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Dormancy in temperate fruit trees – Perspectives for farming in a changing climate

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Contents

Summary

Deciduous fruit trees, which originated in temperate climates, exhibit an inactive period (i.e. dormancy) between late autumn and early spring. This period allows trees to endure the severe winter temperatures usually observed in their native habitat. During dormancy, trees require exposure to chilling and subsequent warm conditions to resume growth (i.e. chill and heat requirement). The exposure to chill triggers physiological modifications inside the buds, restoring the growth mechanisms that were suspended at the beginning of winter. After trees experience enough cold, warm temperatures trigger growth resumption and bloom. To quantify effective accumulation of chill and heat, scientists have developed a number of mathematical models. However, none of these approaches include up-to-date knowledge about the biology of dormancy progression. A process-based model is likely to better represent the entire dormancy phase and to help the temperate fruit industry in preparing for the impacts of climate change. In this thesis, I report on a set of field experiments and assessments of historic weather records. I studied the dormancy phase in temperate fruit trees with a view towards renewed modeling approaches, and I assessed the possible impacts of climate change on the cultivation of temperate fruit trees in a three-year project. A description of these experiments as well as the key results from each study are summarized as follows:

1. In chapter 2, I report on the relationship between chill accumulation and the concentration of non-structural carbohydrates (CHOs) in sweet cherry branches. Results show that the dynamics of starch and hexoses are closely related to dormancy progression. However, our modeling approach using CHOs as predictors of budburst moment performed differently among cherry cultivars, suggesting that other co-occurring processes (e.g. hormonal signaling, genetic expression) must be considered in future dormancy modeling.
2. In chapter 3, I present the calculation of climate-related metrics (i.e. chill availability and spring frost risk) for major agricultural zones of Chile for historic and future climate scenarios using up-to-date methods. Projections suggest that the cultivation of temperate fruit trees in northern-central Chile may face severe obstacles in the near future regarding chill accumulation. In central and southern-central Chile, strategies to ensure dormancy release and budburst may become critical for adequately cultivating deciduous fruit trees.
3. In chapter 4, I report on the comparison of outputs from 13 chill models for historic and future scenarios for nine sites around the globe. In this comparison, we found that chill model selection is the main source of variation in the assessment, more important than the site or future climate scenario. Among all the available approaches, the Dynamic model appears to be the best option for chill estimation due its more credible biological structure. Dormancy researchers, geneticists and other stakeholders should be wary of the high variability between models when working with temperate trees.
4. In chapter 5, I report on the impacts of an unusual drought period during the summer of 2018 on bud dormancy and flowering in an apple orchard in Germany. Results show that under low-chill conditions, buds on non-irrigated trees developed faster than those on irrigated trees. This suggests an impact of summer drought on bud dormancy. We conclude that accounting for the effects of summer drought and warm winters may be necessary for accurately predicting the future phenology of deciduous trees.

Overall, the results of this thesis may be useful for scientists studying the dormancy phase, plant breeders developing new cultivars, stakeholders and authorities making decisions in the fruit industry, and most importantly farmers and orchard managers cultivating deciduous fruit trees.

