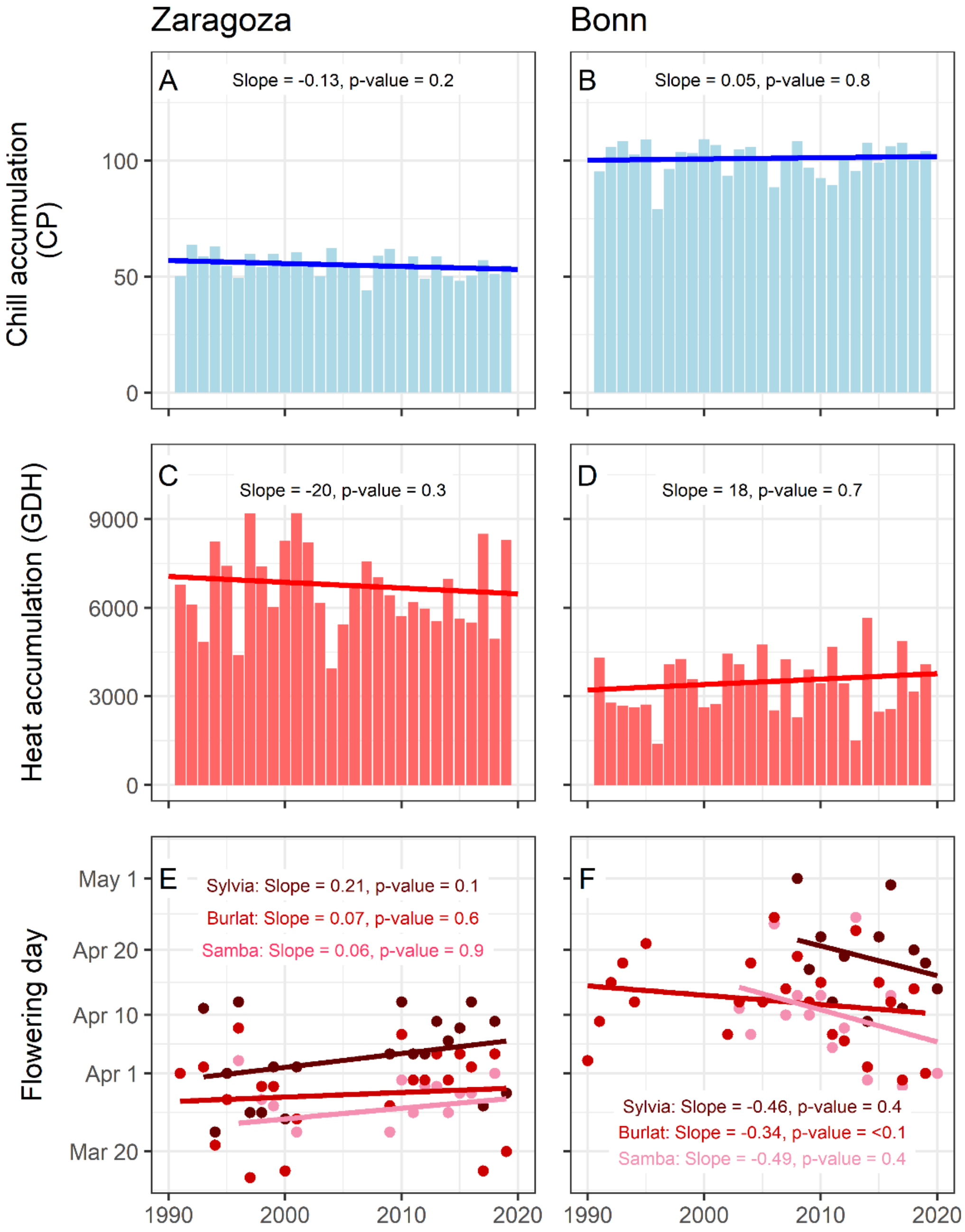

Figure 5. Annual chill accumulation according to the Dynamic Model (in Chill Portions; CP) during the typical chilling period in Zaragoza, Spain (A; chill period: October 1st – January 17th), and Bonn, Germany (B; chill period: October 1st – March 5th), heat accumulation (in Growing Degree Hours; GDH) during the typical forcing period in Zaragoza (C; forcing period: February 3rd – March 28th) and Bonn (D; forcing period: February 11th – April 14th), and flowering dates of three sweet cherry cultivars in Zaragoza (E) and Bonn (F). Colored lines indicate linear trends across datasets shown in the colors of the respective lines, p-values were calculated according to the Kendall test.

Each cultivar presented a location-specific flowering response to chill and heat accumulation. To gain insight on differences between the temperature conditions in Bonn and Zaragoza, we computed flowering date as a function of the mean temperature during the chilling and forcing periods (Fig. 6). In Bonn, the three cultivars showed response surfaces with relatively flat contour lines, indicating that conditions during the forcing phase were the main determinant of flowering dates. The trend in Zaragoza was less clear, but warm chilling phases appeared to exert a bloom-delaying effect that partly compensated the bloom-advancing effect of high forcing temperatures.

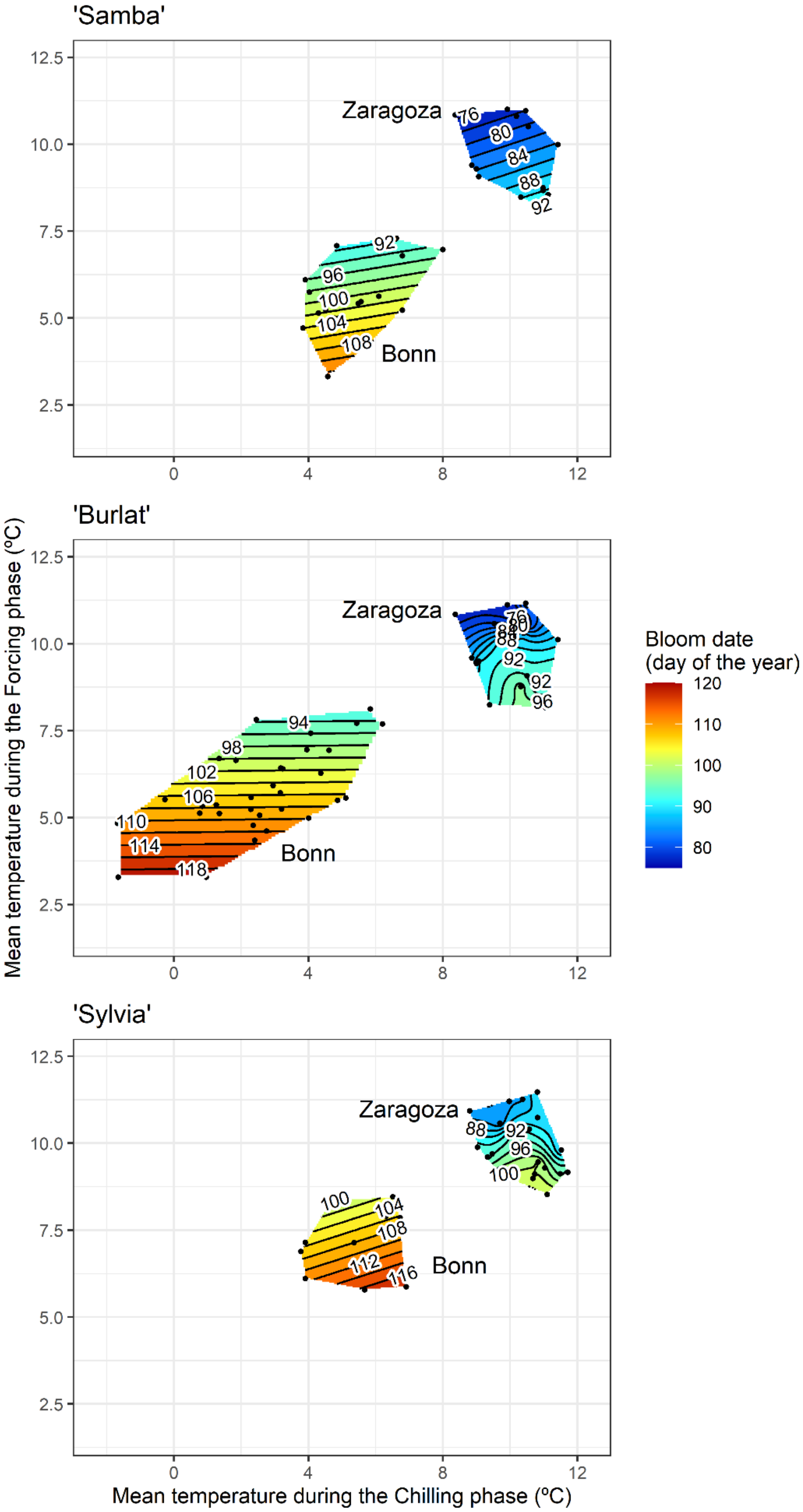
'Samba'
'Burlat'
'Sylvia'
Zaragoza
Bonn
Mean temperature during the Forcing phase (ºC)
Mean temperature during the Chilling phase (ºC)
Bloom date
(day of the year)
120
110
100
90
80
0
4
8
12
2.5
5.0
7.5
10.0
12.5

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Figure 6. Flowering dates as a function of mean temperatures during the chilling and forcing periods as determined by PLS analysis, for the sweet cherry cultivars ‘Samba’, ‘Burlat’, and ‘Sylvia’, in Zaragoza (Spain) and Bonn (Germany). Colors and contour lines represent predicted flowering dates based on observed flowering dates (black dots).

