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Differential responses of trees to temperature variation during the chilling and forcing phases☆

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ABSTRACT

Temperate-zone trees must fulfill cultivar-specific chilling and heat requirements during the dormant period, in order to produce leaves and flowers in the following growing season. Timing and accumulation rate of chill and heat are understood to determine the timing of spring events, but both processes are difficult to observe in dormant tree buds. Where long-term phenological observations are available, Partial Least Squares (PLS) regression offers a statistical opportunity to delineate phases of chill and heat accumulation and determine the climatic requirements of trees. This study uses PLS regression to explore how the timing of spring events of chestnut in China, cherry in Germany and walnut in California is related to variation in the daily rates of chill and heat accumulation, as calculated with horticultural models. Dependent variables were 39 years of flowering dates for chestnuts in Beijing (China), 25 years of cherry bloom in Klein-Altendorf (Germany) and 54 years of walnut leaf emergence in Davis (California, USA). These were related to daily accumulation rates of chill, calculated with the Dynamic Model, and heat, calculated with the Growing Degree Hours Model. Compared to an earlier version of the procedure, in which phenological dates were related to unprocessed temperature data, delineation of chilling and forcing phases was much clearer when using horticultural metrics to quantify chill and heat. Chestnut bloom in the cold-winter climate of Beijing was found to depend primarily on the rate of heat accumulation, while cherry bloom in the temperate climate of Germany showed dependence on both chill and heat accumulation rates. The timing of walnut leaf emergence in the mild-winter climate of California depended much more strongly on chill accumulation rates. Chilling (in Chill Portions = CP) and heat (in Growing Degree Hours = GDH) requirements determined based on PLS regression were 79.8 ± 5.3 CP and 13,466 ± 1918 GDH for chestnut bloom in Beijing, 104.2 ± 8.9 CP and 2698 ± 1183 GDH for cherry bloom in Germany, and 37.5 ± 5.0 CP and 11,245 ± 1697 GDH for walnut leaf emergence in California. Spring phases of cherry in Klein-Altendorf and especially chestnut in Beijing will likely continue to advance in response to global warming, while for walnut in California, inadequate chilling may cause delays in flowering and leaf emergence. Such delays could serve as an early-warning indicator that future productivity may be threatened by climate change. The R package ‘chillR’ makes the method used in this study available for wider use.

1. Introduction

Plants in temperate climates have responded to recent climatic changes (Chmielewski and Rötzer, 2001; Menzel et al., 2006; Parmesan and Yohe, 2003). Spring phases in particular have been affected, with leaf emergence and bloom of many species occurring significantly earlier now than a few decades ago (Fitter and Fitter, 2002), and showing clear advancing trends (Parmesan, 2007). However, there are notable exceptions to these developments (Cook et al., 2012). At some locations, for vegetation at high altitudes (Yu et al., 2010, 2012) and high latitudes (Frich et al., 2002; Kozlov and Berlina, 2002), spring phases have in fact occurred later in recent years, in spite of clear warming trends. Some fruit trees grown in areas that are substantially warmer than their native habitat have also responded with delayed spring phases to extraordinarily warm conditions during the winter (Elloumi et al., 2013). The

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most likely explanation for these exceptions to common trends is the need for climatic cues during plant dormancy (Campoy et al., 2011; Saure, 1985). Especially perennial plants from temperate and cold climates, but also seeds of many annuals, undergo dormancy in winter to avoid frost damage to sensitive tissue (Vegis, 1964). Perennial plants lose their leaves and reduce physiological activity substantially to withstand cold winter temperatures (Saure, 1985). Come spring, plants must emerge from this protective state, develop leaves and flowers, and resume full physiological activity. Temperature has been found to be the most important environmental variable influencing plants' progression through dormancy (Campoy et al., 2011; Luedeling, 2012), possibly modulated by daylength for many species (Blümel and Chmielewski, 2012). It is commonly assumed that two separate temperature responses are responsible for ending dormancy in perennial species: a chilling requirement, which is fulfilled by a phase of cool conditions, and a heat requirement (forcing), which is satisfied by high temperature (Luedeling et al., 2009f).

Depending on the particular climatic requirements of species and the location where they are grown, increasing temperatures may not necessarily have a phenology-advancing effect (Luedeling, 2012). While warming during the heat accumulation (forcing) phase almost certainly advances budbreak and flowering, increasing temperatures during the previous chilling phase may have the opposite effect (Guo et al., 2013; Luedeling and Gassner, 2012; Luedeling et al., in press). If chill accumulates more slowly during the winter, chilling requirements may be fulfilled later, delaying heat accumulation and ultimately leading to later bloom or leaf emergence. This effect can be compensated, at least in part, if warming occurs during both the chill and heat accumulation phases. Heat is then accrued faster in spring, so that plants may be able to make up for delays during the chilling phase (Luedeling, 2012). Strongly delayed spring phases can also be observed, when chilling requirements are not completely fulfilled (Erez, 2000). At least for some species, greater heat accumulation can then compensate for insufficient winter chill (Harrington et al., 2010).

Several recent studies have shown that winter chill has been declining in many temperate and subtropical regions throughout the world and is likely to continue decreasing in the future (Baldocchi and Wong, 2008; Darbyshire et al., 2011, 2013; Luedeling, 2012; Luedeling et al., 2009b,d, 2011a). This trend will almost certainly affect perennials from temperate and cool subtropical locations that depend on winter chill for completion of their annual cycles. For fruit and nut trees in particular, these developments could precipitate into substantial problems in the future that will require many growers to adapt by revising their choice of tree species or cultivar (Darbyshire et al., 2011, 2012; Luedeling et al., 2009b,d). Unfortunately, prominent knowledge gaps currently constrain the ability of researchers and growers to project impacts of climate change on tree phenology (Campoy et al., 2011; Dennis, 2003; Luedeling, 2012). Models commonly used to quantify winter chill are fairly inaccurate (Luedeling and Brown, 2011; Luedeling et al., 2009e), and there is considerable uncertainty about the chilling and heat requirements of species and cultivars. It has also been shown that chilling requirements estimated with common models may not be valid in a different climate or at a location that is different from where the trees' climatic needs were determined (Luedeling and Brown, 2011; Luedeling et al., 2009f). Furthermore, it is unclear, which phases during the dormancy season are relevant for chill and heat accumulation. The main reason for these knowledge gaps is the lack of visible indicators for chill and heat accumulation during dormancy. While some change occurs in buds during this period, it is difficult to detect and does not as yet allow clear differentiation between chill and heat accumulation phases.