Zusammenfassung

Laubabwerfende Obstbäume, die der gemäßigten Klimazone entstammen, weisen zwischen dem Spätherbst und zeitigem Frühjahr eine Periode der Inaktivität auf, die Dormanz genannt wird. Diese Periode erlaubt es den Bäumen, die kalten Temperaturen zu überdauern, welche in ihrem natürlichen Habitat üblicherweise auftreten. Während der Dormanz müssen die Bäume zunächst kalten und anschließend warmen Bedingungen ausgesetzt sein, um das Wachstum fortsetzen zu können (Kälte- und Wärmebedürfnis). Kälte ruft physiologische Veränderungen in den Knospen hervor, sodass die Wachstumsmechanismen, die zu Beginn des Winters ausgesetzt wurden, wieder aktiviert werden. Nachdem das Kältebedürfnis erfüllt ist, lösen warme Temperaturen eine Fortsetzung des Wachstums und das Einsetzen der Blüte aus. Um die tatsächliche Erfüllung von Wärme- und Kältebedürfnis zu quantifizieren, haben Wissenschaftler verschiedene mathematische Modelle entwickelt, jedoch berücksichtigt keiner dieser Ansätze das heutige Wissen über die biologischen Prozesse während der Dormanz. Ein prozessbasiertes Modell bildet die gesamte Dormanzphase wahrscheinlich besser ab und wäre der Obstindustrie der gemäßigten Breiten somit eine große Hilfe bei der Anpassung an die Folgen des Klimawandels. In der vorliegenden Arbeit berichte ich über eine Reihe von Experimenten sowie die Auswertung historischer Wetterdaten. Im Rahmen eines dreijährigen Projektes habe ich mich mit der Dormanz laubabwerfender Obstbäume im Lichte neuer Modellierungsansätze beschäftigt und die möglichen Klimawandelfolgen für den Obstanbau in den gemäßigten Breiten beurteilt. Es folgt eine zusammenfassende Beschreibung der Studien und ihrer Ergebnisse:

1. In Kapitel 2 berichte ich über den Zusammenhang zwischen der Erfüllung des Kältebedürfnisses und der Konzentration nicht-struktureller Kohlenhydrate (CHOs) in Zweigen von Kirschbäumen. Die Ergebnisse zeigen, dass die Dynamiken von Stärke und Hexosen in engem Zusammenhang mit dem Verlauf der Dormanz stehen. Unser Modellierungsansatz unter Verwendung der CHOs zur Vorhersage des Blühzeitpunktes funktionierte für verschiedene Sorten unterschiedlich gut, woraus die Einschätzung folgt, dass Dormanzmodellierung zukünftig weitere gleichzeitig auftretende Prozesse (z.B. hormonelle Steuerung und Genexpression) einbeziehen sollte.
2. In Kapitel 3 präsentiere ich Berechnungen klimabedingter Messgrößen (genauer: Verfügbarkeit von Kältewirkung und das Risiko von Spätfrösten) für wichtige landwirtschaftliche Gebiete Chiles in historischen und zukünftigen Klimaszenarien unter Verwendung moderner Methoden. Die Projektionen zeigen, dass der Anbau von Obstbäumen der gemäßigten Breiten im nördlich-zentralen Chile bezüglich der Erfüllung des Kältebedürfnisses in naher Zukunft ernsthaften Schwierigkeiten ausgesetzt sein könnte. Im zentralen und südlich-zentralen Chile könnten Strategien zur Dormanzbrechung und Blühinduktion für den Anbau laubabwerfender Obstbäume unerlässlich werden.
3. In Kapitel 4 berichte ich über einen Vergleich der Ergebnisse von 13 Chilling-Modellen für historische und zukünftige Klimaszenarien für 9 auf der Erde verteilte Standorte. In diesem Vergleich fanden wir heraus, dass die Wahl des Chilling-Modells hauptursächlich für Unterschiede in den Ergebnissen und wichtiger als der Standort oder das Klimaszenario ist. Von allen verfügbaren Ansätzen erscheint das Dynamische Modell als die beste Option für die Einschätzung von Chilling, da es die biologischen Prozesse am überzeugendsten miteinbezieht. Dormanzwissenschaftler, Genetiker und andere Stakeholder sollten bei der Arbeit mit Bäumen der gemäßigten Breiten die hohe Variabilität zwischen den Modellen beachten.
4. In Kapitel 5 berichte ich über den Einfluss einer außergewöhnlichen Trockenperiode im Sommer 2018 auf Knospendormanz und Blüte in einem Apfelbestand in Deutschland. Die Ergebnisse zeigen, dass sich nach nur geringem Kältereiz die Knospen unbewässerter Bäume schneller entwickeln als Knospen bewässerter Bäume. Dies impliziert einen Einfluss von Sommertrockenheit auf Knospendormanz. Wir schließen daraus, dass es erforderlich sein könnte, die Einflüsse von Sommertrockenheit und warmen Wintern einzubeziehen, um eine präzise Vorhersage der Phänologie von laubabwerfenden Bäumen treffen zu können.