Discussion

The PLS analysis and temperature models were adequate for delineating agroclimatic requirements for flowering of all cultivars in both locations. However, the major differences obtained between locations contradict the commonly held belief that agroclimatic requirements are cultivar-specific traits and genetically determined. These results suggest that the current methodology, applied with the set of models used in this study, is useful for phenology prediction within narrow climatic settings, but it may not produce consistent results across wide temperature gradients.

Site-specific agroclimatic requirements may indicate inaccuracies of agroclimatic models

We observed genetically identical cultivars grown under different climatic conditions. Our results showed that in the cooler region (Bonn, Germany), exposure to chill over a long period (from October to March), followed by relatively low exposure to heat (from February to April) before flowering, promoted an advance in flowering time. In the warmer region (Zaragoza, Spain), the bloom-advancing effect of chill accumulation lasted over a shorter period, and buds flowered after high heat exposure. This resulted in very different estimates of agroclimatic requirements in both locations, which may hint at site-specific instead of cultivar-specific climatic needs. Site-specificity of agroclimatic requirements has also been observed in multi-site experimental studies in Mediterranean climates (Campoy et al., 2012; Gannouni et al., 2017; Viti et al., 2010). Whether these results truly contradict the widely held belief of genetically determined, cultivar-specific chilling requirements is difficult to say, however, because inaccuracies in the agroclimatic models may also introduce major errors in inter-site comparisons (Fernandez et al., 2020).

Our analysis indicated that the cultivar ‘Burlat’ accumulated 59 ± 5 CP in Zaragoza (Spain), which is substantially more than an earlier estimate of 38 ± 4 CP that was derived experimentally at the same location (Fadón et al., 2021a). Conversely, we determined lower heat requirements than those indicated experimentally (5945 ± 570 GDH vs 9345 GDH) (Fadón et al., 2021a). These differences can be explained by differences in methodology. Experimentally derived estimates reflect minimum chill accumulation for flowering, whereas PLS regression analysis is based on identifying periods during which chill has a bloom-advancing effect (Luedeling and Gassner, 2012). Other estimates for 'Burlat' derived with experimental methodology were 48 CP in Bordeaux (southern France) (Campoy et al., 2019), and 86 CP in Murcia (southern Spain) (Alburquerque et al., 2008). The latter result is in line with what we obtained in Bonn (Germany), 87 ± 6 CP, except that the heat requirement was much higher in Murcia (8750 GDH) (Alburquerque et al., 2008) than in Bonn (3273 ± 548 GDH). This wide range of estimates for observations in different climatic settings may indicate that the ability of both models to approximate the agroclimatic conditions experienced by fruit trees differs strongly between the locations.

For our study location near Bonn, PLS regression analysis has been used previously to determine the agroclimatic requirements for the cherry cultivar 'Schneiders späte Knorpelkirsche' (Luedeling et al., 2013). The estimated chilling requirement that resulted from this work was 45.7 ± 5.4 CP, a much lower value than for the cultivars analyzed in the present study (between 87 ± 6 CP and 105 ± 6 CP). Estimated heat requirements were similar, with our results ranging between 3233 ± 546 GDH and 4343 ± 435 GDH, while previous studies indicated 3473 ± 1236 GDH (Luedeling et al., 2013).

Failure of PLS regression to delineate temperature response phases for multi-location dataset

PLS regression analysis has emerged as an established method to determine the chilling and forcing periods in temperate fruit trees (Benmoussa et al., 2017b, 2017a; Delgado et al., 2021; Díez-Palet et al., 2019; Fadón et al., 2021b; Guo et al., 2015b; Martínez-Lüscher et al., 2017). Since an appropriate level of interannual variability of input data is needed for the analysis to be responsive to temperature signals, this analysis requires long-term phenology records, with early estimates indicating a need for about 20 years of data (Luedeling et al., 2013; Luedeling and Gassner, 2012). Our preliminary analysis showed that daily temperatures resulted in maximum chill accumulation with very little variation during some winter months. This impression was later confirmed by PLS analysis, which failed to identify model coefficients for days during these months as important. Similar PLS coefficient patterns have been observed in previous studies in temperate regions, confirming that the analysis is not sensitive to conditions during the colder winter months in some regions (Delgado et al., 2021; Díez-Palet et al., 2019; Fadón et al., 2021b; Guo et al., 2014; Luedeling et al., 2013; Martínez-Lüscher et al., 2017). With the intention of overcoming this limitation, we combined data from two locations, with the resulting dataset presenting variable chill and heat accumulation during all winter months. We realized, however, that, at least in this particular combination of locations, the temperature difference between sites was so large that it led to a strong correlation between high temperatures during all months and early bloom dates. This was simply because bloom of all cultivars consistently occurred earlier in Zaragoza, which features warmer conditions than Bonn throughout the whole winter. A certain level of similarity between locations would be needed for PLS analysis to produce results that can be interpreted with regard to chilling and forcing phases. The progression of dormancy should be largely similar during all years, which implies that observational records should be obtained from the same location, or at least from sites with very similar climatic conditions (Luedeling and Gassner, 2012). In this context, it may even be possible that strong warming signals within long time series may compromise the effectiveness of PLS analysis, even for records from a single location.

Phenology response to global warming is location-specific

In Bonn, sweet cherry flowering advanced in recent years, which is in agreement with previous studies that also found such a trend in response to global warming (Chmielewski et al., 2004; Grab and Craparo, 2011; Legave et al., 2013; Martínez-Lüscher et al., 2017). In Zaragoza, in contrast, the late flowering cultivar 'Sylvia' showed a delay in flowering time, while the two early flowering cultivars 'Burlat' and 'Samba' showed no trend. Phenology delays have been observed in temperate nut tree species in Tunisia (Benmoussa et al., 2017b, 2017a). The flowering response to rising temperatures has been described as species-specific (Diggle and Mulder, 2019) and also site-specific (Guo et al., 2015a).

The site-specific response observed in this work relied both on the agroclimatic trends over time and on the relative importance of chill and heat accumulation during the flowering period. We observed opposite phenomena in the two locations. In Bonn, flowering time depended almost exclusively on heat accumulation during the forcing period. Rising temperatures mainly resulted in rising spring heat accumulation, which explained the flowering advances. In Zaragoza in contrast, chill and heat accumulation decreased and the flowering time appeared to depend mainly on conditions during the chilling period, with increasing temperatures resulting in flowering delays. Such site-specific influences of the chill and heat periods on flowering times have also been described for apricots in China (Guo et al., 2015a).

Conclusions

Certain genotypes of sweet cherry thrive in growing regions with very different climatic conditions, displaying differences between locations in their responses to rising temperatures. However, the methodology applied here proved insufficient for accurate capture of phenology responses to temperature. Determining crop viability in different regions or predicting the effect of global warming on phenology is difficult, because such projections usually include conditions that are outside the range of settings in which agroclimatic requirements were estimated. To gain additional clarity on how cultivars in different places respond to future warming, efforts are needed to improve and standardize methods to characterize agroclimatic needs (both empirically and statistically) and refine the models used to quantify them. The recently developed "PhenoFlex" modeling framework parameterizes the Dynamic and GDH models and includes a sigmoidal transition between endodormancy and ecodormancy periods (Luedeling et al., 2021). A still more ambitious objective would be the development of a truly process-based dormancy model based on the best available knowledge on dormancy physiology and the effect of winter temperatures on flowering, yet such a model remains elusive.

