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Boosting statistical delineation of chill and heat periods in temperate fruit trees through multi-environment observations

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ABSTRACT

Global warming has modified the phenology of deciduous species. Temperature during the dormancy phase modulates the timing of bloom in temperate trees. Chill and heat requirements represent the climatic needs of trees during dormancy. Partial Least Squares (PLS) regression allows delineating chilling and forcing phases, which in turn allows estimating the trees' requirements. However, PLS regression requires long-term phenology and weather data, which are scarce in many growing regions. In a two-year experiment, we generated long-term phenology data by exposing potted trees to distinct environments during winter. We obtained records for 66 and 32 experimental seasons in apple and pear, respectively. We recorded tree phenology and hourly temperature. Through PLS regression methods, we determined the impacts of inter-seasonal variation on the outputs, estimated species' dormancy phases and needs (in Chill Portions – CP and Growing Degree Hours – GDH), and assessed the relationship between bloom and temperature during the chilling and forcing phases. Results suggest inter-seasonal variation may be more important than number of seasons for producing valuable outputs. We delineated the chilling phase from October 19 to January 04 for apple and October 19 to December 27 for pear. The forcing period for both species was January 16 – March 26. Median chill and heat requirements were estimated as 43 CP and 14,845 GDH for apple and 31 CP and 11,816 GDH for pear. Bloom was modulated by temperature during both phases under warm conditions. In cold scenarios, bloom was mostly defined by temperatures during the forcing phase. We expanded the reach of the PLS regression method and made it applicable for cultivars lacking long-term phenology data. Our approach helps dormancy researchers improve their procedures to analyze species' responses under possible future conditions. This work may assist farmers and orchard managers in adapting their orchards to face future challenges.

1. Introduction

Deciduous forest and fruit trees enter a dormancy phase in late autumn. This phase enables such trees to tolerate the freezing temperatures frequently observed during winter in their respective habitat of origin (Vegis, 1964). During this period, the absence of leaves or any visible growth allows trees to protect their meristems inside buds and resume growth after the end of the cold season (Faust et al., 1997). Environmental cues such as short photoperiod and low temperatures have been implicated in promoting dormancy establishment in temperate trees (Singh et al., 2018; Singh et al., 2019; Tylewicz et al., 2018). In contrast, dormancy release is usually assumed to be under the exclusive control of temperature (Cooke et al., 2012).

According to Lang et al. (1987), winter dormancy can be classified into endo- and eco-dormancy. Whereas endo-dormancy is associated with the true dormant state and characterized by inactive buds even under favorable growing conditions, eco-dormancy corresponds to the phase by which buds have become responsive to warm temperatures and can resume growth in spring (Lang et al., 1987). Following this classification, researchers have defined the concepts of chill and heat requirements (CR and HR, respectively) to represent the climatic needs of buds for overcoming the dormant state (Lang, 1987; Luedeling, 2012). Together, these requirements drive the timing of budburst and bloom in temperate tree species.

Common approaches developed to model dormancy in temperate trees assume a sequential relationship between the chilling and forcing phases of dormancy (Alburquerque et al., 2008; Ruiz et al., 2007). In this approach, chill and heat accumulation occur during the respective

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dormancy phases (chilling and forcing), with no interaction among them (Ashcroft et al., 1977). A more biologically plausible structure is represented by the overlapping approach (Cannell and Smith, 1983; Harrington et al., 2010; Pope et al., 2014). This approach suggests that chill and heat can compensate for each other during winter, leading to similar bloom dates in different growing seasons. Although Pope et al. (2014) reported that a model structure considering 75% overlap between chilling and forcing performed better than 50% and 25% overlap in explaining phenological data of almond in California, USA, the specific length of the overlapping period remains mostly unclear. An overlapping period between dormancy sub-phases may result from physiological processes occurring between endo- and eco-dormancy. Various changes at cellular and tree level (e.g. changes in cell-to-cell communication, free-water binding molecules, concentrations of carbohydrates) have been linked to the transition from a deep dormant state to eco-dormancy, where buds are responsive to warm temperatures (Fadón et al., 2020). Identifying the periods for chilling and forcing, as well as their interaction during the dormancy phase might help researchers focus on addressing key biological processes related to chill and heat accumulation.

Knowledge of the dormancy-related climatic needs of temperate trees has become a key consideration in orchard planning and management, as well as for breeding programs aiming to develop new cultivars. Chill and heat requirements in temperate fruit species are usually determined experimentally or statistically. The experimental approach relies on exposure of lateral branches, cuttings, buds, or young potted trees to cold temperatures under field or chamber conditions for a certain period, after which the material is transferred into a favorable environment (forcing period, Alburquerque et al., 2008; Campoy et al., 2019; Fadón and Rodrigo 2018; Fernandez et al., 2019; Fernandez et al., 2020a). Chill and heat requirements are then estimated as the combination of chill and heat necessary to reach a particular share of buds (often 50%) in a given developmental state (Fadón and Rodrigo, 2018).

The statistical approach is based on analyzing long-term phenology datasets usually collected under natural conditions (Luedeling and Gassner, 2012). Statistical analysis is often implemented through Partial Least Squares (PLS) regression between a dependent variable (such as bloom dates) and one or more independent variables (such as daily mean temperature or daily chill and heat accumulation, Benmoussa et al., 2017; Guo et al., 2015b; Luedeling et al., 2013a; Luedeling et al., 2013b). When using chill and heat accumulation, PLS regression analysis helps identify the periods of the dormancy phase where bloom is advanced or delayed by chill and heat accumulation (Benmoussa et al., 2017; Guo et al., 2015b). While this general pattern has usually been found in studies using this methodology, pinpointing the exact start and end dates has often been difficult and ultimately based on the researchers' subjective judgment. This judgment call has made the PLS regression procedure difficult to standardize.

Climate change has affected the phenology of many species (Menzel et al., 2006), and it is likely to continue influencing the development of temperate trees in the future (Chmielewski et al., 2012). Global warming is expected to decrease winter chill accumulation as well as increase the availability of heat in several of the world's most important growing regions (Benmoussa et al., 2020; Darbyshire et al., 2013; del Barrio et al., 2021; Fernandez et al., 2020b; Fernandez et al., 2020c; Luedeling et al., 2009). Reliable tools to anticipate the response of trees to such conditions could greatly support farmers in adapting their orchards to future climate conditions (Campoy et al., 2011; Luedeling, 2012). However, for PLS regression to serve as such a tool, the method would have to be provided with long-term datasets (Luedeling and Gassner, 2012) from a wide range of climatic settings. Even if such a dataset could be assembled, it would still be difficult to anticipate future tree responses, especially for warm locations, because long-term datasets collected in the past may not include the kind of future conditions we must prepare for.