Die Ergebnisse dieser Arbeit könnten nützlich sein für Wissenschaftler, die die Dormanz untersuchen, ebenso wie für Pflanzenzüchter, die neue Sorten entwickeln; für Stakeholder und Behörden, die Entscheidungen in der Obstindustrie treffen und vor allem für Landwirte und Plantagenmanager, die Obstbäume anbauen.

Abbreviations and units

DNS	3,5-dinitrosalicylic acid
ABA	abscisic acid
ATPase	adenosine triphosphatase
AIC	Akaike Information Criterion
α	alpha
ANOVA	analysis of variance
an	anthers
approx.	approximately
AUC	area under the curve
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
$CaCl_2$	calcium chloride
CKA	Campus Klein-Altendorf
CO_2	carbon dioxide
°C	celsius degree
$[CR]^2$	Center for Climate and Resilience Research
cm	centimeter
CIAT	Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture)
CDM	Chill Days model
CP	Chill Portion
CR	chill requirement
CFH	Chilling function
CH	Chilling Hour
CHM	Chilling Hours model
CRF	Chilling Rate function
CDC	Climate Data Center

CI	confidence interval
k_c	crop coefficient
cv.	cultivar
DGA	Dirección General de Aguas (General Direction of Water Resources)
DMC	Dirección Meteorológica de Chile (Meteorology Direction of Chile)
DAM	DORMANCY ASSOCIATED MAD-box
DM	Dynamic model
e.g.	*exempli gratia*
ECF	Exponential Chill function
GLM	generalized linear model
GA4	gibberellic acid 4
GAs	gibberellins
GCM	global climate model
GSOD	Global Surface Summary of the Day
g	gravitational force
GHG	greenhouse gas
GDD	Growing Degree Days
GDH	Growing Degree Hours
HR	heat requirement
ha	hectare
HSD	honest significant difference
h	hour
HC	hydrogen cyanamide
H_2O_2	hydrogen peroxide
i.e.	*id est*
IPCC	Intergovernmental Panel on Climate Change
km	kilometer

kf	king flower
lf	lateral flower
L	liter
LCM	Low Chill model
m	meter
m.a.s.l.	meters above sea level
CH_4	methane
µmol m^{-2} s^{-1}	micro molar per square meter per second
µL	microliter
µm	micrometer
mg	milligram
mg g^{-1}	milligram per gram
mL	milliliter
mm	millimeter
mm day^{-1}	millimeter per day
min	minute
MUM	Modified Utah model
NOAA	National Oceanic and Atmospheric Administration
NO	nitric oxide
N_2O	nitrous oxide
n.s.	non-significant
NCM	North Carolina model
ov	ovary
%	percentage
pe	petals
pH	*pondus Hydrogenii*
PUM	Positive Utah model

H+	proton
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
ROC	receiver-operating curve
ET_0	reference evapotranspiration
RDI	Regulated Deficit Irrigation
RCP	Representative Concentration Pathway
RNA	ribonucleic acid
rd	rounded domes
n	sample size
se	sepals
SVP	SHORT VEGETATIVE PHASE
spp.	species *pluralis*
sd	standard deviation
ss	stigmas
st	styles
TCFH	Triangular Chill function from Hänninen (1990)
TCFL	Triangular Chill function from Legave et al. (2013)
tr	trichomes
UM	Utah model
v/v or v:v	volume per volume
$W\ m^{-2}$	watts per square meter
w/v or w:v	weight per volume

Chapter 1

Introduction

Relevance of deciduous fruit production

Cultivation of deciduous fruit and nut trees contributes to the economic development of many countries as well as to global food security. Worldwide, the production of temperate fruits in 2018 accounted for approximately 48% of the total production of fruits (FAOSTAT 2020). In Chile, temperate fruit species such as table grape, walnut, apple, and sweet cherry cultivated in a Mediterranean climate, position the country among the principal exporters of fresh fruit at a global scale (FAOSTAT 2017). In 2016, temperate fruits contributed 38% to the national gross agricultural product of Chile (ODEPA 2017). In many developed and developing countries, temperate fruit trees, grown in temperate as well as in Mediterranean climates, are a key component of agricultural systems.