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Apparent differences in agroclimatic requirements for sweet cherry across climatic settings reveal shortcomings in common phenology models

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Abstract

Temperate fruit trees are widely cultivated across the world's temperate regions. These trees are well-adapted to cold-winter climates through their ability to synchronize their phenology with the seasons. In autumn, they enter a dormant state, which allows them to survive the low winter temperatures and lasts until they resume growth in early spring. We analyzed the agroclimatic requirements (chill accumulation in Chill Portions, CP, and heat accumulation in Growing Degree Hours, GDH) for blooming in three sweet cherry cultivars ('Samba', 'Burlat', and 'Sylvia') grown in distinct climatic settings in Bonn (Germany) and Zaragoza (Spain). We used Partial Least Squares (PLS) regression analysis to relate bloom dates of the three cultivars grown in both locations to local temperatures. In Bonn, the colder location, trees experienced a long period of chill exposure (87-105 CP), which allowed a rapid growth response to warm temperatures (3233-4343 GDH). The flowering dates were mainly driven by conditions during the forcing period. In contrast, in the warmer climate of Zaragoza, chill exposure of the trees was relatively short (48-59 CP). The buds required a long exposure to warm conditions (5444-6988 GDH) to subsequently bloom. In this case, flowering dates were influenced more by exposure to chilling than by conditions during the heat accumulation period. Global warming caused opposite effects on flowering dates depending on location. While in Bonn flowering dates have advanced between 3 and 5 days per decade, bloom dates in Zaragoza did not show such a trend, except for minor flowering delays in 'Sylvia', the late-flowering cultivar. Our results show that the response of the flowering dates to temperature appeared to depend on specific local climatic conditions. Although we applied current methodologies to determine the agroclimatic requirements of these cultivars, our methods were unable to derive consistent estimates of agroclimatic needs across the two locations.

Keywords: Chilling requirements, Dynamic model, Forcing requirements, Growing degree hours model, PLS analysis.

Introduction

In temperate and cold climates, trees enter a dormant state during autumn, which allows them to survive in low winter temperatures and resume growth in early spring, to be active during the climatically favorable seasons of spring and summer (Rohde and Bhalerao, 2007). Temperate deciduous fruit trees are widely distributed across latitudes between 23.5° and 66.5° N/S, especially between 30° and 40°, with only occasional occurrences outside this range. Temperate regions are characterized by a wide temperature range throughout the year, with distinct seasonal changes. Temperate fruit trees have adapted to these annual changes and synchronized their phenology with the seasons. Temperature and photoperiod are the main environmental signals that regulate phenology (Singh et al., 2017). The annual variation of these parameters varies greatly with latitude (Fadón et al., 2020a). How a particular cultivar adapts to the different regions and distinctly responds to the environmental clues remains unclear.

Temperate fruit tree species and cultivars have specific agroclimatic requirements for flowering that must be met to overcome dormancy and resume growth (Richardson et al., 1975). The interaction between these requirements and the local temperature regime determines the timing of spring phenology. Flowering time strongly depends on temperatures during dormancy, in autumn and winter. Dormancy is traditionally divided into two phases, known as endo- and eco- dormancy (Lang et al., 1987). Endodormant trees are not able to grow even under suitable environmental conditions, since a period of exposure to low temperature is necessary to recover the capacity to grow (chilling requirement). After overcoming endodormancy, trees enter the ecodormancy phase. Growth is not immediately resumed, but remains suspended until a specific amount of heat has been accumulated (forcing requirements) (Lang et al., 1987; Rohde and Bhalerao, 2007).

Chilling and forcing requirements appear to be genetically determined (Calle et al., 2020; Castède et al., 2014), constituting an intrinsic trait of each cultivar that plays a key role in the geographical adaptation of temperate fruit trees. The identification of the DORMANCY-ASSOCIATED MADS-BOX genes (DAM) and numerous Quantitative Trait Loci (QTL) associated with phenology are important steps forward in this field (Fan et al., 2010; Jiménez et al., 2010). However, the lack of exhaustive biological or physiological understanding of dormancy prevents accurate delineation of the process (Fadón et al., 2020). This limitation has hampered the development of a process-based model to accurately predict the effect of temperature on dormancy and phenology.

Knowledge of agroclimatic requirements is useful for predicting the suitability of a cultivar in a certain growing region, and it allows anticipating cultivar responses to future climate conditions in a global warming context (Luedeling et al., 2015). However, information on climatic needs is scarce, even for some of the most common cultivars of temperate fruit trees (Fadón et al., 2020b). This scarcity is due, on the one hand, to the cumbersome methodologies involved in determining these climatic needs and, on the other hand, to the low accuracy of models and agroclimatic requirements in approximating the actual behavior of trees (Fernandez et al., 2020; Luedeling and Brown, 2011).

Chill and heat accumulation are usually quantified according to temperature models (Richardson et al., 1974). For chill quantification, the Dynamic model has been identified as the most sophisticated and best-adapted model for Mediterranean climate conditions (Fernandez et al., 2020; Luedeling and Brown, 2011). This two-step model relies on the assumption that low temperatures stimulate the production of a chill precursor compound (which is not mapped to a concrete biological phenomenon), which then needs to be converted into a permanent “chill portion” – a quantification of effective chill accumulation, by a process that is most effective at moderately cold temperatures (Erez et al., 1990; Erez and Couvillon, 1987; Fishman et al., 1987). However, this model is based on a hypothetical biological process.

To quantify heat accumulation, the Growing Degree Hours is the most widely used model. This model considers that only temperatures within a certain range (usually between 4º C and 25º C) contribute to plant reactivation and growth after the chill requirements have been fulfilled (Richardson et al., 1975). This model is not specific for the phenology of temperate fruit trees, but also used for phenological stages of annual crops and even insect growth. Chilling and forcing models are usually combined for the prediction of phenology (Anderson et al., 1986). This approach assumes that chilling and forcing are successive phases, although recent studies suggest that chill and heat interact with each other during part or the whole dormancy period (Darbyshire et al., 2016; Harrington et al., 2010; Pope et al., 2014). Even though this combination approach is extensively used, including in many recent studies, it is worth noting that it does not incorporate recent advances in dormancy research.

The chilling and forcing periods, during which chill and heat accumulations are quantified, can be determined experimentally or statistically. The experimental methodology establishes the minimum chill exposure that is needed for buds to recover the capacity to grow. Shoots are cut and transferred to a growth chamber with warm conditions throughout winter. Bud growth is then monitored after a certain time of exposure to the chamber environment (Brown and Kotob, 1957; Fadón and Rodrigo, 2018). This methodology can easily be adapted to different fruit species and regions by adjusting the shoot sampling frequency, parameters of the growth chamber (i.e. temperature and light regimes) and the time shoots spend in the chamber. Evaluation methods have varied across studies, with researchers observing either vegetative or flower buds, recording bud weights or bud phenology, and using different thresholds for the transition from endodormancy to ecodormancy. This variability in experimental designs has limited opportunities to compare results across studies (Fadón et al., 2020b).

The statistical methodology analyzes long-term phenology records by relating the phenological observations to daily temperatures during the previous months. Partial Least Squares (PLS) regression analysis has emerged as a suitable approach to determine the periods when cold or warm temperatures advance flowering dates (Luedeling et al., 2013; Luedeling and Gassner, 2012). The agroclimatic requirements of numerous temperate fruit tree species in several regions have been estimated using this technique, including apricot in China and the UK (Guo et al., 2015b, 2015a; Martínez-Lüscher et al., 2017), apple and almond in Spain (Delgado et al., 2021; Díez-Palet et al., 2019), sweet cherry in Germany and Spain (Fadón et al., 2021b; Luedeling et al., 2013), and pistachio and almond in Tunisia (Benmoussa et al., 2017b, 2017a). However, PLS regression has several prerequisites that limit its generalization to a broad range of species and climatic conditions. A particular constraint is the method’s dependence on the availability of long-term phenology records. Long-term records are needed for accurate delineation of the chilling and forcing periods, because the PLS methodology relies on sufficient variation in temperature signals throughout the dormancy phase, the effect of which on bloom timing is then evaluated (Luedeling and Gassner, 2012). However, in some regions, even with long-term data, periods that are commonly assumed to be important for chill contribution appear not to have significant effects on bloom timing in the outputs of PLS analysis (Delgado et al., 2021; Díez-Palet et al., 2019; Fadón et al., 2021b; Guo et al., 2014; Luedeling et al., 2013; Martínez-Lüscher et al., 2017).

We aimed to analyze temperature responses of sweet cherry cultivars in two growing areas with very different climatic conditions. To this end, we used PLS regression analysis to analyze long-term phenology observations of the same three sweet cherry cultivars grown in both locations. We examined the feasibility of delineating the chilling and forcing periods for each cultivar based on the combined data set. However, this analysis was unable to distinguish common chilling and forcing phases of each genotype for these two distinct conditions. Data were finally analyzed separately for each of the locations, revealing very different chilling and forcing periods across the two sites. This analysis provided insights on how the phenology of these cultivars varies across different climate conditions, which has implications for their ability to respond to rising temperatures caused by global warming.

Materials and Methods

Plant material, orchard locations and phenology monitoring

We evaluated flowering dates of three sweet cherry cultivars ('Samba', 'Burlat', and 'Sylvia') at two locations, Bonn in Germany and Zaragoza in Spain. Bonn is exposed to westerly Atlantic weather, tempered by the mild buffering climate of the Rhine valley to the east, resulting in an average annual temperature of 9.8°C. August is the hottest month with an average temperature of 17.9°C and January the coldest month at 2.2°C. Annual rainfall is around 600 mm (Blanke and Kunz, 2009). The experimental orchard is located at Campus Klein-Altendorf (50.62° N; 6.99°E; 160 m a.s.l.) (Fig.1), an experimental station of the University of Bonn. The soil is a loess loam (silty loam), with relatively high water retention capacity. Trees of the investigated cultivars were grafted on GiSelA 5 rootstocks and a Tall Spindle Axe (TSA) training system, with fully grown trees reaching a height of about 4 m. The orchard was occasionally irrigated with a sprinkler system.

