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Agroclimatic Requirements of Traditional European Pear (*Pyrus communis* L.) Cultivars from Australia, Europe, and North America

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Abstract: Flowering in temperate fruit trees depends on the temperatures during the previous months; chill is required to overcome endodormancy, and then heat exposure is needed. These agroclimatic requirements are cultivar-specific and determine their adaptability to the growing area and their response to climate change. We aim to estimate the agroclimatic requirements of 16 traditional cultivars of European pears grown in Zaragoza (Spain). We used Partial Least Squares regression analysis to relate 20-year records of flowering dates to the temperatures of the 8 previous months. This approach allowed us to establish the chilling and forcing periods, through which we quantified temperatures with three models for chill accumulation (Chilling Hours, Utah model, and Dynamic model) and one model for heat accumulation (Growing Degree Hours). The results indicated very little difference in the chilling and forcing periods. Chill requirements ranged from 43.9 to 49.2 Chill Portions; from 1027 to 1163 Chilling Units; and from 719 to 774 Chilling Hours. Heat requirements ranged from 6514 to 7509 Growing Degree Hours. Flowering dates were mainly determined by the temperatures during the chilling period. This means that reductions in winter chill caused by global warming in many regions could cause flowering delays or even failures in the fulfillment of chill requirements.

Keywords: chill requirements; Chilling Hours; Chilling Units; Chill Portions; chillR; dormancy; Growing Degree Hours; heat requirements; Partial Least Squares (PLS) regression

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1. Introduction

The flowering time of temperate fruit trees is highly dependent on temperatures during the winter dormancy period [1,2]. Dormancy allows survival in low winter temperatures, and is characterized by the absence of visible growth. It is traditionally divided into three phases. Prior to winter, the newly formed buds are limited in their growth by the influence of other structures of the plant. Once winter arrives, growth capacity is inhibited by internal factors while chill requirements are fulfilled: endodormancy. Then, during ecodormancy, warm temperatures are required for the resumption of growth (heat requirements), leading to flowering [3]. Agroclimatic requirements are cultivar-specific and determine flowering time. Despite their importance in predicting cultivar adaptation to different growing regions, the information available is limited for many fruit tree species and cultivars [4].

In recent years, dormancy and phenology modeling have experienced a renewed interest due to climatic changes in growing regions caused by global warming [2,5]. Rising temperatures are particularly detrimental during the winter [6–9], which leads to alterations in phenological cycles. This fact may compromise the productivity of traditional cultivars, although they have performed well in their growing areas so far. Growers should act to adapt orchards to these new conditions, but the lack of information on agroclimatic requirements makes it difficult to select cultivars adapted to less winter chilling.

The estimation of the agroclimatic requirements consists of determining the periods of endo- and ecodormancy, and the subsequent quantification of chill and heat during these periods. The cumbersome methodologies to determine the dormancy phases and the low accuracy of the temperature models in approximating the real behavior of trees are the key factors of this lack of information [10,11]. Chilling and forcing periods can be determined experimentally or statistically [4]. The experimental determination of the dormancy phases consists of evaluating when the flower buds recover the capacity to grow. For this, bud growth is characterized by shoots sampled sequentially during the winter and subjected to mild conditions in a growth chamber [12–15]. The limitations of this approach are the high variability in the experimental designs and that chill exposure depends on the climatic conditions of the orchard, which makes it difficult to compare results from experiments carried out on different sites. On the other hand, the statistical methodology consists of relating long-term phenology records of flowering dates to daily temperatures during the previous months. Among the statistical methods, Partial Least Squares (PLS) regression has emerged as a suitable statistical approach to delineate the most probable periods for chill and heat accumulation in temperate species [16–18].

The available information on the agroclimatic requirements of European pear (*Pyrus communis* L.) cultivars is especially scarce when compared to other fruit tree species. The agroclimatic requirements have been studied more extensively in stone fruits (*Prunus* sp.) than in pome fruits. Early experimental methodology was described in stone fruits in the middle of the 20th century [13,19–21] and has been applied extensively since, even to pome fruits [14,15]. In pome fruits, agroclimatic requirements have been reported mainly in apple (*Malus domestica* L.) [22,23], European pear [24], and Japanese pear (*Pyrus pyrifolia* L.) [25]. PLS regression analysis has recently been used for both stone fruits [18,26–28] and apple [29,30].

The European pear experienced a great advancement in breeding during the 18th and 19th centuries. Breeding programs in North America, where pear was introduced by early French and English settlers, bred for fire blight resistance and cold hardiness using crossings with other *Pyrus* species [31,32]. Some of these released cultivars, as well as others selected in Belgium, France, and England, are still widely cultivated [33].