We developed an experimental methodology to collect information on tree phenology under a wide range of temperature scenarios. Our objective was to expand the reach of the methodology and make it applicable even for tree cultivars for which no long-term phenology data are available. We collected data from potted trees exposed to a number of artificial climatic conditions during the dormancy season. We developed a procedure to generate long-term phenology data for 66 experimental seasons in apple and 32 experimental seasons in pear trees over the course of a two-year experiment. Using these data, we assessed the importance of the number of seasons or years in the PLS regression analysis, estimated chill and heat requirements for apple and pear trees, and evaluated the relationship between bloom dates and thermal conditions during both the chilling and forcing phases.

2. Materials and methods

2.1. Plant material

We evaluated tree phenology data collected during two consecutive winter seasons in 2018/2019 and 2019/2020. In each winter, we used 99 three-year-old potted trees of apple cv. 'Elstar' and 48 three-year-old potted trees of pear cv. 'Conference'. The apple trees were grafted on 'M9' rootstock and the pear trees were grafted on 'Quince Adams' rootstock. For both winter seasons, we obtained bare-root trees from a local nursery in Rheinbach (Germany) at the end of the previous winter. Before spring, trees were planted in plastic pots (15 L) using a standard nursery soil mixture. After planting, we placed the trees under normal field conditions in an experimental orchard of the University of Bonn at Campus Klein-Altendorf in Rheinbach (50.62° N, 6.99° E). Trees were irrigated and fertilized during spring, summer, and early fall, following normal orchard management practices. We kept all trees under field conditions until the beginning of the experiment in November 2018 and October 2019 for the first and second winter season, respectively. We started the experiment a month earlier in the second season to increase the coverage of temperature variation in early dormancy stages after conducting preliminary PLS assessments with data from the first season. Since the experiment started about one month earlier in the second winter, with trees still showing green leaves, we promoted dormancy establishment by manually defoliating all apple and pear trees on October 11, 2019. It should be noted, however, that our manual defoliation strategy may have altered the normal cycle of carbon reserves as reported by Mesa et al. (2019) for mature productive pear trees. Nonetheless, we assumed that compared to adult trees our trees were not limited regarding carbon source, since they did not bear any fruit during the growing season. The absence of fruits (or any other major sink structure) allowed us to assume that towards the end of the season trees had accumulated sufficient carbon reserves and that manual defoliation did not severely affect dormancy-related processes.

2.2. Experimental winter seasons

In order to evaluate the effects of temperature variation during winter on tree phenology, we used several distinct environments, to which trees were exposed at different times. During the first winter, we used a heated greenhouse (heated by a hot water system and set to maintain a temperature between 5 and 25°C), an unheated greenhouse and ambient conditions outside greenhouses at Campus Klein-Altendorf (CKA). For the second winter, we added four environments located at Campus Endenich of the University of Bonn (50.73° N, 7.07° E), 13 km from CKA. These environments were three experimental chambers constructed with different materials (ethylene-tetrafluoroethylenecopolymer, float glass and frosted glass) and the natural conditions outside these chambers. It should be noted that these different materials used in the chambers only aimed to generate differences in temperature with no further analyses on the relationship between light intensity and bloom. Over the course of each winter season, we frequently transferred the trees across environments (Fig. S1 in supplementary materials). We defined each combination of environments during winter as a treatment or experimental season. In Fig. 1, we show the rate of chill and heat accumulation on a daily basis for each combination of environments used in this study. By using three replicates (three potted trees) per treatment, we were able to obtain 66 combinations of environments for apple and 32 for pear over the course of two winters. One experimental season (treatment 50 S2 in Fig. 1) was exclusive to pear, whereas the remaining 31 experimental seasons in pear were shared with apple.

On November 19 for the first winter season and October 16 for the second season, we transferred the trees from field conditions to the different environments according to the respective treatments they were assigned to (Fig. S1 in supplementary materials). After the initial transfer, additional shifting dates in the winter of 2018/2019 were December 17 and 27, January 14, and February 02 and 14. During the second winter, transfer dates after the initial movement were November 26, January 15, and February 21.

2.3. Temperature and phenology data collection

We recorded high-resolution temperature data (sub-hourly) in each environment using portable data loggers (Tinytag TGP-4500, Tinytag TGU-4500 and EasyLog USB 31) as well as fixed devices in the case of the heated greenhouse and field conditions (local weather station) at CKA. Temperature data were then summarized into hourly records by computing the mean across observations within each hour of the day. Due to technical device problems, we missed records for 15 complete days in the unheated greenhouse at CKA and field conditions at Endenich at the beginning of the second winter season. We filled these gaps following the procedure described in Fernandez et al. (2020c), with minor modifications. In brief, we used data from the weather station located under field conditions at CKA for filling the gaps after bias