Zaragoza features an Arid Cold steppe climate, classified as BSk on the Köppen scale (Köppen, 1900; Kottek et al., 2006). The temperature averages 15.6 °C, with July being the warmest month with an average temperature of 25°C and January the coldest month at 7°C (AEMET, 2021). The long-term mean of annual precipitation is about 362 mm. The soil is classified as fine-loamy and calcareous soil. Trees of the three investigated cultivars belong to the cultivar collection at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) (41.72º N; 0.81º W; 220 m a.s.l.) (Fig.1). The trees were grafted on Santa Lucia 64 rootstock (*Prunus mahaleb*) and pruned to form a Spanish Bush. Irrigation is essential, and the system used is traditional blanket (by gravity).

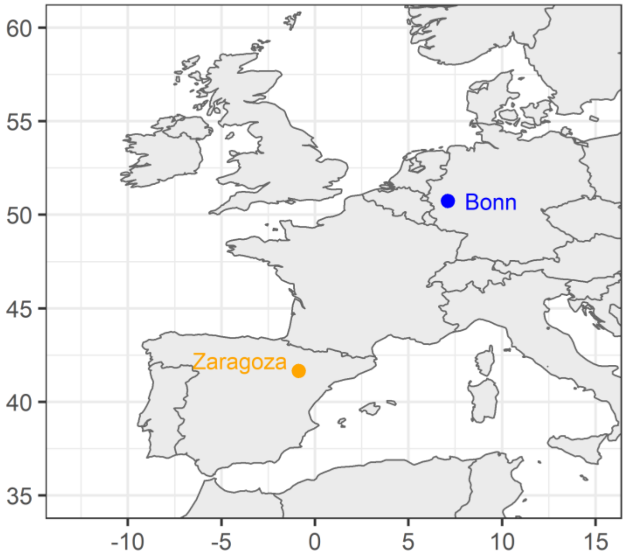


Figure 1. Location of Bonn, Germany and Zaragoza, Spain.

In Bonn, field observations were carried out every two to three days to record three phenological stages according to the BBCH code (Fadón et al., 2015; Meier, 2001). Recorded stages were stage 61 (beginning of flowering), stage 65 (full flowering), and stage 69 (end of flowering), corresponding with stages E, F, and G of the Baggliolini phenology code (Baggiolini, 1952). In Zaragoza, phenology was monitored every two days during spring.

Full bloom was recorded when the F stage of the Baggliolini phenology code (Baggiolini, 1952) was observed as the most frequent stage, corresponding with stage 65 of the BBCH code (Fadón et al., 2015; Meier, 2001). Across the two sites, 38 observations were available for 'Samba', 56 for 'Burlat' and 29 for 'Sylvia' (Table 1).

Table 1. Phenology record availability in Bonn and Zaragoza for the sweet cherry cultivars 'Samba', 'Burlat' and 'Sylvia'.

	Bonn (Klein-Altendorf)		**Zaragoza**	
	Nº years	**Years**	**Nº years**	**Years**
'Samba'	18	2003-2020	20	1993-2001, 2009-2019
'Burlat'	35	1978-1984, 1986-1995, 2002-2019	21	1991, 1993-2001, 2009-2019
'Sylvia'	12	2008-2012, 2014-2020	17	1996-2001, 2009-2019

Chill and heat quantification

Temperature records were obtained from meteorological stations located at the experimental site in Zaragoza (Spain) and at Campus Klein-Altendorf (Germany). Daily minimum and maximum temperatures have been recorded since the 1950s at Campus Klein-Altendorf and since 1990 in Zaragoza.

We used the recorded temperature data to estimate chill and heat accumulation during the respective periods of investigation at both locations. Chill accumulation was computed in Chill Portions (CP), according to the Dynamic Model (Erez et al., 1990; Erez and Couvillon, 1987; Fishman et al., 1987). The Dynamic Model assumes that chill accumulation results from a two-step process, which is mediated by a thermally labile precursor (not yet identified). In the first step, low temperatures stimulate the production of an intermediate product that can be destroyed by warm temperatures. In the second step, this intermediate product is converted into a permanent “chill portion” when a certain quantity has accumulated (for equations, see Luedeling et al., 2021).

Heat accumulation was quantified using the Growing Degree Hours (GDH) Model, a linear model with a base temperature of 4.5ºC and an upper limit of 25ºC. One GDH was defined as one hour at a temperature 1ºC above the base temperature (calculated by subtracting 4.5ºC from each hourly temperature between 4.5ºC and 25ºC), and all temperatures above 25 ºC were assumed equal to 25ºC (Richardson et al., 1974; Richardson et al., 1975; Anderson et al., 1986) (for equations, see Luedeling et al., 2021).

Chill accumulation was calculated over the chilling period defined by Partial Least Squares (PLS) regression (described below) across all years of observation. In a similar manner, heat requirements were quantified between the start of forcing and the flowering date. The final chill and heat requirements were calculated as the mean values across all years for each cultivar, with the standard deviations across all years providing an estimate of uncertainty.

Determination of chilling and forcing periods

Chilling and forcing periods were determined by applying Partial Least Squares (PLS) regression (Luedeling et al., 2013; Luedeling and Gassner, 2012). For this procedure to be successful, the PLS analysis requires some variation in the signal variables (i.e., chill and heat), which can be related to variation in bloom dates. We assessed this variation by relating the temperatures at the study sites to the temperature responses of chill and heat models. First, we produced a plot illustrating the response of the Dynamic Model and the GDH Model to a particular daily temperatures pattern, defined by daily minimum and maximum temperatures. Daily temperature variation patterns are latitude-specific since they are influenced by sunrise and sunset times. We selected an intermediate latitude (46º N) between Bonn and Zaragoza so that the differences in the temperature variation patterns were minimal. We then plotted the daily minimum and maximum temperatures grouped by month during the last 20 years (2000-2020) for both locations over the model response plots.

PLS regression analysis handles highly autocorrelated data better than most other regression approaches and can be used in situations where the number of independent variables substantially exceeds the number of dependent variables. In this case, we used PLS regression analysis to relate sweet cherry bloom dates (one observation per year) to a much larger number of observations of daily accumulated CP and GDH over the previous 8 months of each year. We ran all analyses with 11-day running means of daily CP or GDH to facilitate interpretation of the results.

To delineate the chilling and forcing periods, we examined the PLS regression outputs, considering both the model coefficients and the variable-importance-in-the-projection (VIP) scores. Model coefficients indicate the strength and direction of the influence of temperature on particular dates on bloom dates, whereas VIP values signal for each variable whether it makes an important contribution to the PLS prediction model. We considered all variables with VIP scores greater than 0.8 as important. To identify the chilling period, we examined PLS outputs for extended periods where model coefficients assigned to daily chill accumulation were negative and variables exceeded the VIP threshold. Such outputs mean that early bloom dates are associated with high chill accumulation during the respective periods – a property that is characteristic of the endodormancy phase. For delineating the forcing phase, we used an analogous procedure, except that we focused on negative model coefficients for heat rather than chill accumulation. High heat accumulation during the identified periods is associated with early bloom dates, a pattern that is characteristic of the ecodormancy period.

All analyses were performed using the chillR v.0.70.24 package (Luedeling, 2019) in the R v.4.0.1 programming environment.

Flowering response to temperature variation during the chilling and forcing periods

We characterized the relationship between flowering dates and temperature variation during the chilling and forcing periods for each cultivar and study site. To do so, we plotted the flowering dates as a function of the mean temperature during the chilling and forcing periods identified by the PLS procedure. We used the Kriging technique to interpolate a continuous surface of flowering dates (Guo et al., 2015a; Martínez-Lüscher et al., 2017). Kriging is a Gaussian regression method commonly used in spatial statistics to estimate values at locations where no data are available (Oliver and Webster, 1990). This illustration facilitated the interpretation of responses of flowering dates to multiple climatic drivers. We also demonstrated the direction and magnitude of the effects of chilling and heat accumulation on flowering dates (Guo et al., 2015a).

Results

Flowering periods of the sweet cherry cultivars 'Burlat', 'Samba', and 'Sylvia' in Bonn (Germany) and Zaragoza (Spain)

The sweet cherry cultivars 'Burlat', 'Samba', and 'Sylvia' are successfully cultivated in the North Rhine region (Bonn, Germany) and in the Ebro valley (Zaragoza, Spain). Over the study periods, flowering dates in Bonn occurred between March 22nd and May 1st, while in Zaragoza, the flowering time ranged from March 12th to April 10th, occurring approximately 10-20 days earlier than in Bonn (Fig. 2A). In both locations, the earliest-flowering cultivar was 'Samba' and the latest was 'Sylvia'.

