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# Sweet cherry phenology in the context of climate change: A systems biology approach

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## **Abstract**

**In temperate fruit trees, most key phenological stages are highly dependent on environmental conditions. In particular, correct timing for dormancy and flowering is essential to ensure good fruit production and quality. Consequently, a swiftly-changing environment will put temperate fruit crops at risk in the coming decades. Global changes in environmental conditions include warmer winters and higher risks of frosts in the early spring, which may lead to a wide range of problems in relation to flower and fruit set, sun-scald, cross-pollination or novel host-pest interactions. In addition, hotter summers might affect fruit development and dormancy onset. Considering the time necessary to breed new cultivars better adapted to future climatic conditions, one of the strategies is to develop predictive tool as a support for simulating the behaviour of ideotypes in the context of warmer climatic scenarios. Here, we present a collection of sweet cherry flowering data from European sites. Statistical analyses conducted on 'Burlat' flowering dates collected in Bordeaux reveal the key periods for chill and heat accumulation during dormancy. Additionally, we propose a way of combining different data and approaches, and integrate them into a predictive model as an assisting tool for sweet cherry breeding strategies.**

**Keywords:** *Prunus avium* L., Flowering time, Partial Least Square Regression (PLSR), Modelling

## **INTRODUCTION**

Global climate change has been extensively studied and much evidence now points to a strong impact on biological systems across the globe (Jochner et al., 2016; Parmesan and Yohe, 2003; Root et al., 2003), including changes in the timing of phenophases in fruit trees (Miller-Rushing et al., 2007). Current estimates of advancing phenology for forest and fruit trees, including earlier leaf unfolding and flowering, range between 1.9 and 7.7 days per °C (Jochner et al., 2016; Menzel et al., 2006; Wolkovich et al., 2012). However, the effect of climate change on phenology is very complex and in some locations, spring phases have been observed to occur later (Atkinson et al., 2013; Elloumi et al., 2013), which might reveal the impact of warmer autumn and winter temperatures on lack of chill accumulation during dormancy.

Such alterations might threaten temperate fruit production and may thus affect global economy. As a consequence, it is essential to foresee the changes needed in agriculture and to breed new cultivars better adapted to future climatic conditions. As fruit trees are grown for many years and their breeding processes can take several decades, one of the options is to develop predictive tools that will help simulate the behaviour of ideotypes in the context of warmer climatic scenarios. Here, we propose a way of combining different data and approaches, and integrate them into a predictive model as an assisting tool for sweet cherry breeding strategies.

In particular, a better understanding of temperature response and sensitivity of fruit trees phenology is essential to foresee the changes to come and to have the ability to overcome the effects of climate change on fruit tree productivity. The aim of this study was to provide preliminary information for the identification of trends in sweet cherry flowering response to temperature response. We then discuss options and for the development of predictive tools for sweet cherry phenology.

## **MATERIALS AND METHODS**

Following the collection of a wide range of sweet cherry flowering data across Europe, we used partial least squares (PLS) regression to detect distinct temperature phases as demonstrated on walnuts in California (Luedeling and Gassner, 2012), cherry trees in Germany (Luedeling, Kunz, & Blanke, 2013) and cherry and chestnuts in China (Guo et al., 2013). In this study, we tested the PLS approach to analyse the temperature sensitivity of ‘Burlat’ as reference and the most widely-grown cultivar for sweet cherry, in our experimental orchard (Bordeaux, France) and analysed its flowering response to cold and warm temperatures.

### Flowering data collection

Sweet cherry flowering data for ‘Burlat’ (Table 1) from 22 different locations were collected using two networks: a French national network of experimentation (coordinated by the Centre technique interprofessionnel des fruits et légumes - CTIFL) for the French data and a network of collaborators derived from the European COST Action (FA1104).

Table 1. Countries of data origin and years of cherry flowering observation of the cv. ‘Burlat’ across Europe

Country	Latitude	Number of experimental sites	Years of observation
Belgium	50.55	1	1989-2014
Bulgaria	42.11	1	2006-2011
France	42.76 – 48.47	11	1981-2015
Germany	50.62 – 53.51	2	1978-2015
Italy	44.22	1	1991-2015
Norway	60.32	1	1995-2007
Serbia	43.88	1	1992-2014
Spain	38.04 – 41.70	4	1994-2015
Switzerland	43.22	1	1979-2007

### Partial Least Square Regression

For PLSR analyses, we used a subset of data from Bordeaux (France). In total 180 flowering dates were recorded for ‘Burlat’ at the experimental orchard of INRA Bordeaux research centre located in Toulence near Bordeaux, France (44.58°N, -0.28°E) for 32 years of observation from 1981 to 2012.