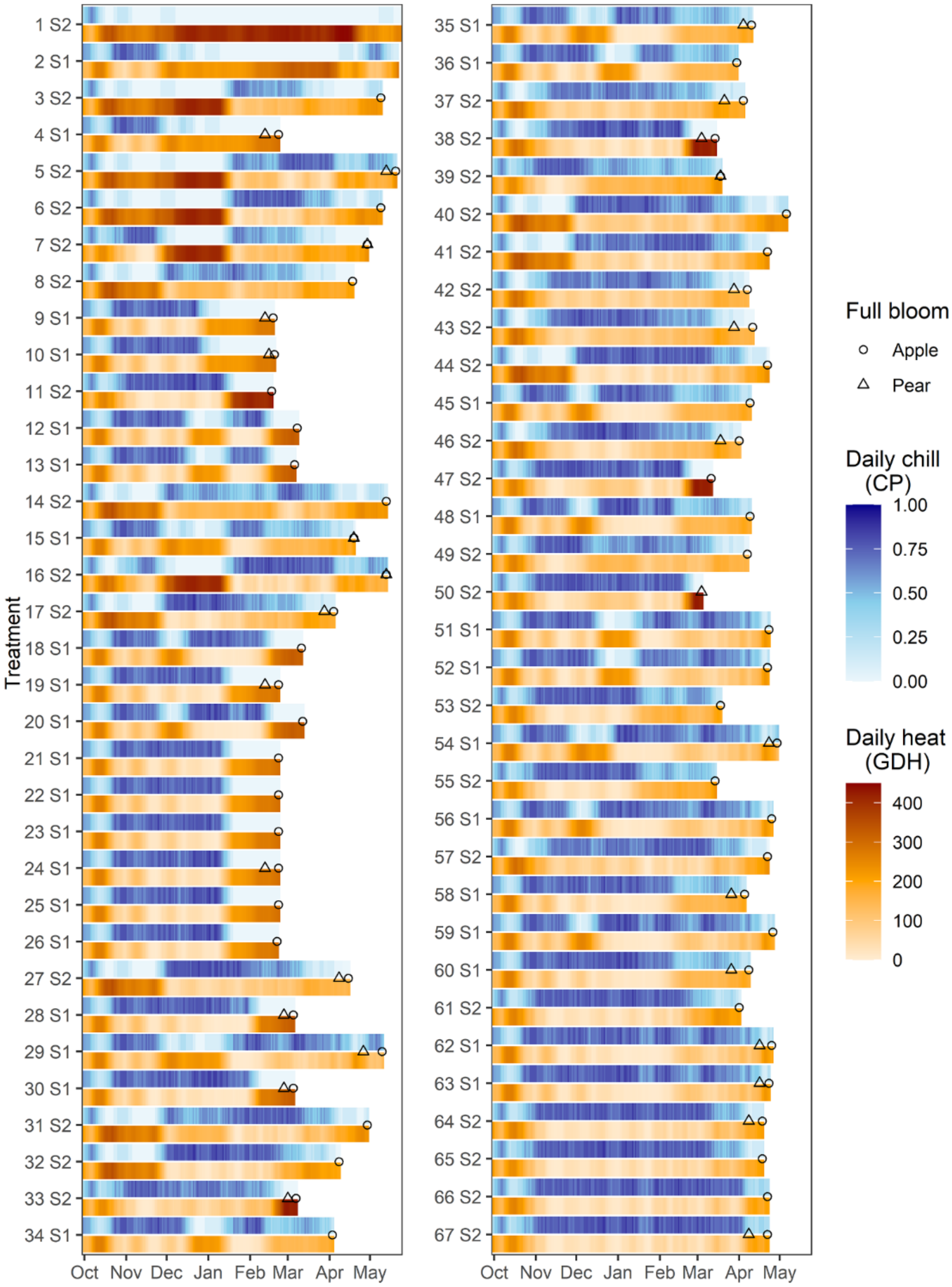

Fig. 1. Schematic illustration of the experimental seasons that consisted of combinations of several distinct environments that trees were exposed to over the course of the winter. We show the combinations of time (x-axis) and daily chill (blue scale in Chill Portions – CP) and heat (red scale in Growing Degree Hours – GDH) accumulation during the first and second winter season (S1 and S2, respectively). The numbers on the y-axis in both panels represent the order of treatments according to increasing chill accumulation (in CP) computed between October 1 and March 31. In the left-hand panel, treatments 21 S1 to 26 S1 show identical chill and heat rates because these treatments showed full bloom before the date planned for the last movement between environments, which would have differentiated the temperature regimes. Additionally, we show the median across replicates (three trees per treatment) for full bloom dates for apple (circle) and pear (triangle) trees.

correction. The bias was computed as the mean difference for each hour of the day between the temperature recorded at the CKA weather station and available temperature records from both the data logger inside the unheated greenhouse at CKA and the data logger recording data under field conditions at Endenich. For records in the unheated greenhouse, values of hourly mean difference bias relative to the weather station were computed using 188 observations (188 values for each hour between 0 and 23). In the case of field conditions at Endenich, this mean bias difference relative to the weather station at CKA was computed using 222 observations for each hour of the day. We then used the computed bias to adjust the hourly records from the weather station at CKA. The bias-corrected temperatures were used to fill the gaps in the datasets of both the unheated greenhouse and the field conditions at Endenich. Additional gaps detected between two close measurement points (due to data extraction from portable devices) were filled through linear interpolation as suggested by Luedeling (2018). These interpolated gaps represented only a minor fraction (~0.7%) of the total intended measurements across seasons and environments. After thus obtaining gap-free hourly temperature records for both winter seasons, we summarized the data into daily observations (minimum, mean and maximum temperatures).

To determine the effects of different temperature regimes on tree behavior, we monitored tree phenology according to the BBCH scale for pome fruit (Meier, 2001). We recorded full bloom date as the moment we observed at least 50% of flowers open with first petals falling (Meier, 2001). For further analyses, we summarized full bloom records into median full bloom dates across replicates (based on three potted trees per treatment). It should be noted that during the second season of the experiment, approximately 30 trees showed few flower buds in spring. For comparison, we performed two versions of our analysis, excluding or retaining these trees. We report only results for the analysis with all observations, since excluding the trees with few flower buds did not strongly affect the results.

2.4. Delineation of chilling and forcing periods using mean temperature and impact of number of seasons on PLS outputs

We used Partial Least Squares (PLS) regression analysis to correlate full bloom dates of both apple and pear trees with daily mean temperature (Luedeling and Gassner, 2012) and determine the most useful treatments for further PLS analyses. We combined the data from both winter seasons to obtain 66 experimental seasons for apple and 32 experimental seasons for pear trees. For each experimental season, we applied an 11-day running mean function to increase the autocorrelation between temperatures of consecutive days and enhance the ability of the PLS regression analysis to reveal phenology response patterns (Luedeling and Gassner, 2012). Major PLS regression outputs are the Variable Importance in the Projection (VIP) statistic and the standardized model coefficients (Guo et al., 2015b). While the VIP statistic helped to identify relevant periods for chill and heat accumulation (consecutive days with VIP ≥ 0.8), the standardized model coefficients were used to differentiate whether these periods corresponded to the chilling (coefficient > 0) or forcing (coefficient < 0) phase (Luedeling et al., 2013b).

Previous studies have suggested to use a minimum of between 15 and 20 years of records for producing valuable PLS regression outputs (Luedeling and Gassner, 2012; Luedeling et al., 2013b). In order to determine the effects of the number of experimental seasons used in the PLS regression analysis on the outputs, we implemented this analysis using a different number of experimental seasons in each model run. We first sorted the experimental seasons from warmest to coolest according to seasonal chill accumulation (computed from October 1 to March 31, Fig. 1) and then started the PLS regression analysis by using the 3 warmest seasons within the dataset. After saving the outputs of this analysis, we progressively added treatments with increasingly more chilling, one treatment at a time. In each model run, we computed the mean standard deviation in mean temperature among the treatments included in the PLS analysis to relate the variation among experimental seasons with the visual interpretation of the results. We repeated the process until the analysis included all 64 experimental seasons in apple and 30 experimental seasons in the pear dataset (the initial number of experimental seasons minus two treatments that did not reach full bloom at the end of the experiment). Finally, we plotted the results separately to determine the impact of the number of experimental seasons on the PLS regression outputs.

We implemented a sensitivity analysis to assess the effect of the sequence of seasons used in our PLS analysis on the results. In addition to sorting the seasons from low to high chill accumulation before using them in the PLS analysis, we assessed a different sequence of seasons to use in the PLS regression. In brief, we defined 3 groups according to the number of experimental seasons (with 5, 10 and 20 experimental seasons) and compared the outputs when selecting these seasons from our ordered list (the first 5, 10 and 20, respectively) as well as when applying a random selection from all available seasons. We then plotted the outputs to decide on the most promising manner of selection. Based on visual interpretation of this analysis (see supplementary materials), we decided to continue with the sequence of ordered experimental seasons as input to the PLS procedure.