Where long-term temperature and phenology records are available, Partial Least Squares (PLS) regression has recently been used to statistically determine and visualize different stages during dormancy progression (Luedeling and Gassner, 2012; Luedeling et al., in press). This method, which is widely used in the fields of chemometrics (Wold, 1995) and hyperspectral remote sensing (Luedeling et al., 2009c; Min and Lee, 2005), allows the establishment of correlations between a large number of independent factors and one or several dependent variables. The procedure is based on latent factors, a form of principal components, which are constructed as linear combinations of the independent variables and then correlated with the dependent variables (Wold, 1995; Wold et al., 2001). In contrast to more conventional regression approaches, this strategy avoids problems with autocorrelation between independent variables, and it works well even when the number of independent variables substantially exceeds the number of observations. PLS regression thus allows the correlation of daily temperatures with spring phenological dates. The resulting models describe the effects of deviation from normal temperature conditions during the winter on phenological dates.

In previous studies, distinct temperature response phases have been detected by PLS regression for walnuts in California (Luedeling and Gassner, 2012; Luedeling et al., in press), for cherries in Germany (Luedeling et al., in press) and for chestnuts in China (Guo et al., 2013). In all of these studies, daily temperatures were used as independent variables. The results from these analyses provided valuable clues about temperature response timing during dormancy. However, they did not consider quantifications of the temperature-dependent effectiveness of chill and heat effects, which have been derived by horticultural researchers from experiments and observations (Anderson and Seeley, 1992; Fishman et al., 1987a,b). In this study, we refine the PLS approach by relating spring phenology dates not directly to day-to-day variation in temperature, but to daily accumulation of chill and heat units according to common horticultural models. This additional analysis step helps define chilling and forcing periods during dormancy more clearly than earlier approaches. We use the resulting delineations to update earlier estimates of chilling and forcing requirements.

2. Materials and methods

2.1. Phenology and temperature records

Three phenology datasets were used in the analysis. Bloom dates of chestnuts were collected from the garden of the former Royal Summer Palace in Beijing, China (40.02°N, 116.33°E, 50 m a.s.l.) (Guo et al., 2013). The dataset contains data for 39 years between 1963 and 2008 (no observations were available for 1969–1971 and 1997–2000). Daily minimum and maximum temperature were sourced from an official weather station in Beijing, just 2.5 km from the observation site.

Bloom data of cherry cv. 'Schneiders späte Knorpelkirsche' were collected at Klein-Altendorf, Germany, at the research station of the University of Bonn (50.40°N, 6.99°E, 160 m a.s.l.). Twenty-five years of observations were available, collected between 1984 and 2008 (no observations were available for 1985) (Luedeling et al., in press). Daily weather data were recorded on the same station (Blanke and Kunz, 2009; Luedeling et al., 2011b).

Phenology data for several walnut cultivars have been recorded at the University of California Davis, USA (38.54°N, 121.78°E, 20 m a.s.l.) since 1953. We selected leaf emergence dates of the cultivar 'Payne' as the spring phenology indicator with the greatest number of available observations. Data for 54 years between 1953 and 2007 were available (no observations for 1996) (Luedeling et al., 2009f). Daily weather data were obtained from the university's weather station, which is located on the same property as the experimental walnut orchard.

2.2. Data processing

For all weather records, short gaps in daily minimum and maximum temperature data were closed by linear interpolation between temperatures on the two days directly before and after the gap. This was not necessary for the records from Beijing or Davis, but for 14 daily values (11 minimum and 3 maximum temperatures) in the data from Klein-Altendorf. Since all common chill models and many heat models require input of hourly rather than daily temperature records, idealized daily temperature curves with an hourly resolution were constructed from the daily temperature extremes. The procedure followed recommendations by Linvill (1990) and was based on a sine curve for daytime warming and a logarithmic decay function for nighttime cooling. These equations require information on sunrise and sunset timing as well as daylength, which were modeled according to procedures given by Spencer (1971) and Almorox et al. (2005), using geographic latitude of the study locations as the only input parameter.

Based on the resulting hourly temperature records, we calculated continuous winter chill and heat accumulation for the entire study periods. For chill quantification, we applied the Dynamic Model (Erez et al., 1990; Fishman et al., 1987a,b), which has consistently been shown to outperform all other common models in most locations (Balandier et al., 1993; Campoy et al., 2011; Darbyshire et al., 2011; Guo et al., 2013; Luedeling et al., 2011b, in press, 2009f). This model is the only commonly used chill model that includes a synergistic effect of moderate temperatures during the chilling phase, which have been shown to increase the rate of chill accumulation in controlled experiments (Couvillon and Erez, 1985).

For heat quantification, we followed the Growing Degree Hours approach by Anderson et al. (1986). Equations for the Dynamic Model are given, for example, in Luedeling and Brown (2011) or Darbyshire et al. (2011) and equations for the Growing Degree Hours model are reproduced in Luedeling et al. (2009f). After producing continuous records of chill and heat accumulation over the entire lengths of the study periods, these were smoothed by applying a 15-day running mean function. This was necessary to ensure that recognizable patterns are produced by the PLS analysis (Luedeling and Gassner, 2012). In the case of the Dynamic Model, the model structure provided a second reason for smoothing of the daily values. An important assumption of the Dynamic Model is that chill accumulation involves two steps. In the first step, an intermediate chill substance or structure is formed by cool temperatures. In the second step, moderate temperatures convert this intermediate product into a permanent Chill Portion, but this step depends on availability of a critical quantity of the intermediate product. In effect, Chill Portions are not formed continuously, even when temperatures are in the optimal chilling range, but their accumulation occurs in steps. Smoothing eliminates the discontinuities in chill accrual that this model structure produces. The need for recognizable patterns, combined with the need to eliminate the stepwise chill accumulation by the Dynamic Model made us extend the running-mean period to 15 days, compared to 11 days used in earlier studies, where PLS analysis was based on unprocessed temperature data (Luedeling and Gassner, 2012). Following smoothing, all hourly chill and heat data were then assigned to phenological years. These years started with the calendar month that succeeded the last month during which bloom or leaf emergence was observed throughout the length of the record. These months were July for chestnuts in Beijing and June for cherries in Germany and walnuts in California.