The order of flowering times across cultivars was consistent in both locations, but bloom occurred earlier in Zaragoza where mean temperatures were about 5ºC higher than in Bonn (Fig. 2B). The temperature differences led to location-specific patterns of daily accumulation of agroclimatic metrics. Chill accumulation started about one month later in Zaragoza than in Bonn, reaching its maximum rate of about 0.75 CP per day during the coldest months (December, January, and February). It is worth noting that despite its warmer climate, chill accumulation in Zaragoza reached higher and more stable levels than in Bonn (Fig. 2C). Spring heat started to accumulate about one month earlier in Zaragoza than in Bonn (Fig. 2D).

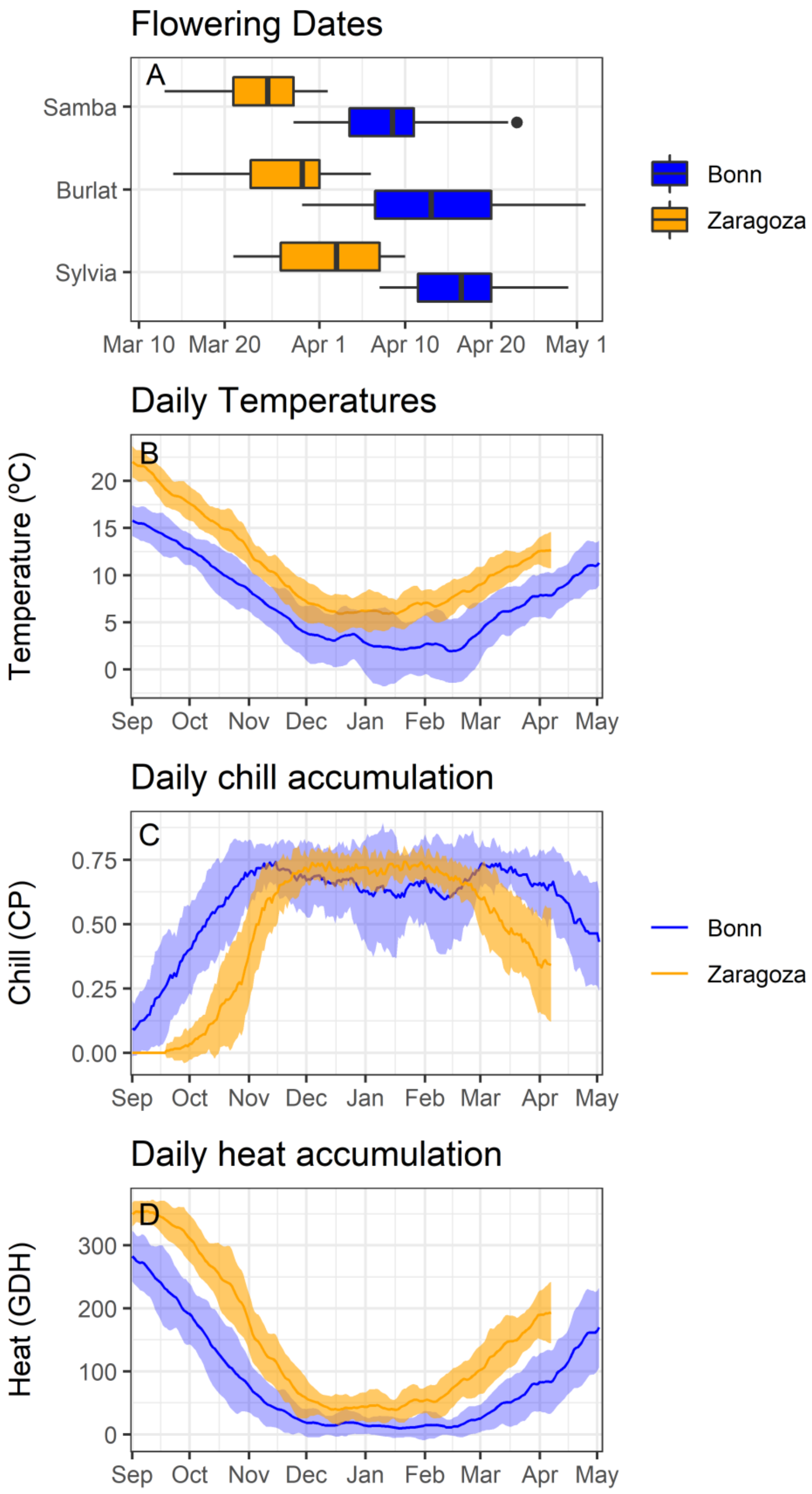


Figure 2. Flowering dates of three sweet cherry cultivars (A) and climatic conditions at Bonn and Zaragoza, including temperatures (B), chill accumulation quantified as Chill Portions - CP (C) and heat accumulation quantified as Growing Degree Hours - GDH (D). In B, C and D, lines indicate means and shaded areas signify standard deviations.

Chilling and forcing periods and agroclimatic requirements

We used PLS regression analysis with daily chill and heat accumulation to delineate the chilling and forcing phases. We first assessed the variation in the signal variables (i.e., chill and heat), which is a prerequisite for the PLS analysis to be sensitive to these metrics (Fig. 3). The months that appeared to have daily minimum and maximum temperatures that resulted in a gradient of chill accumulation were October, November, February, and March in Zaragoza, and October, January, and February in Bonn. We expected PLS to produce useful results for these months, because recorded temperature regimes included both temperature conditions that are effective and conditions that are not effective for chill accumulation (too warm or too cold). With this meaningful variation we expected PLS to produce useful results.

In contrast, months that strongly contributed to chill accumulation, i.e. December and January in Zaragoza, and November and December in Bonn, presented temperature values within a temperature range that was constantly highly effective for chill accumulation. A consistently high daily accumulation resulted in very little variation that could be exploited by PLS analysis. However, considering both locations together, a wider range of daily chill accumulation was covered, which suggested a more accurate delineation of the chilling phase. For daily heat accumulation, there was wide variation in October, November, February and March, both in Zaragoza and in Bonn.

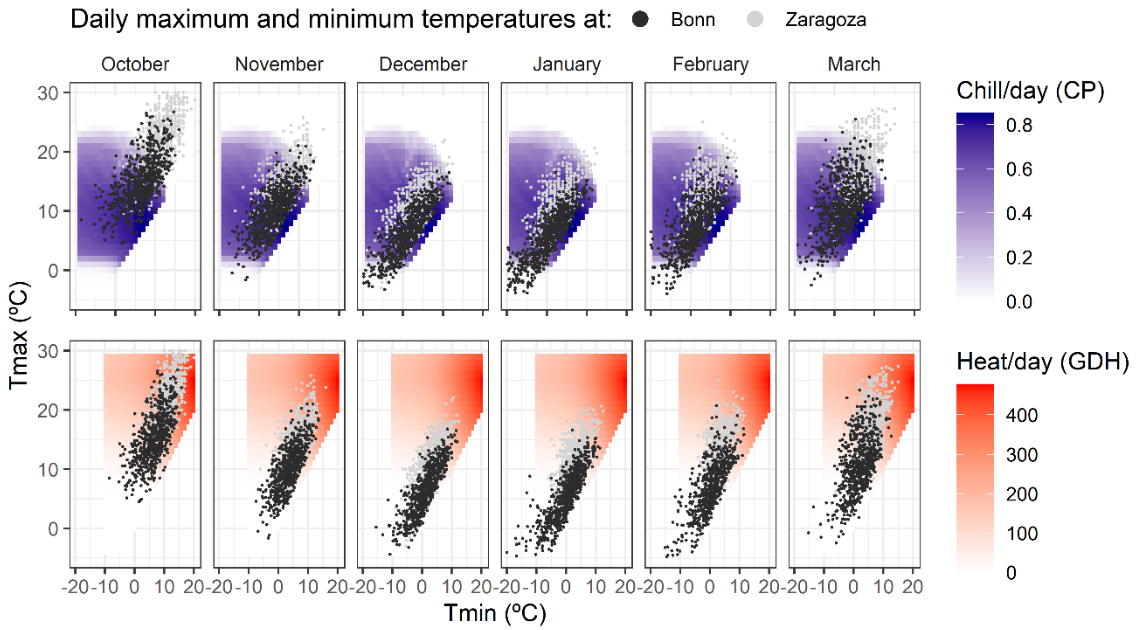


Figure 3. Daily maximum and minimum temperatures grouped by month during the last 20 years (2000-2020) for Zaragoza (light grey) and Bonn (dark gray) in relation to daily chill (CP) and heat (GDH) accumulation (indicated by blue and red gradients, respectively), according to daily minimum and maximum temperatures simulated for a location between Zaragoza and Bonn (at 46º N).