2.5. Delineation of chilling and forcing periods using chill and heat accumulation

After optimizing the selection of treatments that offer an adequate delineation of the chilling and forcing phases of dormancy, we applied PLS regression to relate bloom dates with daily chill and heat accumulation. In this analysis, we used the actual observations of daily chill and heat accumulation without implementing a running mean function. Compared to using mean temperatures in the analysis, the interpretation of the standardized model coefficients is slightly different when using daily chill accumulation. In this version of the analysis, negative coefficients for chill accumulation on particular days are typical of the chilling period. This means that higher chill levels during the chilling phase appear to be related with earlier bloom dates. The interpretation of model coefficients in the case of heat accumulation remains the same as when using mean temperatures. Values of the VIP statistic were interpreted the same way when using both the mean temperature and chill and heat accumulation. It should be noted that we excluded two experimental seasons from the PLS regression analysis, since trees in these treatments did not reach full bloom (see treatments 1 S2 and 2 S1 in Fig. 1).

For this analysis, daily chill was computed in terms of Chill Portions (CP) using the Dynamic Model (Erez et al., 1990; Fishman et al., 1987a; Fishman et al., 1987b), whereas heat accumulation was computed as Growing Degree Hours (GDH) using the Growing Degree Hours model (Anderson et al., 1986). To compute these metrics, we used hourly temperature data recorded for all treatments. Both models were applied by using their respective functions in the chillR package (Luedeling, 2020) for R (R Core Team, 2020).

2.6. Phenology response to mean temperature during chilling and forcing phases

After delineating the chilling and forcing phases through PLS regression analysis, we evaluated the relationship between bloom dates and mean temperatures during both phases. To this end, we classified the treatments into Warm, Mild-warm, Mild-cold, and Cold according to chill accumulation between October 1 and March 31 (Table 1). For the sake of comparison with previous analyses, we included between 15 and 25 experimental seasons in each class. For apple and pear, the category Warm represented data from trees exposed to the 25th and 15th warmest treatments, respectively. Mild-warm and Mild-cold represented data from trees exposed to the 25th to 45th and the 35th to 55th warmest treatments, respectively, for apple. In pear, these classes

Table 1
Description of the classification of experimental seasons we used for assessing the relationship between bloom dates and temperature during the chilling and forcing phase of dormancy.

Species	Class	Experimental seasons in class (from Fig. 1) †	Chill accumulation range (in CP)
Apple	Warm	1 S2 to 25 S1	10.1 – 84.6
	Mild-warm	25 S1 to 45 S1	84.6 – 97.2
	Mild-cold	35 S1 to 56 S1 **	89.3 – 107.1
	Cold	45 S1 to 67 S2 **	97.2 – 128.1
Pear	Warm	1 S2, 2 S1, 4 S1, 5 S2, 7 S2, 9 S1, 10 S1, 15 S1, 16 S2, 17 S2, 19 S1, 24 S1, 27 S2, 28 S1, 29 S1	10.1 – 87.7
	Mild-warm	7 S2, 9 S1, 10 S1, 15 S1, 16 S2, 17 S2, 19 S1, 24 S1, 27 S2, 28 S1, 29 S1, 30 S1, 33 S2, 35 S1, 37 S2, 38 S2	67.6 – 90.5
	Mild-cold	17 S2, 19 S1, 24 S1, 27 S2, 28 S1, 29 S1, 30 S1, 33 S2, 35 S1, 37 S2, 38 S2, 39 S2, 42 S2, 43 S2, 46 S2, 50 S2	80.6 – 98.7
	Cold	29 S1, 30 S1, 33 S2, 35 S1, 37 S2, 38 S2, 39 S2, 42 S2, 43 S2, 46 S2, 50 S2, 54 S1, 58 S1, 60 S1, 62 S1, 63 S1, 64 S2, 67 S2	87.7 – 128.1

† For additional information regarding the experimental seasons see Fig. 1 and Fig. S1

** We removed treatment 50 S2 from the class since the treatment was exclusive to pear

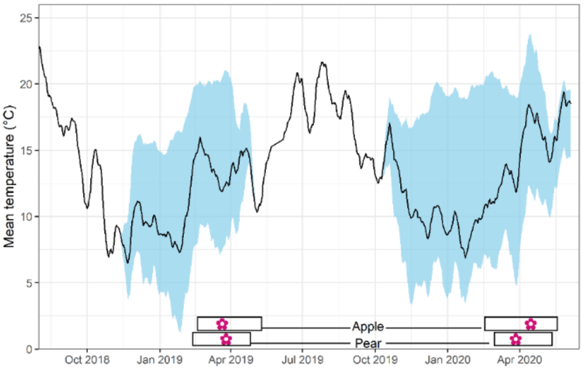

Fig. 2. Temperature observed during the experiment with 198 potted apple and 96 potted pear trees at Campus Klein-Altendorf during the winters of 2018/2019 and 2019/2020. The solid black line shows the mean temperature (using a 15-day running mean function) across treatments (33 each winter), whereas the sky blue shadings represent the range of mean temperature across treatments (temperature variation) for both winter seasons. The rectangles at the bottom indicate the flowering range of apple and pear trees after exposure to the temperature treatments. The pink flowers inside the rectangles indicate the median flowering dates.

represented data from trees in the 5th to 20th and the 10th to 25th warmest treatments, respectively. Finally, the Cold class represented data from trees exposed to the 22 treatments that received the most chill for apple and the 18 treatments with the most chill for pear. For each observation of bloom date in a class, we computed the mean temperature observed during both the chilling and forcing periods and generated a grid of 200 cells within the range of minimum and maximum values of the computed mean temperature in both phases. We interpolated bloom dates across this grid by defining bloom as a function of mean temperature during both the chilling and forcing phases. To this end, we used the Kriging function from the fields package (Nychka et al., 2017). Finally, we visualized the relationship through a surface contour plot.

2.7. *Data processing, PLS regression implementation and further analyses*

All data preparation and processing, the implementation of the PLS regression analysis and figure generation were done in the R programming environment (R Core Team, 2020). All data generated by this study, as well as the analyses we implemented, are available in a public repository (https://github.com/EduardoFernandezC/PLS_experiment_paper).

3. Results

3.1. *Temperature and phenology differences among experimental seasons*

Our experimental seasons produced substantial variation in temperature conditions during the dormancy period of apple and pear trees (Fig. 2). On January 26 for the first winter and January 23 for the second winter, we observed the greatest difference in mean temperature among experimental seasons (Fig. 2). On these days, the difference between the coolest and warmest treatments reached 15.1 and 15.4 °C for the first and second winter, respectively.