The aim of this work is to determine the agroclimatic requirements for flowering of a group of traditional pear cultivars from Australia, Europe, and North America. We used Partial Least Squares (PLS) regression to correlate 20 years of phenology records with the daily accumulation of chill and heat during the months preceding flowering to delineate the chilling and forcing phases. This allowed us to estimate the chill requirements using three chill models (Chilling Hours, Utah, and Dynamic), and the heat requirements using the Growing Degree Hours model.

2. Materials and Methods

2.1. Plant Material and Monitoring of Phenology

Flowering dates were evaluated in 16 pear cultivars from an experimental collection at CITA (Centro de Investigación y Tecnología Agroalimentaria de Aragón), sited in Zaragoza (Spain), at 220 m above sea level and 41°44′30″ N, 0°47′00″ W. The trees were managed in accordance with standard horticultural practices. We studied traditional cultivars obtained from breeding programs in Europe (France, the United Kingdom, and Belgium), the USA, and Australia, which were released from the mid-19th century to the beginning of the 20th century (Table 1). Flowering dates were recorded over 20 years (1986–1989, 1992–2007, 2022). The missing years do not affect the analysis, since phenology is related to the temperatures of the year before. Phenology was monitored every other day during

March and April. For each cultivar on each monitoring day, we recorded three stages of flower development: the earliest, the most advanced, and the most frequent stage, according to the phenological scale of Fleckinger [34]. Full flowering was considered when the most frequent stage was the F stage (full bloom), which corresponds with BBCH stage 65 [35,36]. For each year and cultivar, the median full flowering was used in the analysis as full flowering lasted for several days.

Table 1. Pear cultivars used in this study: country of origin, cultivar name, release year, and breeding program [37,38].

Country	**Cultivar**	**Release Year**	**Breeder/Breeding Program**
Australia	Packham's Triumph	1897	Sam Packham (New South Wales)
France	Alexandrine Douillard	1849	Constant Douillard (Nantes)
	Beurré d´Anjou	1819	
	Général Leclerc	<1950	Fruit Research Station of Angers
	Passe Crassane	1845	Louis Boisbunel (Rouen, Normardie)
	Président Drouard	1876	
	Président Héron	1894	M. Arsene Sannier (Rouen, Normardie)
	Pierre Corneille	1894	
United Kingdom	Conference	1885	Thomas River (Rivers Nursery, Sawbridgeworth)
USA	Grand Champion	1936	
	El Dorado	1925	Seedling (Placerville, California)
	Highland	1974	Robert C. Lamb (New York State Agriculture Experiment Station, Geneva)
	Magness	1960	Howard J. Brooks (United States Department of Agriculture, Beltsville, Maryland)
	Rogue Red	1969	F.C. Reimer, E. Degman, and V. Quackenbush (Oregon State University)
	Sirrine	1954	F. Atwood Sirrine
	Star	1968	

2.2. Delimitation of Chilling and Forcing Periods

Partial Least Squares (PLS) regression analysis was used to determine chilling and forcing periods [16,17]. PLS regression allowed the relation of pear flowering dates with the daily accumulation of chill (in Chill Portions—CP) and heat (in Growing Degree Hours—GDH) that occurred during the 8 months preceding flowering. All analyses were implemented using an 11-day running mean function, which was applied to both daily chill and heat accumulation to facilitate the interpretation of the results. PLS regression analysis produces two main outputs: the model coefficients, which indicate the strength and the direction of the influence, and the variable importance in the projection (VIP) scores, which highlight the importance of each independent variable in a PLS regression model [16,17]. To delineate the chilling and forcing periods, we examined the PLS regression outputs, looking for extended and consistent periods of negative model coefficients for chill and heat accumulation. In both cases, negative model coefficients during the delineated period would indicate that higher levels of chill and heat are associated with earlier flowering dates. We later used both delineated periods to estimate the agroclimatic requirements.

2.3. Determination of Chill and Heat Requirements

Daily temperatures (maximum and minimum temperatures) were registered in a meteorological station placed in the experimental orchard [39]. Hourly temperatures, required for estimating agroclimatic metrics, were derived from an idealized daily temperature curve with the functions from Almorox et al. (2005) [40] and Linvill (1990) [41], implemented in the chillR package for R [42]. This curve depends on the latitude of a place and consists of a sine function for daytime warming and a logarithmic decay function for nighttime cooling.

We computed chill accumulation for the chilling period that was delineated through PLS regression. To this end, we applied the three models (Chilling Hours, Utah, and Dynamic) that are widely used in dormancy-related studies as well as in temperate orchard management. A “Chilling Hour” (CH) is defined as one hour within a temperature range between 0 and 7.2 °C, according to the Chilling Hours model [43]. “Chilling Units” (CU) are computed using different chill effectiveness weights corresponding to various temperature ranges according to the Utah model [44]. “Chill Portions” (CP) are accumulated through a two-step process, in which a chilling precursor is formed in cool conditions and later converted to a permanent CP through a subsequent process that shows optimal effectiveness at mild temperatures, according to the Dynamic model [45].