Considering our analysis of chill and heat model sensitivity in both locations, we ran PLS analyses for each cultivar combining the data from both sites. However, the outcomes appeared to show a continuous forcing period throughout the winter that did not allow distinguishing between chilling and forcing phases. This effect can probably be explained by the substantial temperature differences between the locations, with temperatures always being higher and bloom dates always earlier in Zaragoza than in Bonn. Across the whole dataset, this situation likely led to a strong association between warm conditions in any season and early bloom dates, manifesting in negative model coefficients for almost all days during the dormancy season. Since a joint analysis of the two datasets did not produce useful results, we analyzed the data from the two locations separately to determine the chilling and forcing periods of the three cultivars.

PLS regression analysis resulted in much longer chilling periods in Bonn than in Zaragoza, with average durations of 148 and 106 days, respectively (Fig. 4). In Zaragoza, the typical chilling period ranged from October 2nd to January 22nd for ‘Burlat’ and ‘Samba’. The cultivar ‘Sylvia’ had a shorter chilling phase, from October 6th to January 7th (Fig. 4A). Chill accumulations during these periods were 59 ± 5 CP for ‘Burlat’ and ‘Samba’, and 48 ± 4 CP for ‘Sylvia’. The typical forcing periods started on February 2nd – 3rd and ended at the flowering time for each year (Fig. 4B). Heat accumulation was 5945 ± 570 GDH for ‘Burlat’, 5444 ± 544 GDH for ‘Samba’, and 6988 ± 659 GDH for ‘Sylvia’ (Table 2). The typical forcing period did not follow directly after the chilling period but was preceded by a transition phase, during which model coefficients were neither clearly negative nor clearly positive, and they were not considered important by the VIP analysis. This transition period lasted 12 days for ‘Burlat’ and ‘Samba’, and 26 days for ‘Sylvia’. In Bonn, typical chilling periods ranged from Oct 23rd to Feb 24th for ‘Burlat’, from Oct 2nd to Mar 10th for ‘Samba’, and from Oct 4th to Mar 5th for ‘Sylvia’ (Fig. 4A). Chill accumulation was 87 ± 6 CP for ‘Burlat’, 105 ± 6 CP for ‘Samba’, and 101 ± 6 CP for ‘Sylvia’ (Table 2). Typical forcing periods in Bonn started between February 7th and 15th and lasted until flowering time (Fig. 4B). Heat accumulation was 3273 ± 548 GDH for ‘Burlat’, 3233 ± 546 GDH for ‘Samba’, and 4343 ± 435 GDH for Sylvia’ (Table 2). On average, the chilling and forcing phases overlapped by around 22 days.

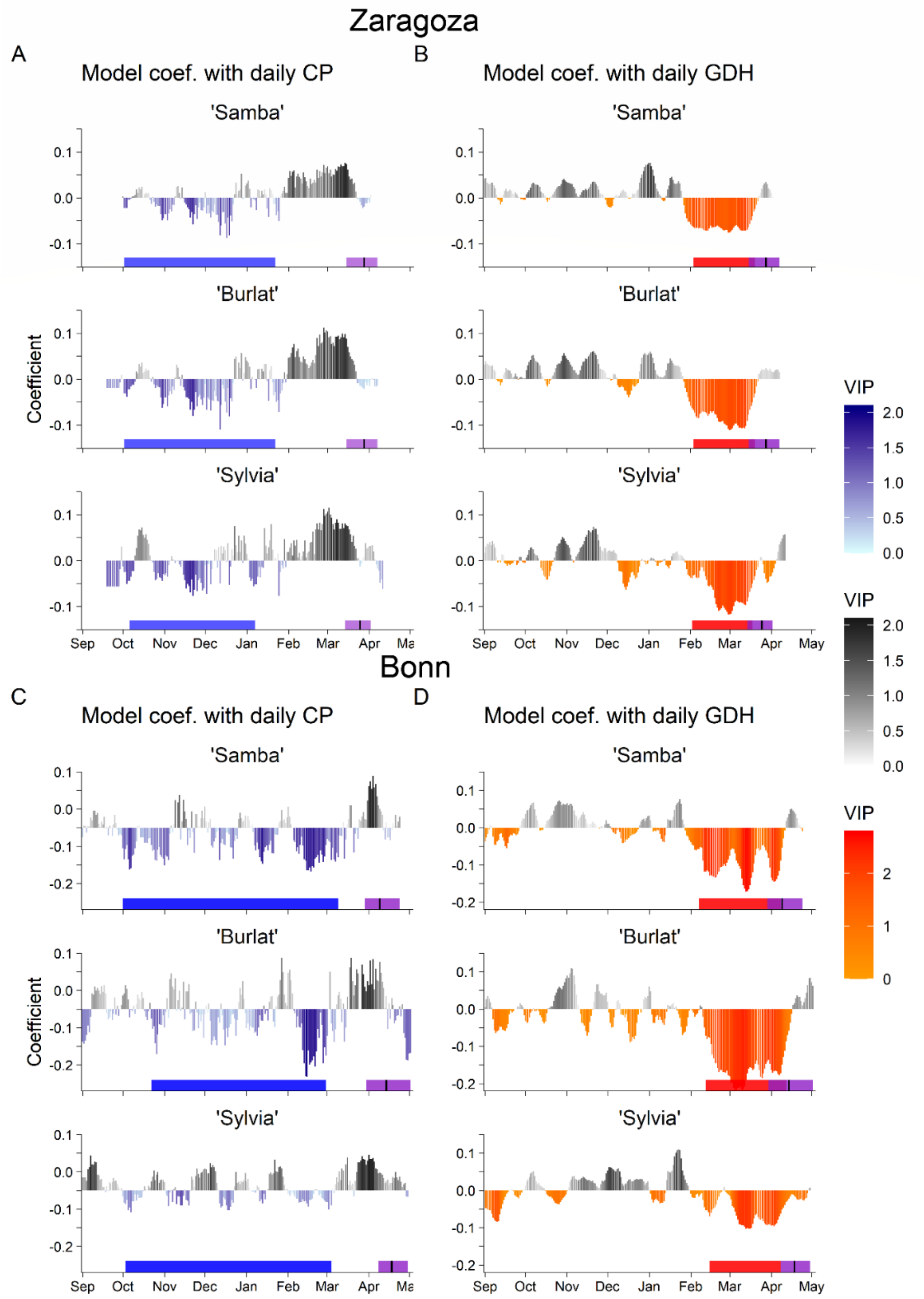


Figure 4. Partial Least Squares regression model of daily accumulation of winter chill (according to the Dynamic Model), daily accumulation of heat (according to the GDH Model), and flowering dates of three sweet cherry cultivars grown in Zaragoza, Spain **(A and B)** and Bonn, Germany **(C and D)**. Bar colors indicate the Variable Importance in the Projection (VIP) (gray scale for positive coefficients, and blue and red scales for negative coefficients for chill and heat, respectively). Rectangles at the bottom of each plot indicate the delineated chilling period (blue), the forcing period (red) and the range of observed flowering dates (purple), for which the median is indicated by the dark purple line.

Table 2. Chilling and forcing periods and temperature requirements of three sweet cherry cultivars grown in Zaragoza and Bonn, derived by PLS regression analysis.

	Zaragoza			
	Chilling		**Forcing**	
Cultivar	**Period**	**Accumulation**	**Period**	**Accumulation**
Samba	Oct 3 – Jan 22	59 ± 5	Feb 3 – flowering	5444 ± 544
Burlat	Oct 3 – Jan 22	59 ± 5	Feb 3 – flowering	5945 ± 570
Sylvia	Oct 7 – Jan 7	48 ± 4	Feb 2 – flowering	6988 ± 659
	Bonn			
	Chilling		**Forcing**	
Cultivar	**Period**	**Accumulation**	**Period**	**Accumulation**
Samba	Oct 2 – Mar 10	105 ± 6	Feb 7 – flowering	3233 ± 546
Burlat	Oct 23 – Feb 24	87 ± 6	Feb 12 – flowering	3273 ± 548
Sylvia	Oct 4 – Mar 5	101 ± 6	Feb 15 – flowering	4343 ± 435

Flowering response to chill and heat accumulation

Once the chilling and forcing periods had been identified, we analyzed temporal trends of chill and heat accumulation, as well as flowering dates (Fig. 5). Chill accumulated during the typical chill period in Zaragoza was about 56 ± 5 CP, tending to decrease by 1.3 CP per decade (Fig. 5A). In Bonn, 100 ± 8 CP were accumulated during the chilling period (Fig. 5B), almost twice the chill amount recorded in Zaragoza, showing no clear trend over time. During the respective forcing period, about 6404 ± 1459 GDH were accumulated in Zaragoza (Fig. 5C), while less heat was accumulated in Bonn (2908 ± 1255 GDH; Fig. 4D). Heat accumulation in the two locations showed opposite trends, which affected phenology. While heat accumulation decreased by 200 GDH per decade in Zaragoza, Bonn showed an increase of 180 GDH per decade (1960-2020). In Zaragoza, the cultivars ‘Burlat’ and ‘Samba’ did not show any change in the flowering dates, but bloom of the late flowering cultivar ‘Sylvia’ has been delayed by 2 days per decade (Fig. 5E). In Bonn, flowering dates have advanced about 3 - 5 days per decade for all three cultivars (Fig. 5F).

