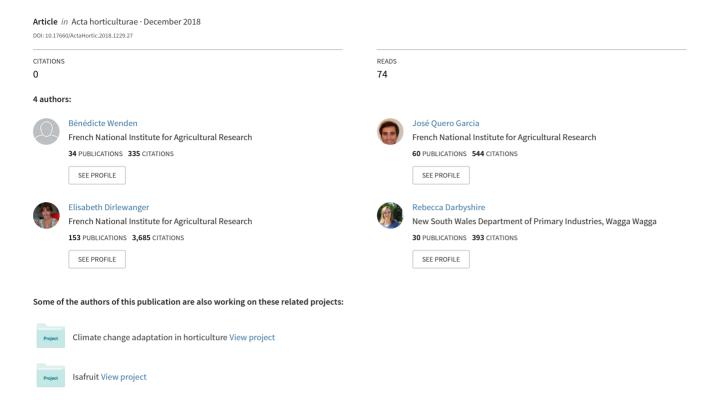
## A comparison of sweet cherry chilling requirements estimated using statistical and biological approaches



# A comparison of sweet cherry chilling requirements estimated using statistical and biological approaches

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#### **Abstract**

Many key phenological stages of temperate fruit trees are highly dependent on environmental conditions. This includes the timing of dormancy release and flowering which are essential to ensure good fruit production and quality. Global changes in environmental conditions including warmer winters and higher risks of frosts in the early spring, may lead to a wide range of problems, including poor flowering, fruit set and cross-pollination, and novel host-pest interaction.

In the context of climate change, one challenge for researchers is to better understand possible impacts on flowering and subsequently to breed fruit trees adapted to future climatic conditions. Predictive models for flowering phenology provide a valuable tool to assist in this process. Here we assessed two methods to determine the chilling requirement, a key parameter to develop a predictive phenology model.

Following the collection of sweet cherry flowering data recorded across Europe, we present an exploration of the chill overlap model for the reference cultivar 'Burlat'. The model was tested and optimised for a wide range of potential chilling requirement values. The best fit was obtained for a critical chilling requirement of 49 chill portions. Using forcing experiments, the chilling requirement was found to vary between the two experimental years (40 and 76 chill portions) and did not correspond to the statistically determined chilling requirement. These results highlight that further investigation is needed for both phenological models and experimental analyses of dormancy and flowering in order to develop more robust models based on biologically-sound parameters.

Keywords: Prunus avium L., phenology, dormancy, modelling

#### INTRODUCTION

In the context of climate change, it is essential to better understand how environmental conditions affect phenology. For temperate fruit trees, increases to temperatures have led to a hastening in flowering time (Chmielewski et al., 2004; Grab and Craparo, 2011; Jochner et al., 2016). On the other hand, increased temperatures have also led to delays in budburst and flowering, especially for earlier species and cultivars (Doi et al., 2008; Legave et al., 2015; Yu et al., 2010). These results confirm that phenology responses to environmental conditions are complex and integrative approaches are thus essential to anticipate future changes. Current challenges include the development of cultivars and a fine-tuning of cultivation practices to adapt to future climate conditions.

Optimal flowering timing relies on ideal winter dormancy onset and release, which are strongly influenced by variations in temperatures. For fruit and nut trees, much effort has been directed to model the control of flowering time by temperature, mainly based on interactions between cold and warm temperatures (Chmielewski et al., 2011; Chuine, 2000; Chuine et al., 2016; Luedeling, 2012). Among them, the sequential model assumes that flowering timing in response to temperature is divided into two consecutive and independent stages. Initially, after dormancy onset, buds accumulate winter chill until a threshold, the chilling requirement (CR) in met. Subsequently, spring heat is accumulated and flowering is triggered when the heat requirement is satisfied. Recent advances have led the way for introducing more physiological processes into models, notably the idea that there is a strong interaction between chill and heat accumulation during dormancy (Pope et al., 2014). Several models were proposed to explain the

compensatory effect between chill and heat accumulation (Chuine, 2000), including the chill overlap model that is supported by biologically-based parameters and performed well against experimental data (Darbyshire et al., 2017, 2016; Pope et al., 2014). For most phenology models, the CR value is an essential parameter but it is commonly estimated from budbreak or flowering dates, thus subjecting the parameterization to large errors, especially for contrasted or unknown climatic conditions (Chuine et al., 2016). Consequently, it seems crucial to use experimental knowledge and data records on dormancy stages to develop improved predictive models.