We observed a wide range of bloom dates in both species during the two winters. Across all treatments, apple trees showed full bloom from February 18 to May 10 (median date for full bloom on March 21) for the first winter and from February 17 to May 19 (median date for full bloom on April 15) for the second winter. The range of flowering dates between the earliest and the latest treatment was 81 days in the winter of 2018/2019 and 92 days in the winter of 2019/2020. In pear, full bloom was recorded across all treatments between February 12 and April 26 (median date for full bloom on March 26) and between February 29 and May 12 (median date for full bloom on March 27) for the first and second winter, respectively (Fig. 2). For both winters, the range between the first and last full bloom observations was 73 days. Trees exposed to warm conditions during both winters (treatments 1 S2 and 1 S1 in Fig. 1 in apple and pear) did not show full bloom.

3.2. *Impact of temperature variation on PLS regression outputs*

Our results suggest that PLS regression outputs are mainly influenced by variation in winter temperature (or chill and heat accumulation) across experimental seasons rather than the total number of seasons used in the analysis. Overall, we observed that using the number of experimental seasons associated with the greatest variation in temperature produced the most valuable PLS regression outputs (Fig. 3A). In apple, we noticed that 10 experimental seasons produced clearly identifiable phases for chill and heat accumulation. This was represented by continuous days displaying VIP values greater than 0.8 between mid-October and mid-January. Regarding pear, 13 experimental seasons were associated with the greatest variation in temperature (Fig. 3B). When we used the same number of seasons for pear, however, the PLS regression analysis suggested an interrupted chilling period characterized by about 15 days of non-relevant VIP values between November and December (Fig. 3A). When using the number of seasons related to the lowest variation in temperature across experimental seasons (4 seasons in apple and 6 seasons in pear), the VIP statistic allowed identifying chilling and forcing phases, but the standardized model coefficients, especially in apple, seemed erroneous (showing values of 0.3 for all days between mid-October and mid-January). For pear, VIP statistic and standardized model coefficients appeared comparable to the results observed when using 13 experimental seasons with the maximum temperature variation across seasons (Fig. 3A). When increasing the number of experimental seasons in the PLS regression analysis to 64 for apple and 30 for pear, temperature variation tended to decrease (Fig. 3B), and the chilling phase became more difficult to recognize (Fig. 3A). In apple, we observed intermediate periods showing VIP values below the importance threshold of 0.8. For pear, the chilling phase was shortened and limited to between mid-November and late December. In contrast, the forcing phase of both species was clearly

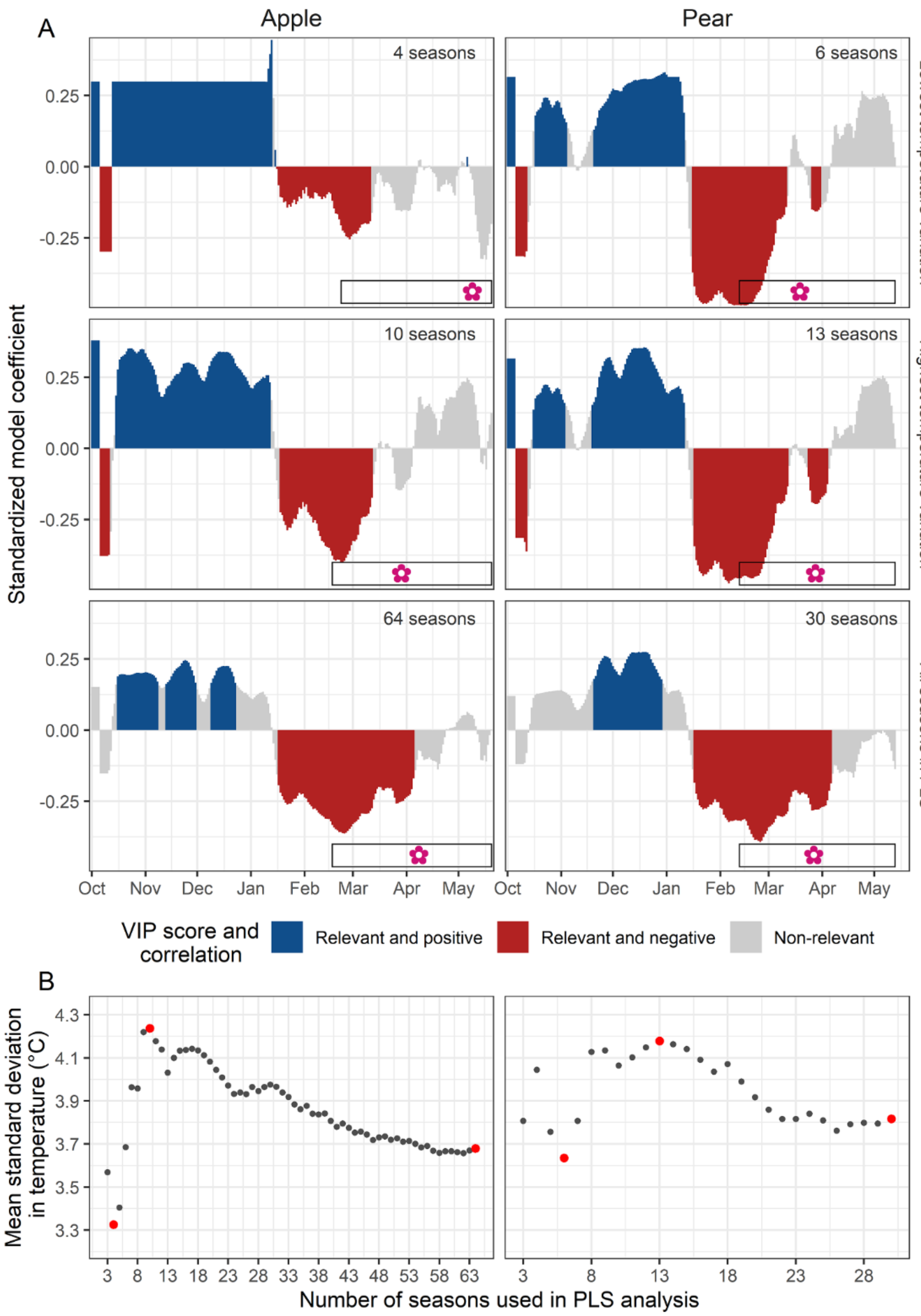

Fig. 3. Results of Partial Least Squares (PLS) regression between apple and pear bloom dates and mean temperature at Campus Klein-Altendorf, Germany. In 'A', we show the results of the PLS regression analysis using varying numbers of experimental seasons (4, 10, and 64 in apple and 6, 13, and 30 in pear) associated with different levels of variation (standard deviation) in mean temperature across experimental seasons when ordered from low to high chill accumulation (red dots in 'B'). In each panel, blue bars represent days with VIP statistic ≥ 0.8 and model coefficients > 0. Red bars indicate days with VIP statistic ≥ 0.8 and model coefficients < 0, and grey bars show days with VIP statistic < 0.8. The rectangle in the bottom-right corner in each panel indicates the range of bloom dates across experimental seasons. The pink flower in these rectangles indicates the median flowering date. In 'B', we present the temperature variation among experimental seasons included in our PLS regressions with increasing numbers of progressively cooler seasons. The y-axis shows standard deviation (in °C) in daily mean temperature, whereas the x-axis represents the respective numbers of seasons used in the PLS regression analysis ordered from low to high chill accumulation (from the 3rd to the 64th experimental season in apple).

recognizable when using all the treatments in the PLS regression analysis.