2.3. Partial Least Squares (PLS) regression

For each species and each phenological season, all daily values of heat and chill accumulation were then related to observed phenological dates. For each year of the record for each species, the resulting datasets consisted of 365 daily chill values (ignoring 31st December in leap years), 365 daily heat values and one phenological date. Thirty-nine such data series were available for chestnuts in Beijing, 54 for walnuts at Davis and 25 for cherries at Klein-Altendorf. Partial Least Squares regression was then used for detecting correlations between the 730 independent (365 daily chill and 365 daily heat values) and one dependent (spring phenology indicators) variables.

In the PLS procedure, latent factors were constructed from all independent variables, and these were then used to build a regression model for explaining variation in the dependent variables (Wold et al., 2001). PLS regression produces two major outputs: the variable-importance-in-the-projection (VIP) statistic, which is calculated for each independent variable, indicates how important variation in the variable is for explaining variation in the dependent variable. A threshold of 0.8 is commonly assumed to signify importance (Wold et al., 2001), and we adopt this threshold for the present study. The second major output contains the model coefficients for all independent variables. Where these are positive (and high VIP scores indicate importance), positive deviation in the independent variable is correlated to late occurrence of a phenological event. If the coefficient is negative, high chill or heat values for the respective day are correlated to early timing of spring events.

2.4. Interpretation of PLS outputs

Based on ecological theory, a high rate of chill accumulation during the chilling period should be related to early bloom or leaf emergence (given similar rates of heat accumulation during the forcing period), because it advances the time when the chilling requirement is fulfilled. Similarly, a high rate of heat accumulation during the forcing period (heat accumulation phase) should advance spring phases. The output of the PLS analysis should thus display the following properties: in the first part of the dormancy season, when chill is accumulated, daily chill contributions should be assigned high VIP values and negative model coefficients, meaning that rapid chill accumulation is related to early spring phenology dates. Following this period, or potentially slightly overlapping, should be a phase, during which the same patterns are shown by the heat accumulation records. Based on these expectations, PLS outputs were interpreted to delineate likely chilling and forcing periods. For these periods mean annual chill and heat accumulation were calculated, providing estimates of chilling and heat requirements of the study species. To illustrate temperature responses of the three species, we plotted spring phenology dates in relation to mean temperatures for the chilling and forcing periods identified by PLS regression. In the resulting three-dimensional plots, surfaces of spring phenology dates were interpolated using the Kriging technique. Kriging is a frequently used method in spatial statistics, in which the spatial dependence between measured data points is described by a semivariogram. This information is then used to estimate values at locations where no measurements are taken (Oliver and Webster, 1990). In our application of this technique, spatial coordinates (e.g. latitude and longitude) are substituted by chill and heat values.

2.5. Data analysis implementation

All analysis steps were implemented in the package ‘chillR’ (Luedeling, 2013; Luedeling et al., in press) for R statistical software (R Development Core Team, 2011). It draws heavily from the ‘pls’

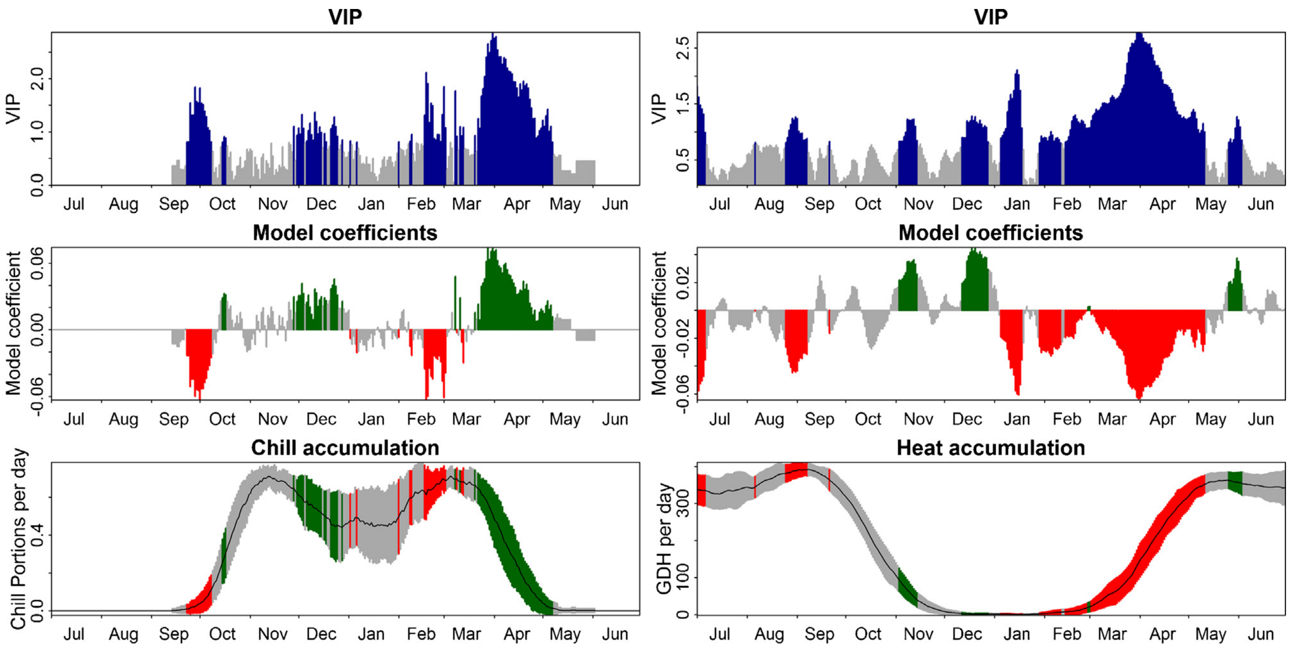

Fig. 1. Results of the PLS regression analysis for chestnuts in Beijing, China. Variable-importance-in-the-projection (VIP; top row), model coefficients (middle row) and accumulation rates (in Chill Portions and GDH = Growing Degree Hours) (bottom row) are shown for daily chill (left) and heat (right) accumulation. Blue bars (black in the print version) in the top row mean that VIP was above 0.8, indicating that the respective variables are important for the model. The same bars are colored green (light gray), when model coefficients are positive, and red (black), when they are negative, in the other rows. In the bottom row, the gray, red (black) and green (light gray) bars indicate the standard deviation of daily chill/heat over the entire length of the temperature records.