To estimate the heat requirements, heat accumulation was quantified between the start of the forcing phase delineated through PLS regression and the flowering date for each year. For this analysis, we used the Growing Degree Hours (GDH) model, which considers temperatures between 4 °ºC and 25 °C as contributing to active growth [46].

The final chill and heat requirements for each cultivar were aggregated by computing the mean values across all years used in the PLS regression analysis. Additionally, we computed the standard deviation to provide an estimate of uncertainty around the mean.

2.4. Chill and Heat Accumulation and Flowering Response

Flowering dates were plotted against the mean temperatures during both the chilling and forcing periods to determine the relationship between flowering dates and the accumulation of chill and heat. The Kriging method was used to interpolate a continuous surface of flowering dates that represented the timing of flowering as a function of mean temperature during the chilling and forcing periods [26,47]. We then visualized the assessed relationship through a surface contour plot.

3. Results

3.1. Chilling Period Delineation

Full flowering dates ranged from mid-March to mid-April for the 16 traditional pear cultivars analyzed (Figure 1 and Table S1). The flowering dates were concentrated between 20 March and 8 April; ‘Pierre Corneille’ was the earliest cultivar and ‘Grand Champion’ was the latest. The variability of the flowering dates for each cultivar depends on the variability of the inter-annual temperatures.

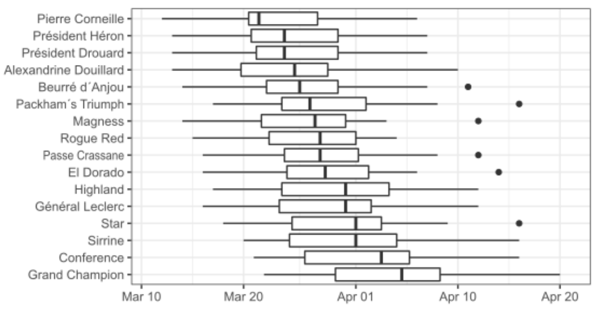

Figure 1. Flowering dates of 16 traditional pear cultivars grown in Zaragoza (northeastern Spain) during the periods 1986–1989, 1992–2007, and 2022. Boxplots represent the 20 years of observations.

In each boxplot, a vertical line shows the median, and the hinges indicate the interquartile range (IQR, percentiles 25th to 75th). On each side of the boxes, the whiskers represent the greatest value located within 1.5 times the IQR.

Chilling periods were delineated through PLS regression analysis, in which daily chill (in CP) was the independent variable and flowering date was the response variable (Figure 2). Chilling period ranged from 61 to 70 days for the cultivars ‘Highland’ and ‘Passe Crassane’, respectively. The start dates occurred between 8 and 12 November and the end dates between 15 and 17 January (Table 2). During these periods, several days clearly contributed to the accumulation of chill in all cultivars, with negative and significant (VIP > 0.8) model coefficients (Figure 2). A few days were identified with model coefficients that were classified as less relevant (smaller VIP score). Since most days during the delineated periods appeared to be consistently correlated with earlier flowering dates, likely indicating a clear dormant state, the chilling periods were considered continuous (horizontal blue rectangles in Figure 2).

Table 2. Average chilling and forcing periods and agroclimatic requirements (mean ± standard deviation) estimated for 16 pear cultivars grown in Zaragoza (Spain) in the 20-year interval 1986–1989, 1992–2007, and 2022. Chill requirements were computed according to the Dynamic (in CP), Chilling Hours (in CH), and Utah (in CU) models. The Growing Degree Hours model (in GDH) was used to estimate heat requirements.