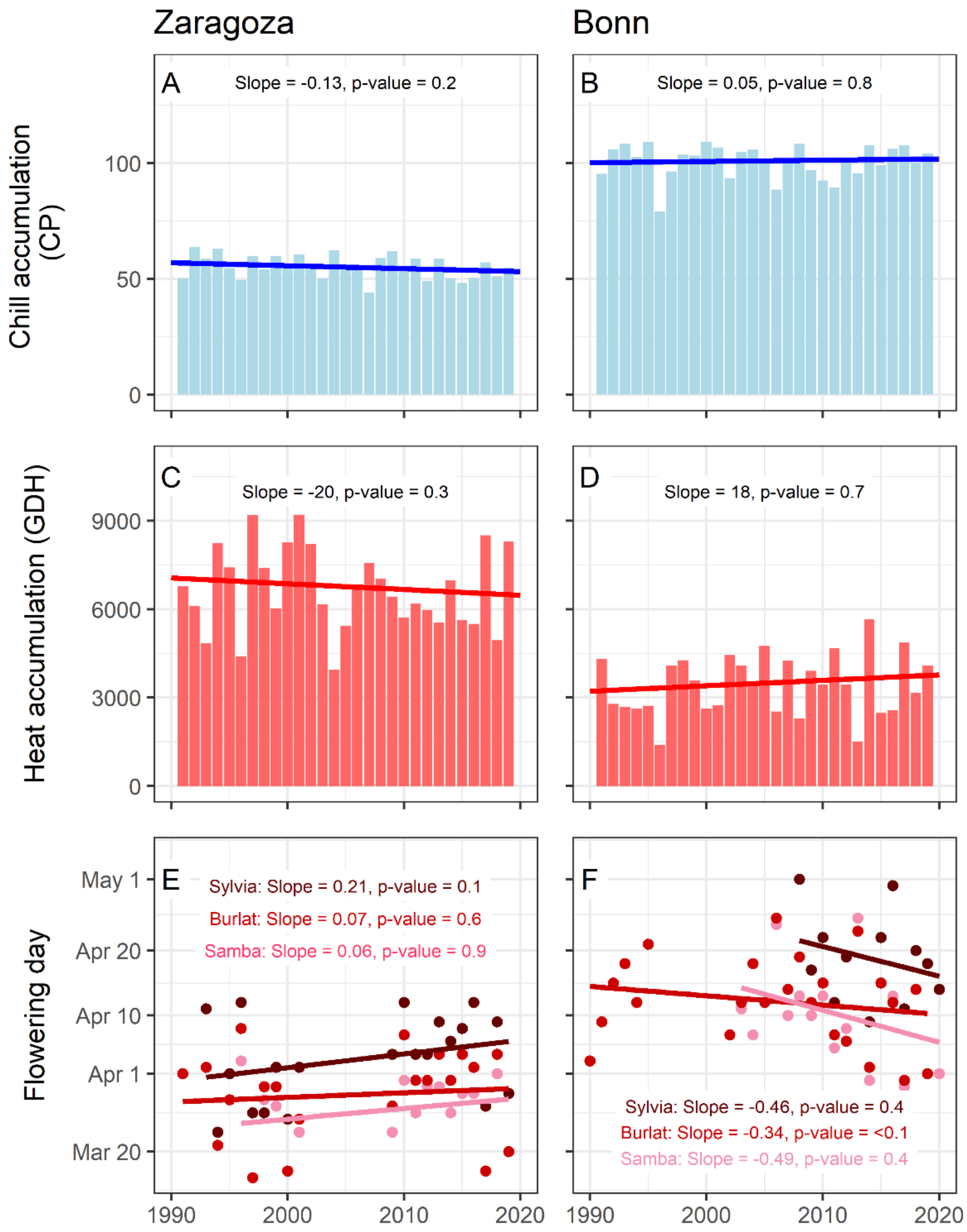
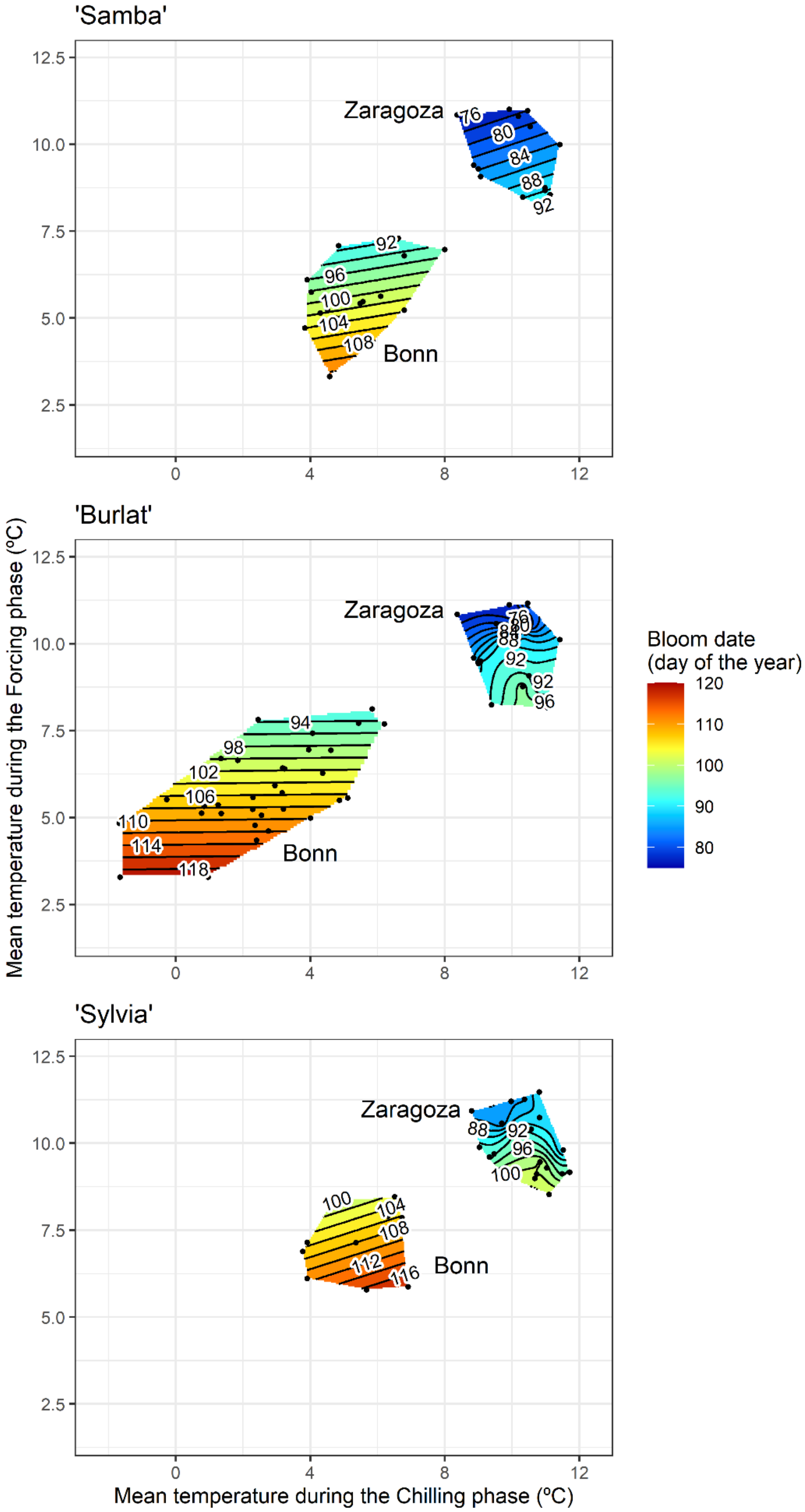


Figure 5. Annual chill accumulation according to the Dynamic Model (in Chill Portions; CP) during the typical chilling period in Zaragoza, Spain (A; chill period: October 1st – January 17th), and Bonn, Germany (B; chill period: October 1st – March 5th), heat accumulation (in Growing Degree Hours; GDH) during the typical forcing period in Zaragoza (C; forcing period: February 3rd – March 28th) and Bonn (D; forcing period: February 11th – April 14th), and flowering dates of three sweet cherry cultivars in Zaragoza (E) and Bonn (F). Colored lines indicate linear trends across datasets shown in the colors of the respective lines, p-values were calculated according to the Kendall test.