package (Mevik et al., 2011) for the PLS analysis and from 'fields' (Furrer et al., 2012) for production of three-dimensional spring phenology plots. Code for reproducing all analyses, as well as an abridged version of the cherry dataset, are provided as supplementary materials to this paper.

3. Results

3.1. Chestnuts in Beijing

For chestnuts in Beijing, the chilling phase was recognizable, but the climate response patterns during the likely chilling period differed slightly from what was expected (Fig. 1). Instead of continuously negative model coefficients, some phases occurred, during which high rates of chill accumulation were related to late occurrence of bloom (green bars in Fig. 1). This response may be related to the frequent incidence of temperatures below the freezing point, which are not typically considered as effective for chill accumulation, or it may indicate shortcomings of the Dynamic Model for chill quantification. It seems likely that two short phases in September/October and February indicate the beginning and end of the chill period. High chill accumulation during these two phases was related to early bloom dates. Between these periods, temperatures may be close to optimal for chill accumulation, so that variation then is of subordinate importance. The heat accumulation phase was clearly visible, showing as a near-continuous period of negative model coefficients for the daily heat variables between early January and early May. Mean bloom dates occurred on 1st June, shortly after the end of this period. Based on the output of the PLS regression, most likely start and end dates are 23rd September and 2nd March for the chilling period and 5th January and 11th May for the forcing period of chestnut in Beijing.

3.2. Cherries in Germany

Compared to results for chestnuts in Beijing, computed patterns were quite similar for cherry in Klein-Altendorf, where two phases of important negative relationships between chill accumulation rates and bloom dates were visible in September and February (Fig. 2). These likely indicate the beginning and end of the chilling period. Important negative coefficients for heat accumulation appeared in February and lasted until mid-April. Mean bloom date of cherry was April 21st, explaining the loss of correlation between high heat and early bloom around this time. The chilling phase likely extended from 16th September to 4th March and the heat accumulation phase from 13th February to 13th April.

3.3. Walnuts in California

The clearest delineation of chilling and forcing phases was achieved for walnuts in California. During what is likely the chilling period, model coefficients were almost continuously important and negative for the daily chill variables. This phase extended from late October to as early as late December (Fig. 3) in the mild climate of Northern California. Subsequently, from mid-January, heat accumulation rates had consistently important and negative model coefficients until early April. After this, heat variables were rarely important. Since walnut leaves emerged in Davis, on average, on March 17th, this is not an unexpected result. Chill was likely accumulated between 25th October to 2nd January and heat from 18th January to 5th April.

3.4. Chilling and heat requirements

Based on the outputs of the PLS regression, likely chilling and forcing periods were delineated (Table 1). The mean numbers of Chill Portions and Growing Degree Hours accumulated between these dates are interpreted as being rough approximations of the species' chilling and heat requirements at the respective locations. According to these criteria, chilling requirements were 79.8 ± 5.3 Chill Portions (CP) for chestnuts at Beijing, 103.5 ± 9.0 CP for cherries at Klein-Altendorf and 37.5 ± 5.0 CP for walnuts at Davis. Required heat was 13,466 ± 1918 GDH for chestnuts in Beijing,