Cultivar	**Chilling**						**Forcing**			
	Period			**Accumulation (Mean ± sd)**			**Period**			**Accumulation (Mean ± sd)**
	Start	**End**	**Duration (Days)**	**CP**	**CU**	**CH**	**Start**	**End (Flowering Date, Average)**	**Duration (Days)**	**GDH**
Alexandrine Douillard	10 Nov	16 Jan	67	47.5 ± 2.5	1027 ± 141	753 ± 117	28 Jan	26 Mar	58	7287 ± 945
Beurré d´Anjou	08 Nov	15 Jan	65	47.7 ± 2.8	1100 ± 100	745 ± 117	30 Jan	03 Apr	62	6619 ± 750
Conference	12 Nov	16 Jan	67	46.5 ± 2.3	1113 ± 186	745 ± 114	1 Feb	29 Mar	60	7347 ± 905
El Dorado	10 Nov	16 Jan	67	47.5 ± 2.5	1163 ± 30	753 ± 117	29 Jan	30 Mar	58	7094 ± 1082
Général Leclerc	10 Nov	16 Jan	65	47.5 ± 2.5	1028 ± 140	753 ± 117	1 Feb	05 Apr	63	7107 ± 890
Grand Champion	12 Nov	16 Jan	65	46.5 ± 2.3	1028 ± 140	745 ± 114	2 Feb	30 Mar	58	7168 ± 776
Highland	12 Nov	16 Jan	61	46.5 ± 2.3	1113 ± 186	745 ± 114	1 Feb	27 Mar	59	7402 ± 853
Magness	16 Nov	16 Jan	68	43.9 ± 2.0	1113 ± 186	719 ± 106	28 Jan	27 Mar	57	7509 ± 1048
Packham´s Triumph	10 Nov	16 Jan	67	47.5 ± 2.5	1163 ± 31	753 ± 117	29 Jan	29 Mar	60	6997 ± 1048
Passe Crassane	08 Nov	17 Jan	70	49.2 ± 2.8	1100 ± 100	774 ± 120	31 Jan	29 Mar	58	6514 ± 673
Pierre Corneille	10 Nov	16 Jan	67	47.5 ± 2.5	1099 ± 99	753 ± 117	29 Jan	24 Mar	55	6519 ± 741
Président Drouard	08 Nov	16 Jan	69	48.5 ± 2.8	1113 ± 186	760 ± 118	29 Jan	25 Mar	56	7289 ± 914
Président Heron	08 Nov	16 Jan	69	48.5 ± 2.8	1163 ± 30	760 ± 118	29 Jan	26 Mar	57	6935 ± 1042
Rogue Red	08 Nov	16 Jan	69	48.5 ± 2.8	1028 ± 140	760 ± 118	28 Jan	27 Mar	59	7170 ± 656
Sirrine	09 Nov	16 Jan	68	48.0 ± 2.8	1163 ± 30	757 ± 118	30 Jan	01 Apr	62	7075 ± 902
Star	09 Nov	16 Jan	68	48.0 ± 2.8	1100 ± 100	757 ± 118	1 Feb	31 Mar	59	6573 ± 637