Short gaps in daily temperature data were closed by linear interpolation, as described in Luedeling et al. (2013). As proposed in Luedeling and Gassner (2012), an 11-day running mean of daily temperatures was constructed, which smoothes the temperature curve and eases the interpretation of PLS regression (PLSR) outputs.

In addition, the R package ChillR (Luedeling, 2013) which includes all procedures described in the different PLSR studies on fruit trees was used (Guo et al., 2013; Luedeling and Gassner, 2012; Luedeling et al., 2013). These functions were used to analyze the response of sweet cherry cv. ‘Burlat’ flowering dates to variation in mean daily temperatures during the 365 days of the year, from July to June.

The major outputs of the PLS analysis are the variable importance in the projection (VIP) and standardized model coefficients. Periods with VIP scores higher than the threshold set to 0.8 as previously described (Luedeling and Gassner, 2012), and high absolute values of model coefficients represent the relevant phases, when temperatures have a significant effect on flowering dates in ‘Burlat’. Positive coefficients in the chilling phase indicate that higher temperatures during the given period delay flowering, or that temperatures lower than the

average hasten flowering, whereas negative coefficients reveal that warmer temperatures lead to earlier flowering dates.

## RESULTS AND DISCUSSION

### Flowering data collection

Flowering data were checked for consistency and long time series for the sweet cherry cv. 'Burlat' confirmed a relation between flowering dates and latitudes, with flowering periods being significantly later in Northern growing sites (Figure 1). Although the dataset is not homogeneous regarding the record length, they represent a valuable asset for phenological studies, phenology models having been successfully tested and optimized using data sourced from different sites (Andreini et al., 2014; Darbyshire et al., 2016).

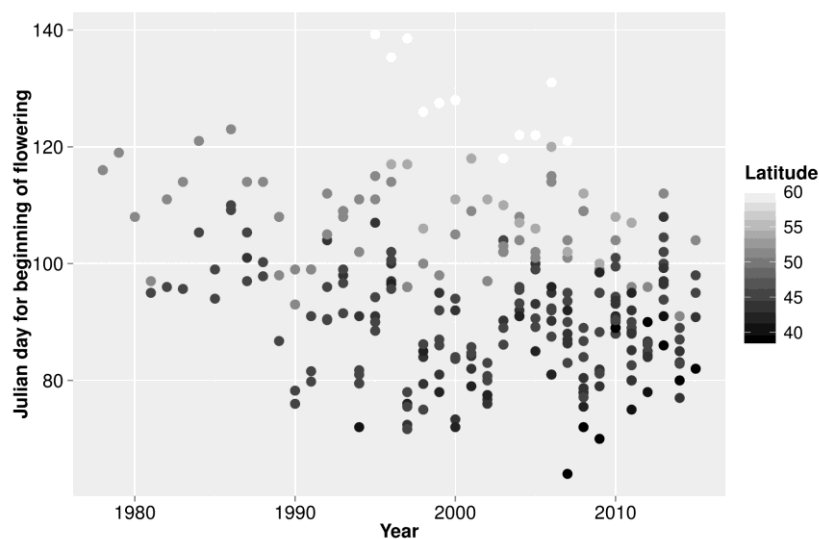


Figure 1. Average date of beginning of flowering of the sweet cherry cv 'Burlat' collected from 23 experimental sites across Europe.

### Flowering dates response to temperatures: example of Bordeaux (France)

In order to test the possibility to extract information on the flowering sensitivity to temperatures, we fitted a PLSR model for 'Burlat' flowering dates against average daily temperatures. PLSR coefficients give information on the strength and the cold/warm effect of temperatures on flowering time. However, only the periods characterized by a VIP threshold value greater than 0.8, are considered to have a significant effect of the temperatures on flowering time. Although the coefficients varied greatly over the 365 days, two main distinct groups of significant coefficients were identified (Figure 2).

The timing of flowering is directly dependent on a proper dormancy release and bud growth, which are controlled by environmental factors, mainly temperature in sweet cherry. Winter bud dormancy can be divided into two main stages: endodormancy followed by ecodormancy (Lang et al., 1987). During endodormancy, even under optimal climatic conditions, the plant ceases to grow until sufficient accumulation of cold temperatures, namely chilling requirements, to trigger dormancy release. Subsequently, ecodormancy starts when buds are able to grow but low temperatures inhibit bud break. Flowering occurs when temperatures increase and heat requirements for bud growth are satisfied. Consequently, for endodormancy, we expected to clearly identify chilling periods defined by positive coefficients with highly significant VIP in the PLSR results. However, there was no relevant pattern in autumn and winter temperatures except for one distinct peak with VIP values greater than 0.8 at the end of September

(Figure 2). This lack of significant cold effect validates the absence of limitation in terms of cold temperature during the major part of winter, meaning that the low winter temperatures in Bordeaux appear to be sufficient to fulfill 'Burlat' chill requirements every year. The high statistical coefficients in September may indicate significant effects of particularly cold temperatures before dormancy onset with the physiological mechanisms remaining obscure at the moment.