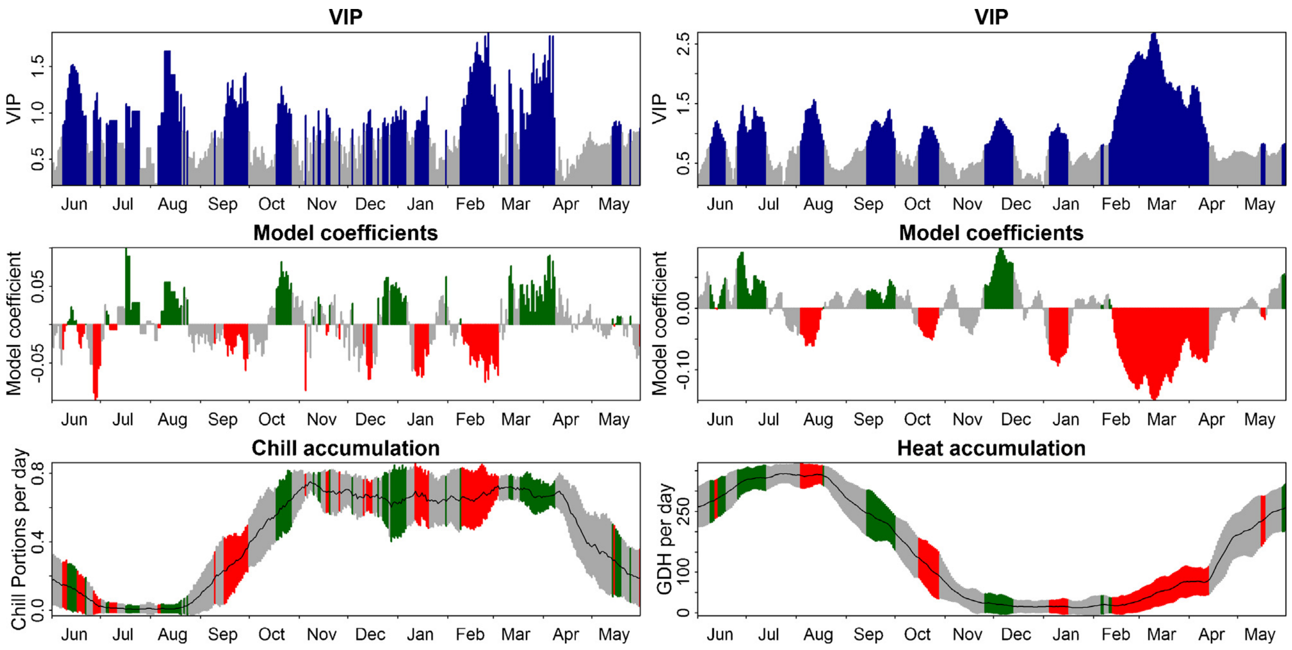

Fig. 2. Results of the PLS regression analysis for cherries in Klein-Altendorf, Germany. Variable-importance-in-the-projection (VIP; top row), model coefficients (middle row) and accumulation rates (in Chill Portions and GDH = Growing Degree Hours) (bottom row) are shown for daily chill (left) and heat (right) accumulation. Blue bars (black in the print version) in the top row mean that VIP was above 0.8, indicating that the respective variables are important for the model. The same bars are colored green (light gray), when model coefficients are positive, and red (black), when they are negative, in the other rows. In the bottom row, the gray, red (black) and green (light gray) bars indicate the standard deviation of daily chill/heat over the entire length of the temperature records.

2698 ± 1183 GDH for cherries at Klein-Altendorf and 11,245 ± 1697 GDH for walnuts at Davis.

3.5. *Spring phenology responses to chilling and forcing temperatures*

Relating the timing of phenological responses to mean temperatures during the chilling and forcing periods revealed important differences between the species, which provided information about their sensitivity – at the particular sites analyzed here – to warming during dormancy. These responses could range between two extremes: (1) spring phenology is only determined by temperature during the forcing phase, with warming causing an advance; and (2) spring phenology is only determined by temperatures during the chilling phase, with warming causing a delay. In the plots of Figs. 4–6, the first case would be shown as horizontal contour lines and clear color gradients in vertical direction. In the second extreme case, with a dominant influence of temperatures during chilling, vertical contour lines and horizontal color gradients would be expected.

Chestnuts in Beijing (Fig. 4) and cherries in Germany (Fig. 5) were responsive to temperature variation during both the chilling and forcing periods, indicated by diagonal contour lines in the respective figures. Flowering occurred earliest, when a cool chilling phase was followed by a warm forcing phase, placing these dates in the upper left corner of the plots. Conversely, the bottom right edge of the area spanned by conditions observed in the past was characterized by late flowering dates. For these species at these locations, projections of future phenology should consider both effects.

For walnuts in California, temperatures during the chilling period were more clearly related to leaf emergence dates than conditions during the forcing period, indicated by strong color gradients in horizontal direction in Fig. 6. Even during some years with very warm springs, leaves did not emerge particularly early, when the chilling phase was not cool enough. Walnuts in California thus appear more likely to show delayed spring phenology in response to warming than cherries in Germany or chestnuts in Beijing.

4. Discussion

4.1. *Usefulness of the analysis procedure*

Compared to results from earlier studies (Guo et al., 2013; Luedeling and Gassner, 2012; Luedeling et al., in press), the inclusion of chill and heat models in preparing data for PLS analysis allowed much clearer delineation of chilling and forcing phases. PLS results from the earlier studies showed some relationships between summer temperatures and spring phenology in the following year, which the present study does not highlight. While these may be real effects, possibly related to dormancy induction

Table 1

Estimated start and end dates of the chill and heat accumulation periods of chestnuts in Beijing, China, cherries in Klein-Altendorf, Germany and walnuts in Davis, CA, USA, and estimates of their chilling and heat requirements. CP = Chill Portions; GDH = Growing Degree Hours. For each climatic requirement, the standard deviation of historic accumulations between the given dates is provided as an approximation of the error.

Species/location	Chilling period			Forcing period		
	Start	End	Requirement	Start	End	Requirement
Chestnut/Beijing	23rd Sep	2nd Mar	79.8 ± 5.3 CP	5th Jan	11th May	13,466 ± 1918 GDH
Cherry/Klein-Altendorf	16th Sep	4thMar	104.2 ± 8.9 CP	13th Feb	13th Apr	2698 ± 1183 GDH
Walnut/Davis	25th Oct	2nd Jan	37.5 ± 5.0 CP	18th Jan	5th Apr	11,245 ± 1697 GDH