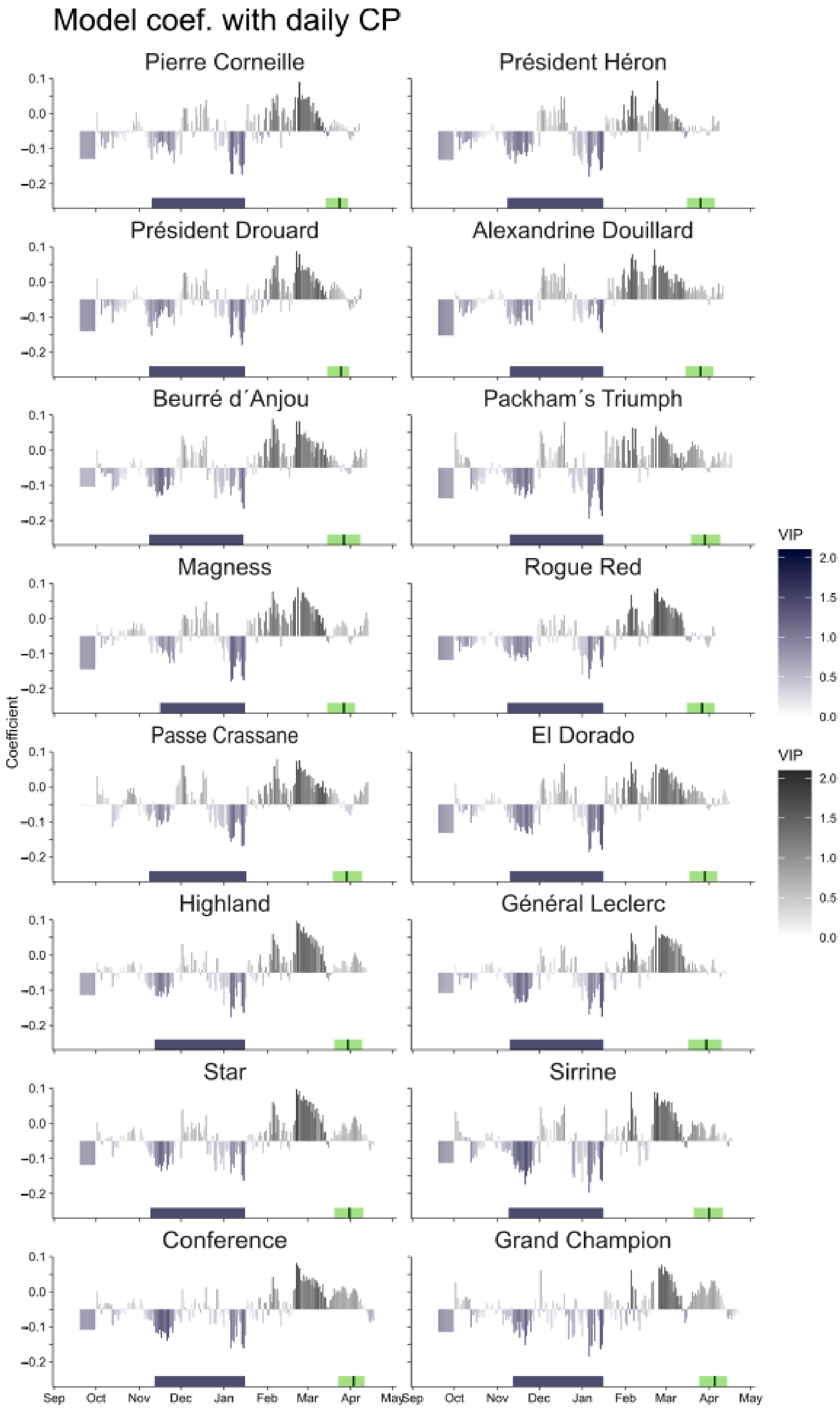

Figure 2. Partial Least Squares regression analysis between flowering dates of 16 cultivars of pear grown in Zaragoza (northeastern Spain) and daily accumulations of winter chill in Chill Portions (CP) according to the Dynamic model. The direction of the vertical bars in each plot indicates days with positive or negative model coefficients, and the color of the bar represents the variable importance in the projection (VIP) score (with blue for negative coefficients and gray for positive coefficients). Horizontal thick bars at the bottom of each plot indicate the delineated chilling (blue) and the range of observed flowering dates (green). In the bars showing the flowering range, the dark purple vertical line indicates the median flowering date in the 20 years analyzed.

3.2. Forcing Period Delineation

Forcing periods were delineated through PLS regression analysis with daily heat (in GDH) as the independent variable and flowering date as the response variable (Figure 3). Forcing periods ranged from 55 to 63 days for the cultivars 'Pierre Corneille' and 'Général Leclerc', respectively (Table 2). Start dates occurred between 28 January and 2 February and flowering dates between 20 March and 8 April (red rectangles in Figure 4).

The forcing period did not start immediately after the chilling period, as a transition phase was observed in which the coefficients were neither clearly negative nor clearly positive and were not considered significant by the VIP analysis. This transition period occurred in January, and ranged between 12 and 16 days.