Each cultivar presented a location-specific flowering response to chill and heat accumulation. To gain insight on differences between the temperature conditions in Bonn and Zaragoza, we computed flowering date as a function of the mean temperature during the chilling and forcing periods (Fig. 6). In Bonn, the three cultivars showed response surfaces with relatively flat contour lines, indicating that conditions during the forcing phase were the main determinant of flowering dates. The trend in Zaragoza was less clear, but warm chilling phases appeared to exert a bloom-delaying effect that partly compensated the bloom-advancing effect of high forcing temperatures.



'Samba'
Zaragoza
76
80
84
88
92
92
96
100
104
108
Bonn
'Burlat'
Zaragoza
76
80
84
88
92
92
96
94
98
102
106
110
114
118
Bonn
'Sylvia'
Zaragoza
88
92
96
100
100
104
108
112
116
Bonn
12.5
10.0
7.5
5.0
2.5
0
4
8
12
Mean temperature during the Forcing phase (ºC)
Mean temperature during the Chilling phase (ºC)
Bloom date (day of the year)
120
110
100
90
80

Figure 6. Flowering dates as a function of mean temperatures during the chilling and forcing periods as determined by PLS analysis, for the sweet cherry cultivars ‘Samba’, ‘Burlat’, and ‘Sylvia’, in Zaragoza (Spain) and Bonn (Germany). Colors and contour lines represent predicted flowering dates based on observed flowering dates (black dots).

Discussion

The PLS analysis and temperature models were adequate for delineating agroclimatic requirements for flowering of all cultivars in both locations. However, the major differences obtained between locations contradict the commonly held belief that agroclimatic requirements are cultivar-specific traits and genetically determined. These results suggest that the current methodology, applied with the set of models used in this study, is useful for phenology prediction within narrow climatic settings, but it may not produce consistent results across wide temperature gradients.

Site-specific agroclimatic requirements may indicate inaccuracies of agroclimatic models

We observed genetically identical cultivars grown under different climatic conditions. Our results showed that in the cooler region (Bonn, Germany), exposure to chill over a long period (from October to March), followed by relatively low exposure to heat (from February to April) before flowering, promoted an advance in flowering time. In the warmer region (Zaragoza, Spain), the bloom-advancing effect of chill accumulation lasted over a shorter period, and buds flowered after high heat exposure. This resulted in very different estimates of agroclimatic requirements in both locations, which may hint at site-specific instead of cultivar-specific climatic needs. Site-specificity of agroclimatic requirements has also been observed in multi-site experimental studies in Mediterranean climates (Campoy et al., 2012; Gannouni et al., 2017; Viti et al., 2010). Whether these results truly contradict the widely held belief of genetically determined, cultivar-specific chilling requirements is difficult to say, however, because inaccuracies in the agroclimatic models may also introduce major errors in inter-site comparisons (Fernandez et al., 2020).

Our analysis indicated that the cultivar ‘Burlat’ accumulated 59 ± 5 CP in Zaragoza (Spain), which is substantially more than an earlier estimate of 38 ± 4 CP that was derived experimentally at the same location (Fadón et al., 2021a). Conversely, we determined lower heat requirements than those indicated experimentally (5945 ± 570 GDH vs 9345 GDH) (Fadón et al., 2021a). These differences can be explained by differences in methodology. Experimentally derived estimates reflect minimum chill accumulation for flowering, whereas PLS regression analysis is based on identifying periods during which chill has a bloom-advancing effect (Luedeling and Gassner, 2012). Other estimates for 'Burlat' derived with experimental methodology were 48 CP in Bordeaux (southern France) (Campoy et al., 2019), and 86 CP in Murcia (southern Spain) (Alburquerque et al., 2008). The latter result is in line with what we obtained in Bonn (Germany), 87 ± 6 CP, except that the heat requirement was much higher in Murcia (8750 GDH) (Alburquerque et al., 2008) than in Bonn (3273 ± 548 GDH). This wide range of estimates for observations in different climatic settings may indicate that the ability of both models to approximate the agroclimatic conditions experienced by fruit trees differs strongly between the locations.

For our study location near Bonn, PLS regression analysis has been used previously to determine the agroclimatic requirements for the cherry cultivar 'Schneiders späte Knorpelkirsche' (Luedeling et al., 2013). The estimated chilling requirement that resulted from this work was 45.7 ± 5.4 CP, a much lower value than for the cultivars analyzed in the present study (between 87 ± 6 CP and 105 ± 6 CP). Estimated heat requirements were similar, with our results ranging between 3233 ± 546 GDH and 4343 ± 435 GDH, while previous studies indicated 3473 ± 1236 GDH (Luedeling et al., 2013).

Failure of PLS regression to delineate temperature response phases for multi-location dataset

PLS regression analysis has emerged as an established method to determine the chilling and forcing periods in temperate fruit trees (Benmoussa et al., 2017b, 2017a; Delgado et al., 2021; Díez-Palet et al., 2019; Fadón et al., 2021b; Guo et al., 2015b; Martínez-Lüscher et al., 2017). Since an appropriate level of interannual variability of input data is needed for the analysis to be responsive to temperature signals, this analysis requires long-term phenology records, with early estimates indicating a need for about 20 years of data (Luedeling et al., 2013; Luedeling and Gassner, 2012). Our preliminary analysis showed that daily temperatures resulted in maximum chill accumulation with very little variation during some winter months. This impression was later confirmed by PLS analysis, which failed to identify model coefficients for days during these months as important. Similar PLS coefficient patterns have been observed in previous studies in temperate regions, confirming that the analysis is not sensitive to conditions during the colder winter months in some regions (Delgado et al., 2021; Díez-Palet et al., 2019; Fadón et al., 2021b; Guo et al., 2014; Luedeling et al., 2013; Martínez-Lüscher et al., 2017). With the intention of overcoming this limitation, we combined data from two locations, with the resulting dataset presenting variable chill and heat accumulation during all winter months. We realized, however, that, at least in this particular combination of locations, the temperature difference between sites was so large that it led to a strong correlation between high temperatures during all months and early bloom dates. This was simply because bloom of all cultivars consistently occurred earlier in Zaragoza, which features warmer conditions than Bonn throughout the whole winter. A certain level of similarity between locations would be needed for PLS analysis to produce results that can be interpreted with regard to chilling and forcing phases. The progression of dormancy should be largely similar during all years, which implies that observational records should be obtained from the same location, or at least from sites with very similar climatic conditions (Luedeling and Gassner, 2012). In this context, it may even be possible that strong warming signals within long time series may compromise the effectiveness of PLS analysis, even for records from a single location.

Phenology response to global warming is location-specific

In Bonn, sweet cherry flowering advanced in recent years, which is in agreement with previous studies that also found such a trend in response to global warming (Chmielewski et al., 2004; Grab and Craparo, 2011; Legave et al., 2013; Martínez-Lüscher et al., 2017). In Zaragoza, in contrast, the late flowering cultivar ‘Sylvia’ showed a delay in flowering time, while the two early flowering cultivars ‘Burlat’ and ‘Samba’ showed no trend. Phenology delays have been observed in temperate nut tree species in Tunisia (Benmoussa et al., 2017b, 2017a). The flowering response to rising temperatures has been described as species-specific (Diggle and Mulder, 2019) and also site-specific (Guo et al., 2015a).

The site-specific response observed in this work relied both on the agroclimatic trends over time and on the relative importance of chill and heat accumulation during the flowering period. We observed opposite phenomena in the two locations. In Bonn, flowering time depended almost exclusively on heat accumulation during the forcing period. Rising temperatures mainly resulted in rising spring heat accumulation, which explained the flowering advances. In Zaragoza in contrast, chill and heat accumulation decreased and the flowering time appeared to depend mainly on conditions during the chilling period, with increasing temperatures resulting in flowering delays. Such site-specific influences of the chill and heat periods on flowering times have also been described for apricots in China (Guo et al., 2015a).

Conclusions

Certain genotypes of sweet cherry thrive in growing regions with very different climatic conditions, displaying differences between locations in their responses to rising temperatures. However, the methodology applied here proved insufficient for accurate capture of phenology responses to temperature. Determining crop viability in different regions or predicting the effect of global warming on phenology is difficult, because such projections usually include conditions that are outside the range of settings in which agroclimatic requirements were estimated. To gain additional clarity on how cultivars in different places respond to future warming, efforts are needed to improve and standardize methods to characterize agroclimatic needs (both empirically and statistically) and refine the models used to quantify them. The recently developed “PhenoFlex” modeling framework parameterizes the Dynamic and GDH models and includes a sigmoidal transition between endodormancy and ecodormancy periods (Luedeling et al., 2021). A still more ambitious objective would be the development of a truly process-based dormancy model based on the best available knowledge on dormancy physiology and the effect of winter temperatures on flowering, yet such a model remains elusive.

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