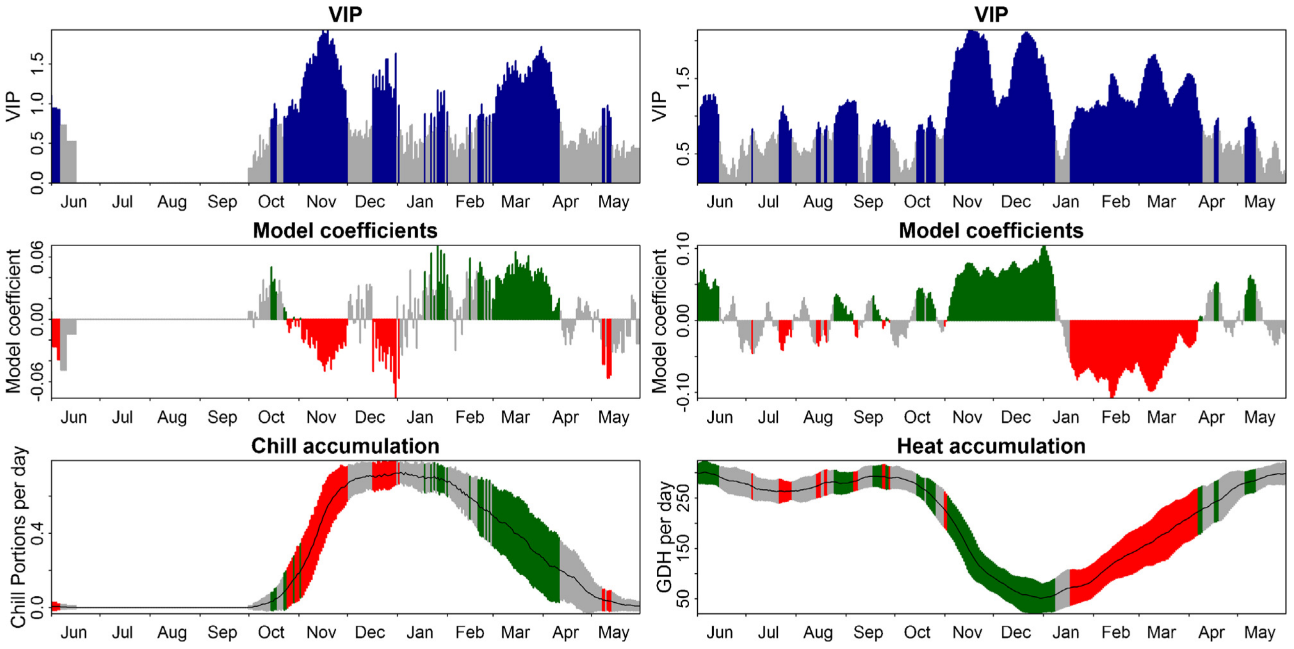

Fig. 3. Results of the PLS regression analysis for walnuts at Davis, California. Variable-importance-in-the-projection (VIP; top row), model coefficients (middle row) and accumulation rates (in Chill Portions and GDH = Growing Degree Hours) (bottom row) are shown for daily chill (left) and heat (right) accumulation. Blue bars (black in the print version) in the top row mean that VIP was above 0.8, indicating that the respective variables are important for the model. The same bars are colored green (light gray), when model coefficients are positive, and red (black), when they are negative, in the other rows. In the bottom row, the gray, red (black) and green (light gray) bars indicate the standard deviation of daily chill/heat over the entire length of the temperature records.

(Tanino et al., 2010), they are unlikely to indicate the beginning of chill accumulation. Use of Chill Portions in the analysis effectively restricted the detection of chill effects to the cooler parts of the year, because high temperatures preclude accumulation of chill during the summer. This seems reasonable for estimating chilling requirements. Some significant effects of heat during the summer months were still indicated by the PLS regression. These may be related to dormancy initiation, but should have little to do with the breaking of ecodormancy. These effects are therefore not discussed further. Another advantage of substituting temperature with a chill model was the better handling of low temperature effects. When pure temperatures are used as inputs for the PLS procedure during

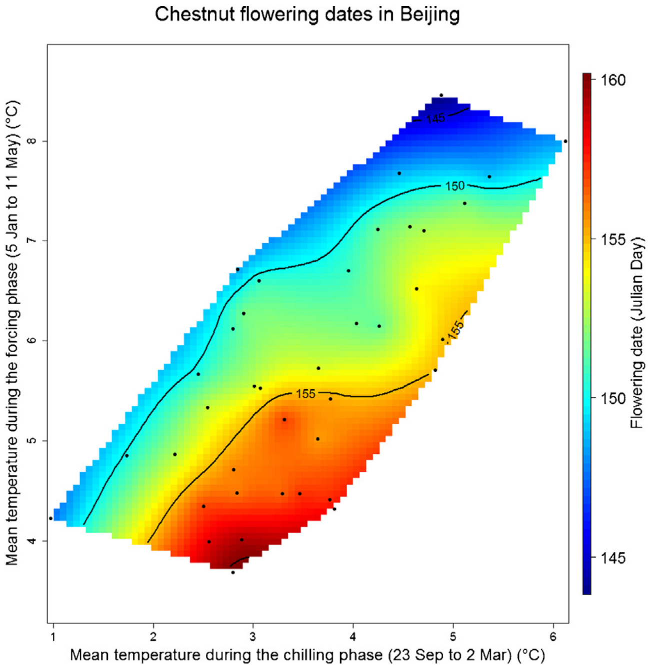

Fig. 4. Relationship of flowering dates of chestnuts in Beijing with mean temperatures during the chilling (*x*-axis) and forcing (*y*-axis) periods. Colors (shades of gray in the print version) and contour lines indicate the timing of phenological events, expressed in Julian Days (days of the year). Black dots indicate observed data points.

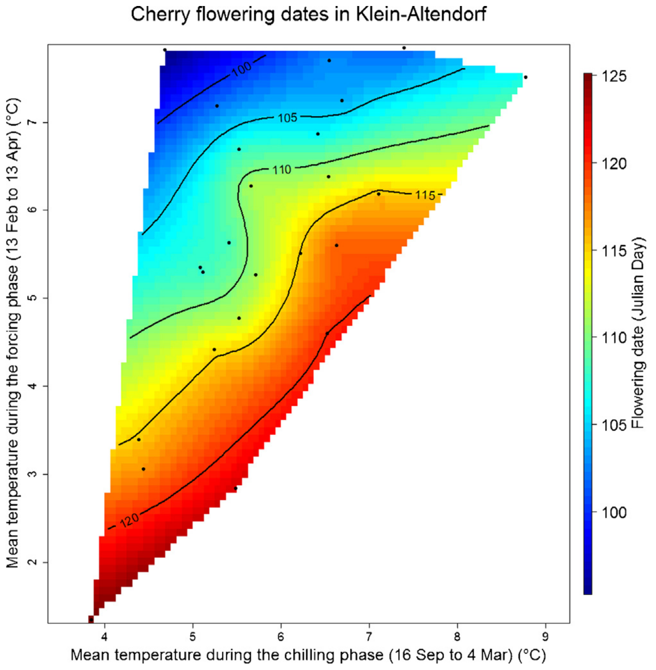

Fig. 5. Relationship of flowering dates of cherries in Germany with mean temperatures during the chilling (*x*-axis) and forcing (*y*-axis) periods. Colors (shades of gray in the print version) and contour lines indicate the timing of phenological events, expressed in Julian Days (days of the year). Black dots indicate observed data points.