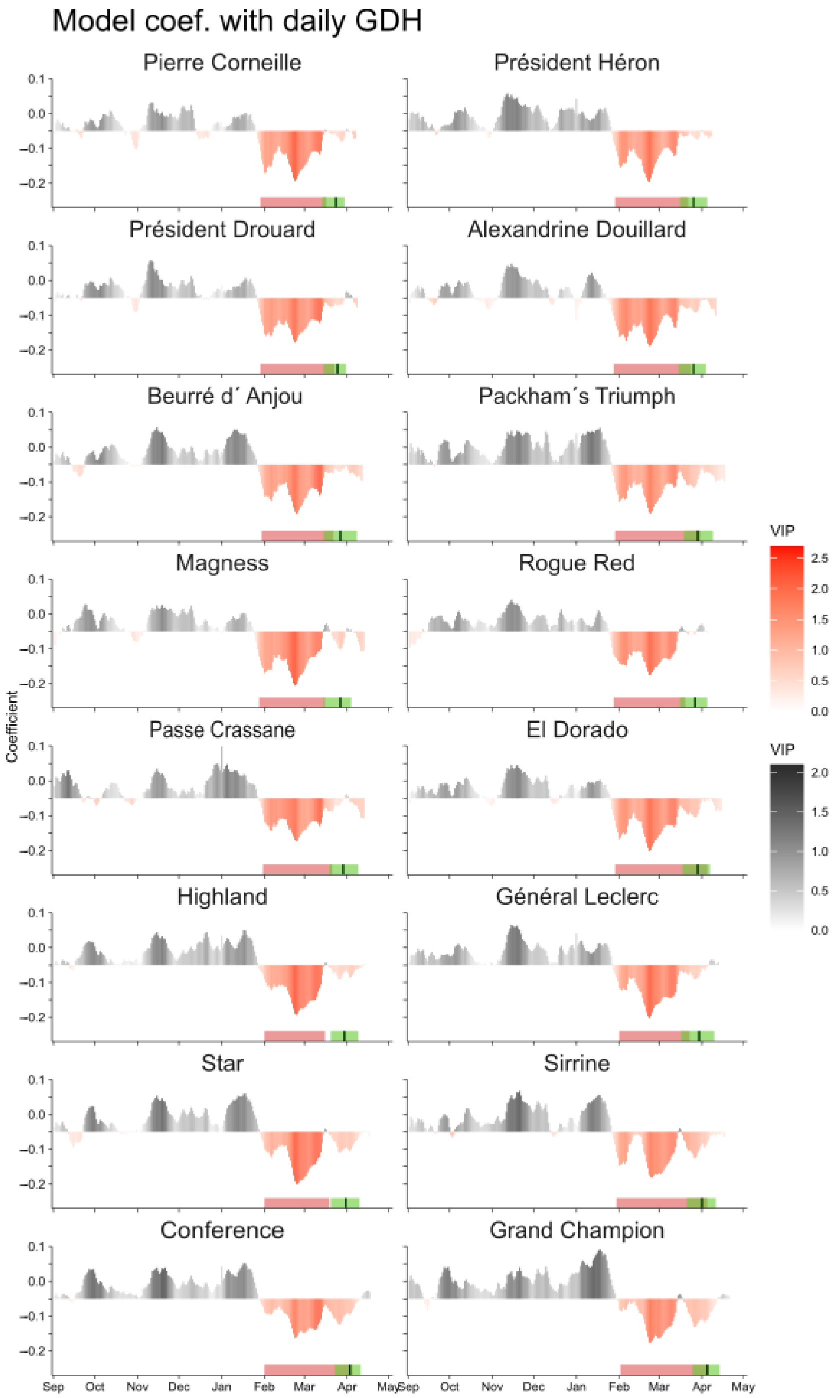

Figure 3. Partial Least Squares regression analysis between flowering dates of 16 cultivars of pear grown in Zaragoza (northeastern Spain) and daily accumulations of heat in Growing Degree Hours

(GDH). The direction of the vertical bars in each plot indicates days with positive or negative model coefficients, and the color of the bar represents the variable importance in the projection (VIP) score (with red for negative coefficients and gray for positive). Horizontal thick bars at the bottom of each plot indicate the delineated forcing periods (red) and the range of observed flowering dates (green). In the bars showing the flowering range, the dark purple vertical line indicates the median flowering date in the 20 years analyzed.