3.3. *Delineating the chilling and forcing phases from daily chill and heat accumulation*

After identifying the treatments that resulted in the best delineation of chilling and forcing phases when using mean temperature as independent variable, we delineated chilling and forcing phases for apple and pear trees through PLS regression analysis between bloom dates and daily chill and heat accumulation (Fig. 4). Using the number of seasons representing the greatest variation in mean temperature (10 seasons in apple and 13 seasons in pear), we determined chilling periods of October 19 - January 04 for apple and October 19 - December 27 for pear. The forcing period for both species was between January 16 and March 26. We identified a transition period between the chilling and forcing phases as January 05 - January 15 for apple and December 28 - January 14 for pear. These periods were characterized by days with VIP values < 0.8 and standardized model coefficients below zero.

Chill accumulation during the delineated chilling period in apple ranged from 3 (percentile 5% – P5%) to 53 (percentile 95% – P95%) Chill Portions (CP), with a median of 29 CP. These values were estimated using the 10 experimental seasons associated with the highest variation in temperature when analyzed through PLS regression. The wide range (3 to 53 CP) is explained by three out of the 10 experimental seasons only accumulating about 3 CP. These treatments were characterized by late exposure to chilling conditions after being in the heated greenhouse until mid-January (treatments 3 S2, 5 S2, and 6 S2 in Fig. 1). When excluding the three extreme low-chill treatments in apple, chill accumulation ranged from 25 (P5%) to 54 (P95%) CP, with a median of 43

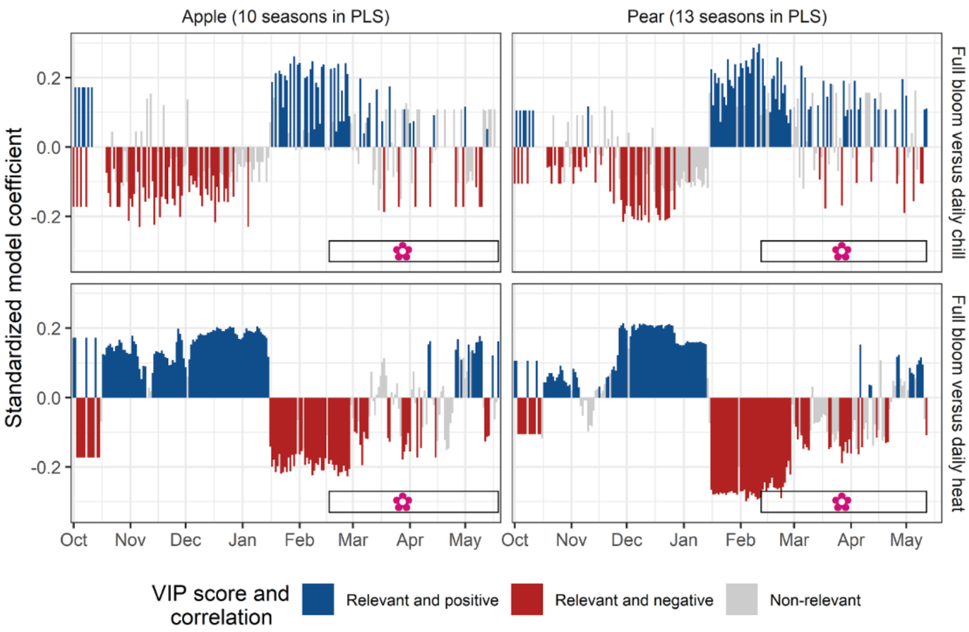

Fig. 4. Partial Least Squares (PLS) regression analysis between daily chill (upper panels) and heat (bottom panels) accumulation and median full bloom dates (stage BBCH 65 across three replicates per treatment) for apple and pear (columns). We show results for 10 experimental seasons in apple and 13 experimental seasons in pear, representing the highest variation (standard deviation) in mean temperature observed across seasons. In each panel, blue bars represent days with VIP statistic ≥ 0.8 and model coefficients > 0. Red bars indicate days with VIP statistic ≥ 0.8 and model coefficients < 0, and grey bars show days with VIP statistic < 0.8. The rectangle in the bottom-right corner in each panel indicates the range of bloom dates across experimental seasons. The pink flowers in these rectangles indicate the median flowering dates.