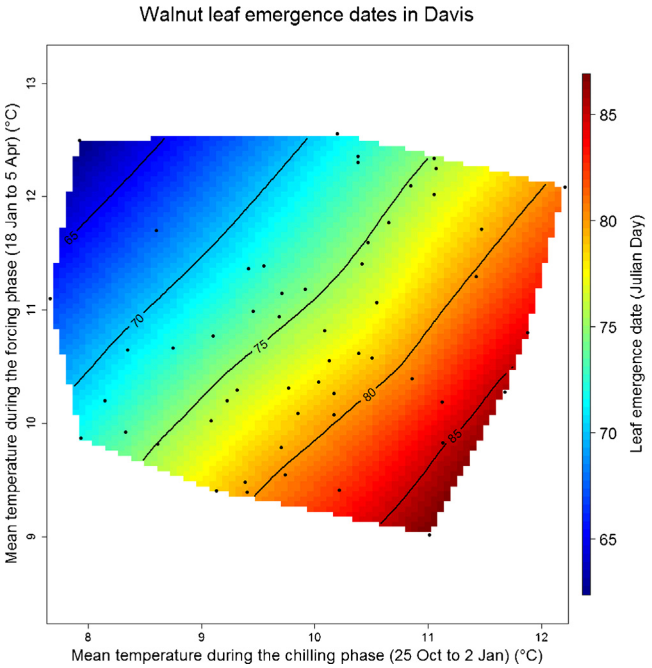

Fig. 6. Relationship of leaf emergence dates of walnuts in California with mean temperatures during the chilling (*x*-axis) and forcing (*y*-axis) periods. Colors (shades of gray in the print version) and contour lines indicate the timing of phenological events, expressed in Julian Days (days of the year). Black dots indicate observed data points.

periods with temperatures below the freezing point, high temperatures should have a positive effect on chill accumulation rates, because no (or little) chill is assumed to accrue during freezing conditions. This effect would then run counter to what may happen during warm winters, when warming should reduce chill accumulation rates. PLS analysis based on temperature alone cannot distinguish between these effects, leading to unclear relationships between winter temperatures and spring phase timing. Use of a chill model translates temperature into what horticultural scientists have come to understand as chill effectiveness for the trees. It seems more reasonable that spring phase timing should be related to such an indicator rather than to temperature, and this is reflected in clearer delineation of temperature response phases in the PLS outputs.

Even when using chill and heat units calculated by horticultural models, PLS regression still indicated importance of certain phases that were clearly outside the range where effects of chill or heat appear plausible. For example, between March and May, high chill accumulation rates were related to late bloom of chestnuts in Beijing. This is almost certainly not a chill-related effect, but rather due to a phenology-advancing effect of a high heat accumulation rate. Since chill and heat are inversely correlated, the temperature response appears both on the chilling plot (high chill related to late bloom) and on the heat plot (high heat related to early bloom). Since the temperature ranges, at which chill and heat accumulate, overlap substantially, this effect can probably not be avoided with the current methodology. Interpretation of PLS results should therefore be guided by at least a conceptual understanding of major temperature responses during dormancy, rather than on consideration of every variable that was labeled as important according to the VIP scores.

4.2. *Phenology responses to temperature during dormancy*

For walnuts in California, results of the PLS procedure corresponded very closely to what one might expect, based on commonly applied concepts about plant dormancy. High chill accumulation rates during October through December were related to early leaf emergence. About two weeks after this time window, a strong phenology-advancing effect of heat accumulation became apparent, which remained effective approximately until leaves emerged. The short period of unclear effects between the chilling and forcing phases was probably due to variation between years in timing of the transition from one phase to the other. These results are in line with the commonly used sequential chill-heat model of dormancy progression, in which defined chilling requirements are first fulfilled, followed by accumulation of a defined amount of heat. There was no indication that the chill or heat models were inadequate for walnuts in California.

Results for the other two species, chestnuts in Beijing and cherries at Klein-Altendorf, were less in line with expectations. It seems plausible that the beginning and end of the chilling phase were defined by negative important model coefficients for chill accumulation, signifying an advance in spring phases in response to high chill accumulation rates. Yet this response was not visible for the remainder of what appeared to be the chilling period. Correlations between chill accumulation then and spring phase timing were inconsistent and mostly unimportant. We suspect that this finding is related to problems of using the Dynamic Model for quantifying chill in Beijing and Klein-Altendorf. During the winter, these locations frequently experience temperatures below the freezing point, which rarely occur in the regions, for which the Dynamic Model was developed (Israel and other subtropical locations). It therefore seems likely that the effects of cold temperatures are not adequately described. It is also possible that chill accumulation rates are homogeneous across a wider range of temperatures than the Dynamic Model assumes. If chill accumulation is actually similar within a certain temperature range, but appears to vary according to the model, this variation cannot be expected to influence phenology timing. There may be other reasons for the lack of correspondence of model results with expectations, but given the knowledge gaps in quantifying temperature responses during dormancy, we will refrain from further speculation. In spite of these difficulties in interpreting PLS results, estimates of plant response timing nevertheless seem plausible. The PLS results also provide entry points for future improvement to chill models, as well as for physiological and genetic studies on chill and heat sensing by trees.

In contrast to the chilling phase, the forcing phase was very clearly characterized by consistently important negative model coefficients for the heat accumulation rate. This is in accordance with the expectation that fast heat accumulation should advance spring phenology. It may also indicate that heat accumulation is better approximated by the Growing Degree Hour model than chill accumulation by the Dynamic Model.

For both chestnuts in Beijing and cherries in Klein-Altendorf, apparent chilling and forcing periods overlapped substantially, by 57 and 20 days, respectively. This finding indicates that chill and heat accumulate in parallel during part of the dormancy period, and that parallel phenology models are more appropriate than sequential approaches for both species in their respective locations.