PLSR results show a significant peak with positive statistical coefficients in July (Figure 2). Rather than a positive cold effect on flowering time, it is likely that during this period, warm if not hot temperatures negatively affect dormancy onset. Jonkers (1979) demonstrated a tendency for dormancy to deepen when the temperatures, at which the apple and pear buds had been formed

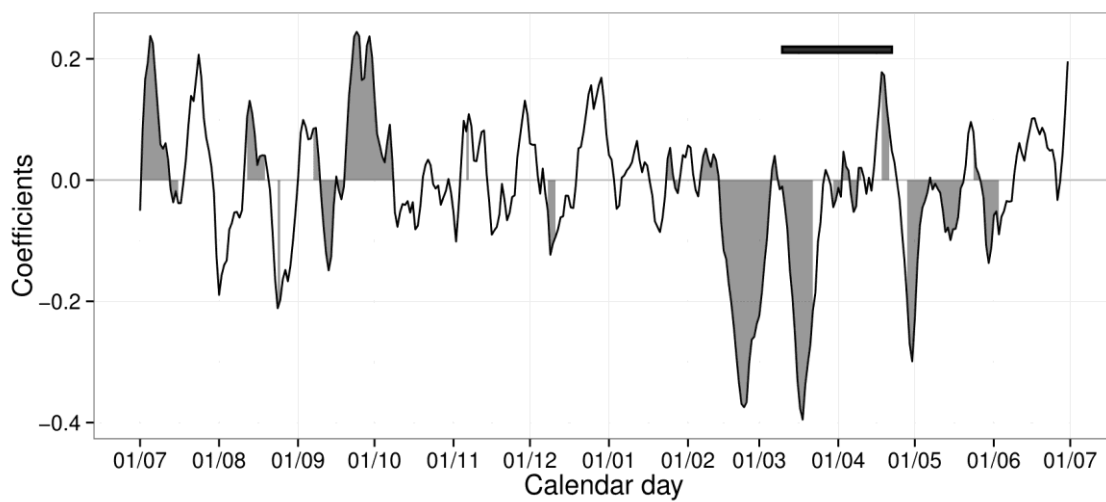


Figure 2. Results for the model coefficients of Partial Least Squares (PLS) regression for beginning of flowering dates in sweet cherry cv. 'Burlat' in Bordeaux (France), in correlation with mean daily temperature, averaged over 11 days, from July 1<sup>st</sup> (Year N-1) to June 30<sup>th</sup> (Year N). Grey areas indicate that the Variable Importance on Projection (VIP) is greater than 0.8, corresponding to the threshold chosen for significant effect of the temperatures on flowering time. Positive coefficients indicate that warm temperatures during the given period delay flowering, or that cold temperatures hasten flowering, whereas negative coefficients reveal that warmer temperatures lead to earlier flowering dates. The top black horizontal line indicates the flowering dates over the observation years (1981-2012).

were too high.

Around February and March, negative coefficients with high absolute values and VIP values exceeding 0.8 corresponded to a critical phase, when warm temperatures were needed to hasten ('force') flowering in sweet cherry cv. 'Burlat'. This is in agreement with the heat requirement period, related to the ecodormancy. Highly significant results confirm that climatic conditions mostly affect flowering time through temperatures at the end of winter. These results are consistent with the forcing phase previously identified in Bonn (Germany) for the sweet cherry cv. 'Schneiders spate Knorpelkirsche' (Luedeling et al., 2013).

### **Towards predictive models and tools for sweet cherry flowering time**

In the current context of climate change, breeding strategies for fruit trees aim at obtaining cultivars well adapted to future climatic conditions, while sustaining good production. Precise phenological modelling represents a valuable tool as a support to select the best genotypes but also to assess the risks for production areas. Predictive models for chill availability project an increase in this delaying effect of mild winters as temperature increase becomes more pronounced (Luedeling, 2012; Vitasse et al., 2011), especially in warmer locations (Guo et al., 2015). Consequently, phenology models can predict the regions that will become unfavourable in terms of optimal conditions for growth and production (Ford et al., 2016) or highlight the future safe areas (Darbyshire et al., 2013). This approach represents a valuable decision-making assistance for cherry production, notably for the choice of cultivars adapted to upcoming climatic conditions in threatened areas (Measham et al., 2014).