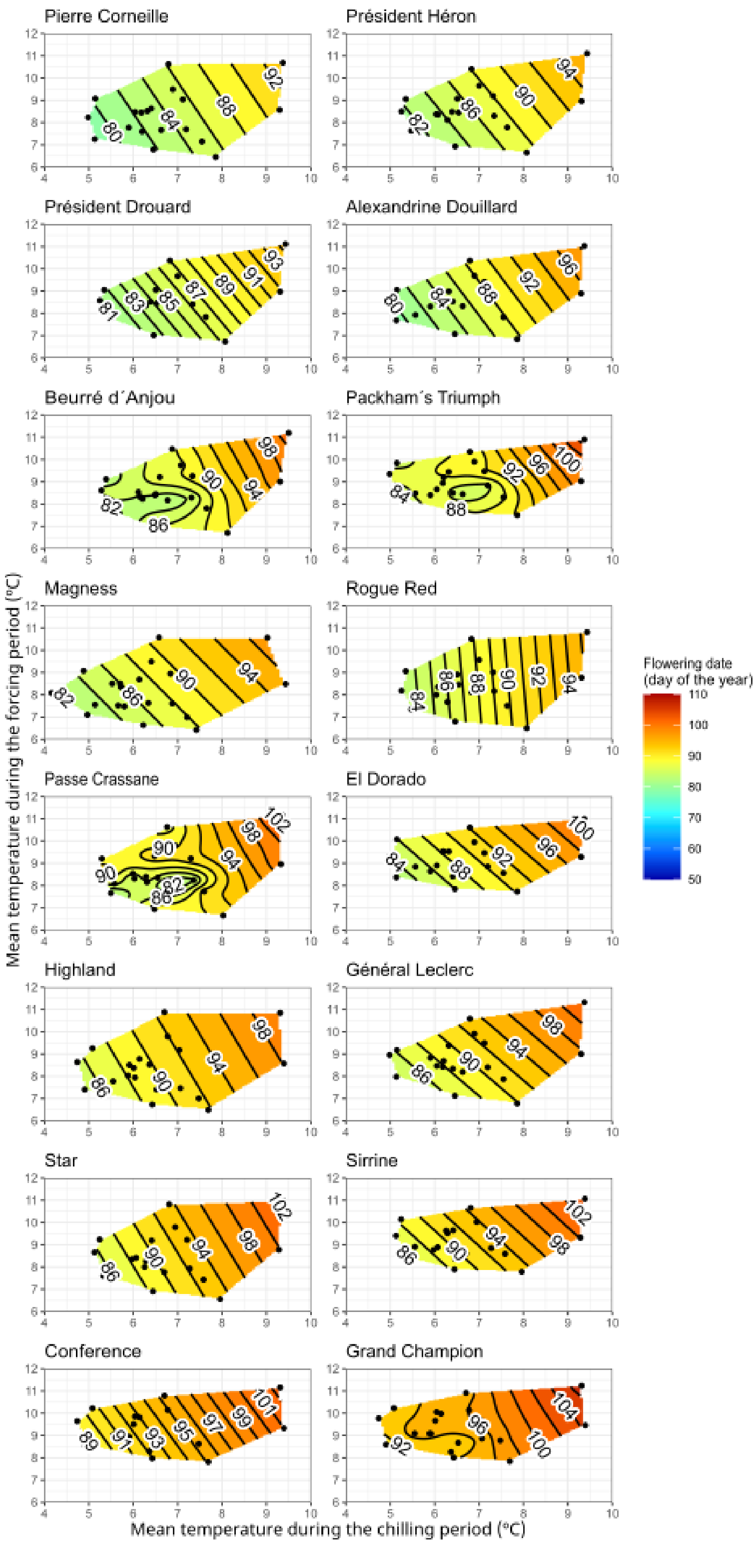

Figure 4. Flowering dates as a function of mean temperature during chilling and forcing periods as determined through PLS regression analysis of 20-year data for 16 pear cultivars grown in Zaragoza

(Spain). The contour lines and the color surface show the expected flowering dates based on actual phenological data (black dots).

3.3. Chill and Heat Requirements for Flowering

The agroclimatic requirements of the 16 pear cultivars were estimated based on the chilling and forcing periods identified through PLS regression. We used three chill models (Dynamic, Chilling Hours, and Utah) and one heat model (Growing Degree Hours model). The chill requirements presented low variability between the cultivars analyzed: from 43.9 ± 2.0 CP to 49.2 ± 2.8 CP when calculated with the Dynamic model, from 1027 ± 141 CU to 1163 ± 30 CU with the Utah model, and from 719 ± 106 CH to 774 ± 120 CH with the Chilling Hours model. Chill requirements did not correlate with the flowering dates; 'Magness' presented the lowest chill requirements but mid-period flowering dates, similar to 'Passe Crassane', which showed the highest chill requirements. For heat requirements, the range was from 6514 ± 673 GDH for 'Passe Crassane' to 7509 ± 1048 GDH for 'Magness'. Heat requirements were also not correlated with flowering dates.

To analyze the flowering response to temperatures during the chilling and forcing periods, flowering was defined as a function of mean temperature during both of these periods (Figure 4). The response surface showed contour lines with a pronounced negative slope, suggesting that flowering was mainly triggered by the temperatures during the chilling period. Earlier flowering occurred after the winters with the lowest temperatures; in contrast, late flowering took place in the seasons that presented the highest temperatures during the winter.

4. Discussion

The agroclimatic requirements for flowering (chill and heat requirements) were estimated for a group of traditional European pear cultivars with PLS regression analysis. The chill requirements ranged from 43.9 ± 2 to 49.2 ± 2.8 CP and the heat requirements between 6514 ± 673 GDH and 7509 ± 1048 GDH. The ranges of these results agree with the chill and heat requirements of other fruit tree species grown in the same area, such as sweet cherry [4,18].

All of the cultivars analyzed presented similar agroclimatic requirements, showing very low variability between cultivars compared to wider ranges reported for cultivars of various *Prunus* sp., both for species with low-chilling cultivars such as almond, peach, and Japanese plum (< 20 CP) as well as species with high-chilling cultivars such as European and Japanese apricot, peach, and sweet cherry (> 70 CP) [4]. This low variability found in the pear cultivars analyzed could be due to the fact that all of them were traditional cultivars released in areas with similar cold climates. Those breeding programs probably did not aim to obtain a wide range of flowering dates. Previous genetic analysis revealed relatively low genetic diversity among these and other cultivars [48]. For breeding purposes, a source of diversity could be obtained from landraces from warm areas such as Tunisia [49] and Sicily [24]. As these are adapted to warm regions, they would probably present strong differences in this trait, such as those identified in the temperature requirements of Tunisian and foreign almond cultivars [27].