4.3. *Chilling and heat requirements*

Species differed significantly in their chilling and heat requirements. The highest chilling requirement was identified for cherries in Germany, with 104.23 ± 8.94 Chill Portions. This requirement is higher than an earlier estimate based on a PLS analysis of temperature data alone, which indicated the need to accumulate 68.6 ± 5.7 Chill Portions (Luedeling et al., in press). We believe that the new figure is closer to the truth, because processing of temperatures into Chill Portions allowed for a much clearer identification of chilling phases than the procedure used in the earlier study. It also explains better the clearly visible effect of temperatures during the chilling phase shown in Fig. 5. If the chilling requirements of cherries cv. ‘Schneiders späte Knorpelkirsche’ at Klein-Altendorf were less than 70 CP, effects of temperature during late fall/early winter on flowering dates would probably be less pronounced, given typical seasonal chill totals of 140–150 CP (Luedeling et al., 2009a). Our estimates are substantially higher than chilling requirements reported in Spain, where different cultivars needed between 30.4 and 57.6 Chill Portions (Alburquerque et al., 2008). These differences are not surprising, since cultivars grown in Spain are adapted to the local mild-winter climate. Heat requirements according to the present analysis (2698 ± 1183 GDH) were comparable to those in the previous study (3473 ± 1236 GDH). The updated values are lower than heat requirements of 7326–9450 GDH reported from Spain (Alburquerque et al., 2008). While the high heat requirements in Spain may be adaptations to the local climate, compensation of suboptimal chill accumulation by higher heat requirements (Harrington et al., 2010) could also be occurring there.

For walnuts in California, chilling requirements were lower than what was found in earlier studies. Using a very different statistical approach, Luedeling et al. (2009f) estimated a chilling requirement of 66.1 CP and a heat requirement of 5139 GDH for leaf emergence of cv. ‘Payne’ walnuts at the same location. This is a substantially higher chilling requirement than the 37.5 ± 5.0 CP and a lower heat requirement than the 11,245 ± 1697 GDH found in the present study. We suspect that these differences arise from the assumptions made in the earlier analysis. The assumption of completely fixed chilling and heat requirements that must be fulfilled strictly in sequence might have been too simplistic. We have more confidence in the PLS regression for delineating temperature response phases. Using PLS based on unprocessed temperature data indicated a chilling requirement of 45.7 ± 5.4 CP (Luedeling et al., in press), which was comparable to the estimate from the present analysis. We see no support in our results for recent claims by Pope et al. (2013) that ‘Payne’ walnuts in California have an even higher chilling requirement than the 45.7 CP ± 5.4 CP derived from our earlier study. A recent study on climatic requirements of different walnut cultivars in Iran (Vahdati et al., 2012) quantified chilling requirements under controlled cool conditions (4 °C) using the Chilling Hours model, so that results cannot be compared to climatic needs determined under orchard conditions (Luedeling et al., 2009f). Heat requirements reported by Vahdati et al. (2012) ranged between approximately 10,200 and 15,200 GDH for lateral and terminal buds, making them comparable to our results.

To our knowledge, no earlier estimates of the chilling and heat requirements of chestnuts in Beijing are available, making our results of 79.8 ± 5.3 CP and 13,466 ± 1918 GDH the first indications of the climatic needs of these trees in the Chinese capital.

4.4. *Temperature-sensitive periods and implications for climate change projection*

While responses of spring phases to heat accumulation rates were consistently important throughout the forcing phase, responses to rates of chill accrual were limited to shorter time windows for chestnuts in Beijing and cherries at Klein-Altendorf. Critical phases were relatively short periods at the beginning and end of the chilling phase, with little relationship found for conditions between these points in time and the timing of spring phases. The most likely explanation of this result seems to be that temperatures were almost always close to optimal for chill accumulation. Apparent variation in chill accumulation rates according to the chilling model may not reflect actual differences in chill effectiveness. As long as temperatures during the chilling phase remain within this optimal range, increasing temperatures should have only a small effect on the speed of chill accumulation, concentrated on the beginning and end of this phase. Buds appeared responsive to heat accumulation rates for much longer periods, leading to clear dominance of the effects of heat in determining the timing of spring events. For chestnuts in Beijing, the responsive phases during chill accumulation that were identified by PLS regression were the shortest among all species analyzed, yet temperature during these phases still showed a clear relationship with bloom dates (Fig. 4).

Walnuts at Davis were responsive to the rate of chill accrual throughout the chilling phase. This indicates that typical temperatures during this phase may currently be higher than ideal. Any warming, or rather any decrease in chill effectiveness, was correlated with earlier leaf emergence dates. This finding clearly indicates that walnuts in California may respond strongly to future climate change. A hypothesis emerging from our results is that warming between October and December should strongly delay the time of chilling requirement fulfillment and ultimately cause later leaf emergence. Implications of such developments for tree productivity seem likely, but more research will be required before robust quantitative projections are possible.

5. Conclusions

Using PLS regression between heat and chill units as independent factors and phenological dates as dependent variables produced very useful results. For all species, it delineated chilling and forcing phases that appeared more plausible and differentiated better between temperature response phases than previous approaches. The method should thus be useful for approximation of chilling and heat requirements of temperate trees, wherever long temperature and phenology records are available. The R package ‘chillR’ (Luedeling, 2013) makes our method easily applicable to datasets of similar nature.

Prospective tree responses to increasing temperatures differed substantially. Bloom of chestnuts in Beijing responded primarily to temperature variation during the forcing phase. Responses to variation in chill accumulation rates were limited to short periods at the beginning and end of the chilling period. This indicated that spring phase timing for these trees will depend primarily on spring temperatures for the foreseeable future. The response of cherries in Germany to variation in chill accumulation rates was slightly stronger. This hints at a stronger though still quite weak influence of weather conditions during chill accumulation. With further warming, we expect this effect to start modulating the currently dominant tendency toward early spring phases. Since chilling and forcing periods overlapped for chestnuts in Beijing and cherries at Klein-Altendorf, a parallel approach to phenology modeling appears appropriate. For walnuts at Davis, heat accumulation appeared to begin after fulfillment of the chilling requirement, so that a sequential chill-heat model could be sufficient for modeling the phenology of this species in California.

Among the species analyzed in this study, walnuts in California seem most responsive to warming during the chilling phase. In spite of much lower chilling requirements compared to chestnuts in China and cherries in Germany, California walnuts are thus the most likely among the species analyzed to display delayed phenology in response to recent and future warming. Particularly for species that, like walnuts in California, are grown in locations that are warmer than their native habitats, close monitoring of phenology responses to warming is recommended. Such observations might serve as early-warning indicators of adverse impacts of climate change.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agrformet.2013.06.018.

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