In this study, we evaluated the chill overlap model for the sweet cherry cultivar 'Burlat', first without *a priori* knowledge of the chilling requirement and then compared this finding with observations of dormancy release determined by forcing experiments.

#### **MATERIALS AND METHODS**

#### Flowering and meteorological data

Dates of beginning of flowering, corresponding to 5-10% open flowers (BBCH 61; Meier, 2001; Fadón *et al.*, 2015) were used to optimize the chill overlap model. Flowering data for the sweet cherry reference cultivar 'Burlat' were obtained from the dataset collected for 17 contrasted European sites (Wenden *et al.*, 2016; Figure 1). For all sites, daily minimum and maximum temperatures were extracted from the ENSEMBLES interpolated gridded dataset E-OBS (v13.1; Haylock *et al.*, 2008). The 'chillR' package for R (Luedeling, 2013) was used to calculate chill accumulation in chill portions (CP) from October 1<sup>st</sup> (Dynamic model; Fishman *et al.*, 1987). Heat accumulation was calculated in growing degree hours (GDH; Richardson *et al.*, 1974).

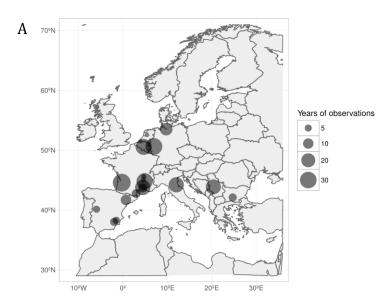
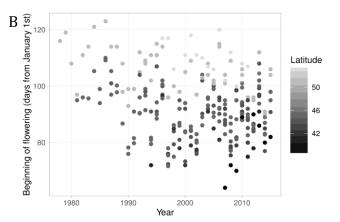


Figure 1. Flowering data for the sweet cherry reference cultivar 'Burlat' used for the model parameterization. (A) Experimental sites and record length for flowering dataset; (B) Dates for beginning of flowering (expressed in day of the year, from January 1st) over the 17 sites defined by their latitude.



#### Chill overlap model parameterization and evaluation

In the chill overlap model, a critical accumulation of chill,  $C_r$  is required before heat is effective for bud development. After this threshold has been met, the amount of accumulated heat  $H_a$  required for flowering depends on the amount of chill accumulated  $C_a$  over a defined portion of the heating phase.

The chill overlap model was defined by Pope et al. (2014) as:

$$H_a = \beta_1 + \frac{\beta_2}{e^{(\beta_3 \times C_a)}} \tag{1}$$

where  $C_a$  and  $H_a$  represent the accumulation of chill and heat prior to the date of beginning of flowering and after the chill requirement  $C_r$  has been met. In their study, Pope et al. (2014) provided interpretation of the parameters;  $\beta_1$  corresponds to the lowest heat accumulation at which flowering is possible, defined as the heat requirement  $H_r$ ;  $\beta_2$  is the amplitude between the optimal heat  $H_0$  and the heat requirement  $H_r$ .

As in Pope et al. (2014) and Darbyshire et al. (2016), we tested different hypotheses on the proportion of overlap between chill and heat accumulation: 25, 50, 75 and 100% overlap periods. We chose to evaluate a wide range of potential  $C_r$  values (0-90 CP). The models were fitted and selected as described in Darbyshire et al. (2016) on all available data (n=260).

For each value of  $C_r$ , the best candidate models were selected and evaluated using root mean square error (RMSE).

### **Dormancy release assessment**

Dormancy phenotyping was carried out on cut branches from 'Burlat' trees, sampled between November 1<sup>st</sup> and March 15<sup>th</sup> for two seasons (2014/2015, 2015/2016). The trees were grown in the experimental orchard of the Institut National de la Recherche Agronomique (INRA)-Bordeaux research center, located in Toulenne (France, 44°34'N 0°16'W). Three fruiting branches were randomly collected every two weeks and placed in water under forcing conditions (25°C, 16h light/8h dark). After 10 days, the phenological stage of the flower buds was observed. The date of dormancy release was estimated when 50% of flower buds reached BBCH stage 53 (Meier, 2001).

#### **RESULTS and DISCUSSION**

## Model evaluation for the range of $C_r$

Although previous experiments have been conducted to determine the chill requirements of sweet cherry cultivars (Alburquerque et al., 2008; Castède et al., 2014; Kuden et al., 2012), our models were evaluated without *a priori* knowledge for  $C_r$  values. Performance, defined statistically by RMSE, for the best candidate models is presented in Figure 2 for the whole range of tested  $C_r$  values (0 – 90 CP). The best performing models were characterized by 25% chill overlap and a chill requirement around 50CP. The best parameter set was estimated for  $C_r$  equal to 49 CP (Table 1, Figure 3) but RMSE was calculated below 6 days for 27 models ( $C_r$  range of 38 – 63 CP).