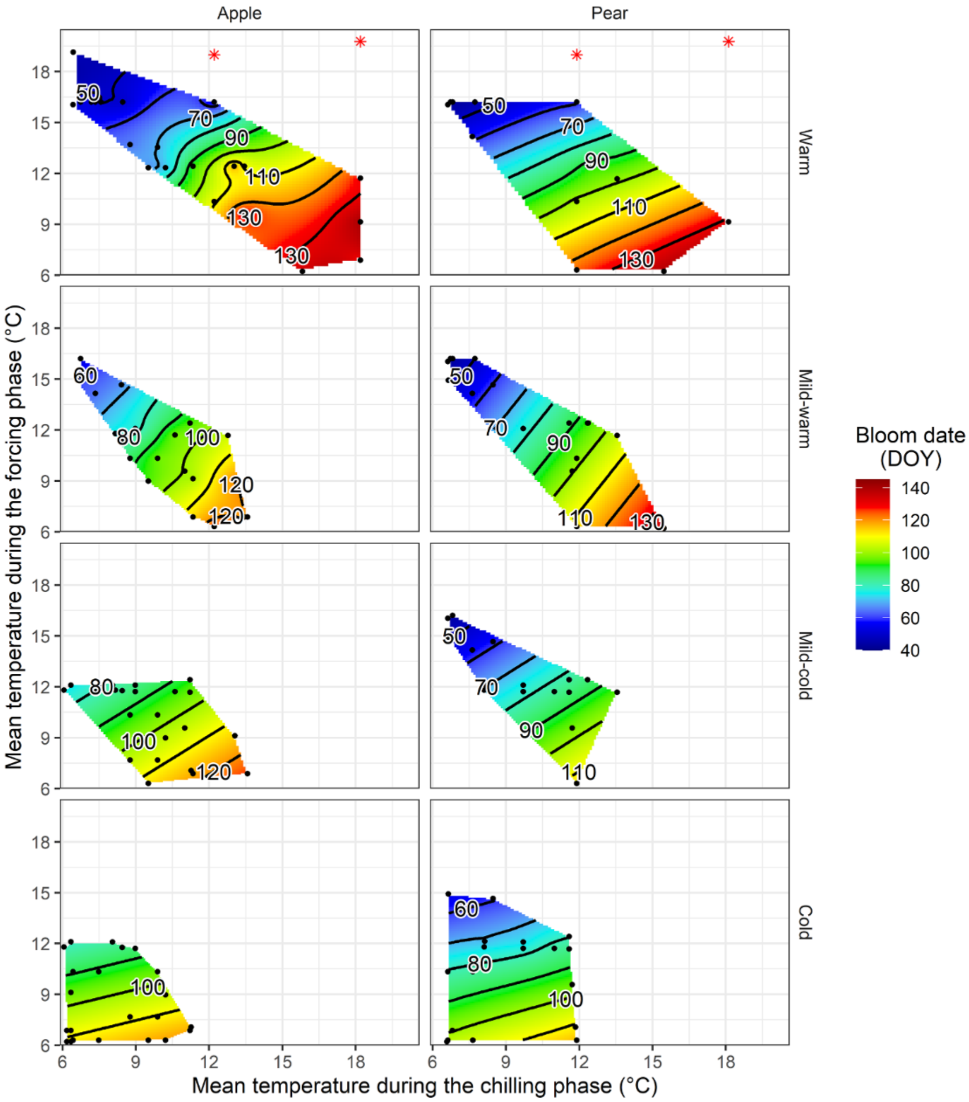

Fig. 5. Full bloom dates of three-year-old potted apple and pear trees plotted as a function of mean temperatures during the chilling (x-axis) and forcing phases (y-axis). Separate analyses were done for four sets of experimental seasons according to the chill accumulated by the trees during the winter period. In each panel, colors indicate full bloom dates (in day of the year – DOY). Contour lines represent the combination between mean temperatures during the chilling and forcing phases that yield similar full bloom dates. Black dots represent the actual bloom date observations. Red asterisks in the Warm class represent treatments that had not reached full bloom by the end of the experiment.

CP. Heat accumulation in the proposed forcing phase reached a median of 10,567 GDH, ranging from 4,913 (P5%) to 21,231 GDH (P95%). After excluding the three treatments previously indicated, heat accumulation ranged from 9,444 (P5%) to 22,973 (P95%) GDH (median of 14,845 GDH).

Regarding pear, chill accumulation in the respective phase across 13 experimental seasons representing the highest variation in temperature ranged from 11 (P5%) to 50 CP (P95%) with a median of 31 CP. Similar to apple, treatment 5 S2 in pear (Fig. 1) accumulated only about 3 CP during the delineated chilling period but received sufficient chill exposure later in the season. Median heat accumulation during the forcing phase in pear was estimated as 9,444 GDH (from 3,549 – P5% to 14,845 – P95%). When excluding the extreme low-chill treatment, chill accumulation ranged from 21 (P5%) to 50 (P95%) CP (median of 31 CP), whereas heat accumulation showed a median of 11,816 GDH (from 3,527 – P5% to 14,845 – P95% GDH).

3.4. *Bloom date response to mean temperature during chilling and forcing*

Overall, we detected a similar trend across species when plotting full bloom dates as a function of mean temperature during both the chilling and forcing phases (Fig. 5). Under low-chill conditions (Warm and Mild-warm classes), the relationship between the mean temperature during both phases and bloom dates was less linear in apple compared to pear. Under Warm conditions (between 12 and 18 °C in both phases), two treatments in apple and pear trees showed no bloom (Fig. 5).

The range of bloom dates differed greatly when different sets of treatments were analyzed. Under Warm conditions, trees showed bloom from February 12 to May 12 in the case of pear and February 18 to May 19 for apple. This range narrowed when colder treatments were analyzed. In the Cold class, bloom dates of pear ranged from February 26 to April 25 and March 10 to April 28 for apple. Similarly, the slope of the contour lines tended to decrease when colder classes were used in the analysis (Fig. 5).

4. Discussion

The experimental setup allowed us to generate a diverse collection of winter seasons that trees were exposed to during the dormancy period. Besides the variation in temperature across treatments, our experiment helped simulate diverse winter conditions that may represent conditions experienced currently or in the future in various growing regions of the world. For instance, treatment 3 S2, which consisted of late chill exposure (starting in mid-January), may represent future conditions for Mediterranean climate areas such as northern-central Chile, Tunisia, and Australia. In these regions, increasing temperatures might not only reduce seasonal chill accumulation (Benmoussa et al., 2020; Darbyshire et al., 2013; Fernandez et al., 2020b; Fernandez et al., 2020c; Luedeling et al., 2009) but also delay the beginning of the chilling period. Other treatments simulated intermittent warm periods within the dormancy phase, a situation that may arise in subtropical regions cultivating temperate fruit trees, such as southern Brazil (Anzanello et al., 2014). On the other hand, the treatments that were only exposed to field conditions may represent the current or even near-future winter conditions for the temperate region near Rheinbach, Germany. In this region, the winters of 2018/2019 and 2019/2020 were particularly warm compared to previous years, especially in February. The gradient of winter conditions generated a wide range of phenological responses in apple and pear trees. These data allowed us to delineate the chilling and forcing phases of apple and pear, as well as explore possible limitations of the PLS regression method, which have been mentioned in earlier studies analyzing long-term phenology records from several temperate fruit tree species (Benmoussa et al., 2017; Guo et al., 2013; Luedeling et al., 2013b). Together with the PLS regression approach, this experimental setup may offer an improved procedure for estimating climatic needs for new cultivars, for which no long-term observational records are available.

The chilling and forcing phases were clearly delineated using just a small share of the total number of experimental seasons (10 in apple and 13 in pear). This subset resulted in easily interpretable outputs of the PLS regression analysis between daily mean temperature and median bloom date. Luedeling and Gassner (2012) estimated that more than 20 years of phenology data are required for producing recognizable temperature response patterns. Subsequent studies, therefore, focused on analyzing long-term phenology data. For instance, 25 years of data were analyzed by Luedeling et al. (2013b), 33 years by Benmoussa et al. (2017), 46 years by Guo et al. (2013), and 52 years by Martinez-Lüscher et al. (2016). In our approach, however, using the total number of experimental seasons (comparable to 64 years of data for apples) tended to make the results of the PLS regression analysis less clear, e.g. leading to interruptions in the estimated chilling period. This number of experimental seasons was associated with a reduction in the temperature variation within the dataset (standard deviation reduced from 4.3 to 3.7 °C) compared to using 10 experimental seasons in apple and 13 experimental seasons in pear. It should be noted that we observed little difference in the output figures from pear when using the number of seasons associated to the highest and lowest variation in temperature across seasons. We hypothesize this may be explained by the difference between highest and lowest temperature variation (about 0.3 °C) being smaller compared to apple (about 1 °C), for which the difference was clear when using the seasons associated to highest and lowest temperature variation. Overall, our results may suggest that a smaller number of years with adequate variation within the dataset may be sufficient for delineating the chilling and forcing phases of temperate fruit trees through PLS regression. We highlight, however, that our experiment analyzed the response of young potted trees, which may differ from the response of adult trees.