Previous data on the agroclimatic requirements of European pears are very scarce, and comparisons with our results have shown large differences. For example, 47 European pear cultivars were experimentally analyzed in the early 1960s, grouping them into four qualitative categories but without estimates of chill requirements [50]. Another study of 45 cultivars in Kent (UK) that included monthly sampling and growing cameras at 15 °C resulted in lower values than our estimates for the cultivar 'Magness' (617 CH/891 CU vs. 719 CH/1113 CU) and higher estimations for the cultivars 'Conference' (1422 CU/1159 CH vs. 1113 CU/745 CH), 'Packham´s Triumph' (1422 CU/1159 CH vs. 1163 CU/753 CH), and 'Pierre Corneille' (2335 CU/2103 CH vs. 1099 CU/753 CH) [51]. Previous data on heat requirements are only available for 17 cultivars, among them 'Conference', which needed

4755 GDH for flowering in 1976, a lower value than our estimate (7347 ± 905 GDH) [51]. The large differences observed between our results and previous ones could be due to the fact that the few available studies date back to the 1960s and 1970s, and therefore both the experimental methodology for determining dormancy and the models to quantify chilling temperatures differ from the approaches commonly used at present.

Recent studies on dormancy in pears combined experimental and statistical approaches. The exposure of potted trees to distinct environments during winter resulted in multiple experimental seasons with a wide range of flowering dates [52], generating sufficient data in a two-year experiment to be analyzed with PLS regression [53]. The agroclimatic requirements of the cultivar 'Conference' resulted in 31 CP and 11,816 GDH, resulting in lower chill requirements and higher heat requirements than our results (46.5 ± 2.3 CP and 7347 ± 905 GDH). These differences could be due to the fact that the synthetic years obtained with the multi-environment experiment explore more extreme conditions, despite generating long phenological records.

Based on the slope of the response of flowering dates to the temperatures during the chilling and forcing periods (Figure 4), the temperatures during the chilling period were the main factor in determining the flowering dates of the European pear cultivars analyzed in Zaragoza in the period 1984–2022. This has been previously reported for sweet cherry cultivars at the same location [18], but is contrary to what was reported in Germany, where temperatures during the forcing period further conditioned the flowering date [54]. The effect of the chilling and forcing periods on flowering is both cultivar- and location-specific, and provides information on cultivar adaptation and behavior under different conditions, which can anticipate the response of cultivars to the effects of global warming. In a previous study, we proposed a four-step scale that relates the phenological response of trees to the effects of climate change on orchard viability according to the relative importance of the chilling and forcing phases in phenology [18]. Stage (i) corresponds to the majority of native temperate woody species, for which phenology is usually conditioned by temperatures during the forcing period. Rising temperatures cause an advance in phenology, which is likely the first observable effect of global warming on phenology. Then, at stage (ii), rising temperatures would not cause changes to phenology, as the advancing effect of rising temperatures during the spring is cancelled out by the delays in overcoming dormancy resulting from the lack of chilling during the winter. Then, even higher temperatures could provoke stage (iii), in which phenological delays are caused by the late release from endodormancy. In this stage, the temperatures during the chilling phase determine the flowering dates almost exclusively. Finally, further increases in temperature can completely prevent dormancy release, and in stage (iv), seriously compromise the annual cycle of the tree. According to this classification, the pear cultivars analyzed in this work would be grouped at stages (ii) and (iii).

Research on dormancy in fruit trees, in addition to providing information on the adaptation of each cultivar to each growing area, aims at a deeper understanding of the physiology and genetics of the dormant process to ultimately develop a process-based methodology and model for phenology prediction. Advances are at different stages depending on the species, but very few studies have focused on dormancy in the European pear. Significant breakthroughs were achieved in *Prunus* sp., e.g., the establishment of the role of the *DAM* genes in the regulation of dormancy [55–57], the relationship of the dynamics of starch accumulation in the ovary primordia with the accumulation of chill and heat in sweet cherry [58], or male meiosis as a biomarker for chilling fulfillment in apricot [59,60]. Further research on dormancy in the European pear is necessary to find biomarkers that help to delimit the dormancy phases and estimate the agroclimatic requirements of cultivars.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Flowering dates (day of the year) of 16 traditional pear cultivars grown in Zaragoza (northeastern Spain) during the period 1986–1989, 1992–2007, and 2022.

Author Contributions: Conceptualization, E.F. and J.R.; methodology, E.F; formal analysis, E.F.; investigation, E.F.; resources, P.E., J.M.A.S., and J.R.; data curation, M.T.E.; writing—original draft preparation, E.F.; writing—review and editing, E.F., M.T.E., P.E., J.M.A.S., and J.R.; supervision, J.R.; funding acquisition, P.E., J.M.A.S., and J.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original data is available in the Table S1.

Conflicts of Interest: The authors declare no conflicts of interest.

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