At another biological scale, predictive tools based on genetic and physiological knowledge of the response to environmental conditions may allow accurate simulation of the behaviour of given genotypes under particular climatic scenarios. These models will help designing ideotypes, i.e. “model plants”, and in particular adjusting the optimal allele combinations, under future climatic conditions, such as warmer winters or wet springs, as it has been reviewed for cereals (Rötter et al., 2015). Overall, predictive modelling approaches represent powerful tools to assist the breeding strategies in overcoming the complexity of all mechanisms involved in the temperature responses. Phenology models are very valuable for assessing the impact of temperature but at the moment they are limited by their lack of transferability between sites and cultivars (Linkosalo et al., 2008; Luedeling and Brown, 2011). Therefore, the future approaches for predictive models should be based on more precise phenological observations such as dormancy release dates, more environmental parameters including photoperiod (Chmielewski and Götz, 2016) and molecular mechanisms (Satake et al., 2013). We propose a process to build such predictive models for sweet cherry phenology based on a wide range of data and hypotheses (Figure 3).

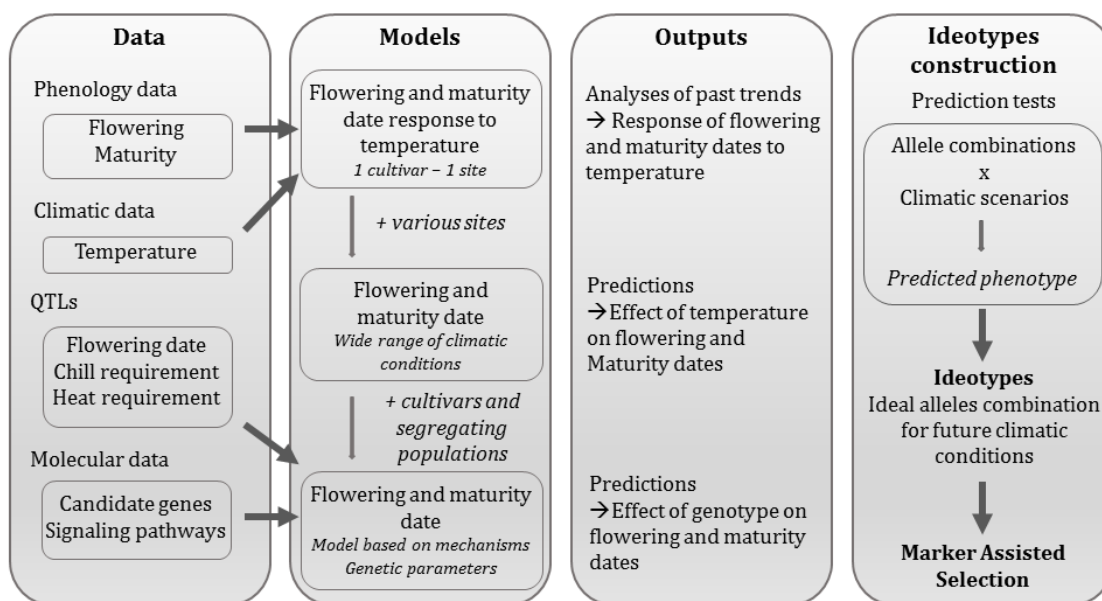


Figure 3. Integrating phenology, genetic, molecular and climatic data into a predictive tool as a support for ideotype design and marker assisted selection.

Based on exploratory analyses of phenology data, including the PLS regression, current phenological models can be improved with the addition of the key periods during which the temperatures have a significant effect on flowering and maturity. Subsequently, the analyses of flowering data for several sites, characterized by highly contrasted climates, should lead to predictive models that can be used for a wide range of climatic conditions (Figure 3). However, in order to apply predictive models to various cultivars, genetic data are indispensable in order to avoid re-parameterizing for each new genotype. Several approaches were already proposed to simulate traits based on genetic and molecular determinisms including gene-based (Messina et al., 2006), QTL-based (Yin et al., 2005) and multiscale models (Chew et al., 2014). In sweet cherry, a multiyear analysis of flowering time and chilling and heat requirements led to the identification of QTLs (Castède et al., 2014) and genomic and molecular studies conducted in woody perennials have identified candidate genes and signalling pathways involved in the control of phenology (Ríos et al., 2014). These data, combined with knowledge acquired through on-going experiments, will support new hypotheses for the development of predictive models based on genetic and/or molecular parameters. Such integrative models will support the evaluation of cultivar suitability and the ideotype design under climate change, but will also provide key information for marker assisted selection (Figure 3).

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