On the other hand, excessive temperature variation within the dataset may produce some challenges for the effectiveness of this method in delineating the chilling and forcing phases of dormancy. Excessive variation arises from major differences between seasons, which may lead to shifts or strong differences in the duration and timing of chilling or forcing phases. In such cases, a particular period may be part of the chilling phase in one season and associated with the forcing phase in another season (Luedeling and Gassner, 2012). Such an effect may explain the results we observed when using the total number of experimental seasons in our approach, which included treatments that spent the beginning of the cold season in the greenhouse and only moved to chilling conditions late in the season. A similar phenomenon may arise when merging datasets from climatically different locations, as well as in long-term datasets that include substantial temperature changes in response to climate change. The interrupted chilling periods we observed in some of the analyses (especially between mean temperature and bloom dates) may arise from the method's limitation in considering strong shifting of phases across seasons as well as from a biological response of trees to warm spells during winter. From an ecological perspective, these interrupted chilling periods may suggest a potential acclimation of trees to short-term conditions. Further research is, however, still required to elucidate if these results arise from the limitations of the method or are a genuine response of trees to warm periods during dormancy. Researchers and practitioners using the PLS regression method should therefore be aware of the conditions under which the data were collected when interpreting their results.

The chilling period started on October 19 for both species and lasted until January 4 for apple and December 27 for pear. While there are no recent studies on pears, a recent study using the apple cv. 'Elstar' in the same location determined the end of the chilling period around January 11. The authors used the traditional experimental methodology of budgrowth determination after transferring shoots to warm conditions at various points in time during the winter (Fernandez et al., 2020a). Between January 05 and 15 for apple, and between December 28 and January 14 for pear, we detected a period of non-relevant days (with VIP

< 0.8), with increasing model coefficients (from about -0.2 to 0.2). A number of studies have suggested that during some part of the dormancy phase, chill and heat can compensate for each other and therefore the effectiveness of chill and heat accumulation may gradually drop and increase, respectively (Harrington et al., 2010; Pope et al., 2014). However, the extent of this relationship as well as its implications on the phenology of temperate fruit trees remain unclear.

We estimated that apple cv. 'Elstar' requires 43 CP and 14,845 GDH for bloom, and pear cv. 'Conference' needs 31 CP and 11,816 GDH for reaching the same development stage. These values were calculated after excluding treatments that only accumulated about 3 CP during the chilling phase of both species delineated through PLS regression. In the excluded treatments, chill exposure occurred after the chilling period delineated by the PLS analysis, but this situation may not have been reflected in the overall PLS regression output, because the remaining seasons in the analysis (the other 7 treatments) obscured the effect of warm seasons in the data set. In the case of apple, our HR estimations differed widely from previous studies reporting that only 5,000 GDH are required to reach full bloom in the same cultivar and location (Fernandez et al., 2020a). This difference may have resulted from Fernandez et al. (2020a) estimating HR under field conditions, which might be substantially cooler compared to our 10 experimental seasons representing the highest variation in temperature. Regarding CR estimations for this cultivar, our results are slightly lower compared to the 50 CP estimated by Fernandez et al. (2020a) in the same location working on adult trees with a traditional shoot experiment. In a PLS regression study, Diez-Palet et al. (2019) reported comparable results to those obtained in our experiment. The authors reported chill requirements ranging from 40 CP to 54 CP depending on the cultivar (their set of cultivars did not include cv. 'Elstar'). On the other hand, our estimate for the chill requirement is considerably lower compared to data determined under Australian climate conditions (Parkes et al., 2020). In that study, Parkes et al. (2020) determined the chill requirements of apple cultivars (also not including cv. 'Elstar') as between 55 and 80 CP by evaluating bud growth in shoots exposed to warm temperatures after a period of cold exposure. We are not aware of studies reporting chill (in CP) and heat requirements (in GDH) for pear trees that we could compare with our results.

Current knowledge supports the hypothesis that temperature during both the chilling and forcing phases of dormancy affect the moment of bloom in temperate trees (Guo et al., 2015a; Luedeling et al., 2013a). Our contour plots suggest that under warm conditions (Warm class) bloom dates depend on temperature during both phases. Under cold conditions (Cold class), the moment of bloom is mostly defined by temperatures during the forcing phase. The slope of the relationship tended to flatten when evaluating cooler treatments, and the range of bloom dates decreased considerably from warm to cold conditions. These results support the theoretical framework proposed by Guo et al. (2015a), who suggested that increasingly steeper slopes indicate that temperature variation during the chilling period gains influence on spring phenology, as temperatures during dormancy increase. Knowing the nature of the impact of temperature during chilling and forcing on bloom dates in a specific climate may help farmers focus on the main factor that is driving bloom timing, as well as generate management strategies that may be needed to overcome dormancy-related production problems.

5. Conclusions

Our experimental procedure helped boost the statistical approach usually applied for determining the climatic needs of temperate fruit trees. Together with PLS regression analysis and possibly in a single season, our experimental setup can offer an opportunity for breeders to obtain comprehensive estimates of the climatic needs of newly released cultivars, for which long-term records are unavailable. Providing farmers with reasonable estimates, obtained after exposing the trees to current as well as possible future environmental conditions, is likely to improve the transferability of these new developments into different growing regions that face similar climatic challenges. Similarly, farmers and orchard managers currently cultivating species and cultivars in temperate and Mediterranean regions can also benefit from this approach, since it allows analyzing the response of trees to unusually warm conditions. Finally, this experimental setup may be a promising tool for the development of improved phenology models that remain valid under future scenarios. These models are likely to perform better compared to currently available alternatives since their development should include data recorded under current conditions and conditions that are similar to projected future temperature regimes. Projecting climate change impacts on tree phenology with such models may help farmers and orchard managers adapt their systems to face future challenges.

Author contributions

Eduardo Fernandez, AK, and EL conceived the research idea. Eduardo Fernandez and PK implemented the experiment with the assistance of Erica Fadón and HD. Eduardo Fernandez and PK collected and analyzed the data. EL and AK guided and supervised Eduardo Fernandez and PK during the experiment. All authors discussed the results. Eduardo Fernandez and PK wrote the first draft with all remaining authors commenting on and improving the final version.

Declaration of Competing Interest

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

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Supplementary materials

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