Surprisingly, in contrast with findings for almond (Pope et al., 2014) and apple trees (Darbyshire et al., 2017, 2016), the best statistical estimation for  $\beta_1$  was a value of 0 (Table 1), with the mathematical assessment thus suggesting that in sweet cherry, buds might not need any heat accumulation for flowering. This result reflects the statistical parameterization but does not correspond to any biological interpretation. However, it would be interesting to further analyze flowering data from Northern experimental sites, especially Norwegian orchards, to test this hypothesis.

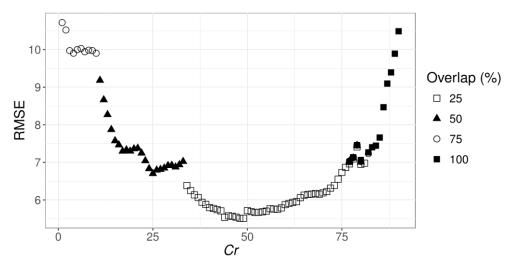


Figure 2. RMSE evaluation of the best candidate models for each value of chill requirement ( $C_r$ ) for all data. RMSE is measured in days.

Table 1. Parameters for the best fitting model

$C_r$	Overlap	$eta_1$	$eta_2$	$B_3$	RMSE (days)
49 CP	25 %	0	10 481	0.020	5.51

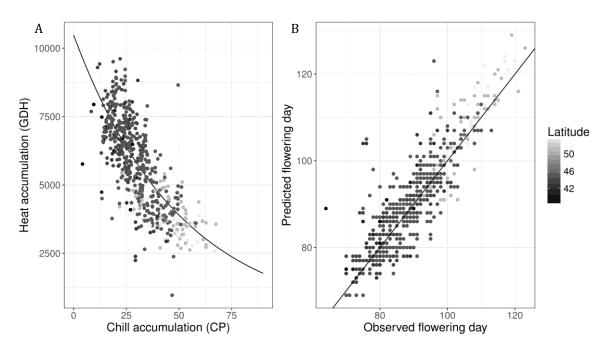


Figure 3. Experimental data compared to the predictions by the best performing model ( $C_r$  = 49 CP, overlap = 25 %) for the 17 sites defined by their latitude. (A) Heat and chill accumulation from 49 CP as predicted (line) and calculated for observed data (points); (B) Observed and predicted flowering days (in days from January 1<sup>st</sup>).

## **Experimental estimation of chill requirements**

Based on forcing experiments, the  $C_r$  for 'Burlat' was estimated as 40 and 76 CP in 2015 and 2016 respectively. Such variation in  $C_r$  has been previously shown in sweet cherry,

depending on cultivar and climatic conditions (Alburquerque et al., 2008; Cortés and Gratacós, 2008; Measham et al., 2017). Therefore, it appears that it is not currently possible to experimentally estimate the value of the  $C_r$  parameter for the chill overlap model or indeed other phenology models.

#### CONCLUSION

In this study, we proposed an analysis of chilling requirement (CR) in sweet cherry for the reference cultivar 'Burlat' using two methods: statistical optimization of the Chill Overlap model and commonly used forcing experiments over two winters. Considering the size of the dataset and the diversity of climates for the different experimental sites, the Chill Overlap model performed well with a RMSE of 5.5 days for the best fitting candidate model. Our approach which did not use *a priori* information regarding  $C_r$ , found the best model fit was obtained for a critical chill requirement of 49 chill portions, which did not correspond to the values estimated in forcing experiments (40 and 76 chill portions). In addition, the performance of chill and heat sub-models needs to be assessed, as proposed in Darbyshire et al. (2017). This analysis highlighted that both methods produced inconsistent CR values according to the underlying theory (a  $\beta_1$  value of 0 for the chill overlap model, and divergent CR values obtained from forcing experiments). Adequate understanding and modelling of the complexity of the mechanisms underlying dormancy control by temperature are still obstacles to efficient predictive approaches. Overall, further investigation is needed for both phenological models and experimental analyses of dormancy and flowering in order to develop more robust models based on biologically-sound parameters.

#### **ACKNOWLEDGEMENTS**

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