The dormancy phase

During a normal season, temperate fruit and forest trees enter a dormant phase (i.e. dormancy) in late autumn or early winter. This phase enables trees to tolerate the freezing temperatures observed in their respective habitat of origin (Vegis 1964). Dormancy allows trees to endure unfavorable winter conditions and resume productivity in the subsequent growing season (Faust et al. 1997). The absence of any visible growth in a bud characterizes the dormant period of deciduous trees (Fig. 1-1). During this phase, rigid structures known as bud scales surround and protect meristems, which are the principal growth units of buds. Dormancy manifests itself by suspension of all activities of meristems in buds or other plant structures, which become unable to undergo cell division and expansion (Rohde and Bhalerao 2007). Over the years, dormancy has continuously attracted the attention of both scientist and managers of deciduous fruit orchards (reviewed by Fadón et al. 2020).

Figure 1-1. Representation of dormant buds on a sweet cherry twig

According to Lang et al. (1987), winter dormancy can be classified into endo- and eco-dormancy. Additionally, the authors described the phase of para-dormancy as a type of summer dormancy in which buds remain inactive because of the inhibitory activity of near apical buds (e.g. apical dominance, Fig. 1-2, Lang et al. 1987). Whereas endo-dormancy is associated with the true dormant state and is 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). Although some modifications (i.e. the addition of two endo-dormancy sub stages) in the nomenclature by Lang et al. (1987) have been suggested (Kaufmann and Blanke 2017), this is the most commonly used terminology for the dormancy phase of temperate fruit trees.

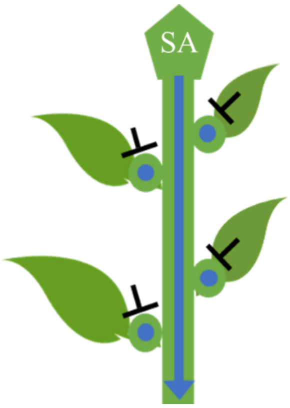

Figure 1-2. Apical dominance scheme. The shoot apex (SA) is dominant over the axillary buds. According to this model, polar auxin transport (blue arrow) from the apex inhibits bud growth by inhibiting auxin (blue dots) export from the axillary buds. Scheme adapted from Smith and Samach (2013)

Similar to other biological processes, environmental cues trigger and regulate dormancy in temperate trees. Current evidence suggests that dormancy-related processes are driven mainly by thermal and photoperiodic fluctuations (Singh et al. 2018; Singh et al. 2019; Tylewicz et al. 2018). Whereas photoperiod has been mostly associated with dormancy establishment in poplar trees (Kozarewa et al. 2010), temperature is usually considered the principal driver during the entire dormancy phase of temperate fruits (Alburquerque et al. 2008; Cooke et al. 2012; Egea et al. 2003). Most research efforts have therefore focused on the regulatory effect of temperature on the response of buds or the whole tree.

The need for chilling and forcing temperatures in dormant trees

Dormancy assures that trees only resume active growth when the cold season is over and temperatures are conducive to growth and development. This suggest that buds or other plant structures can sense and record exposure to both chilling and forcing conditions. During endo-dormancy, buds need exposure to a species-specific (or even variety-specific) period of cold temperatures to re-activate the internal mechanisms that are suspended during winter (Campoy et al. 2011; Faust et al. 1997; Luedeling 2012). Evidence suggests that after receiving this minimum exposure to cold conditions, buds become responsive to warm temperatures, which trigger cellular activity and promote the resumption of growth and development (Faust et al. 1997). The need for both chill and heat to overcome the dormant state and reach a given development stage, such as bloom, has led to the development of the concepts chill and heat requirement (CR and HR, respectively, Luedeling 2012). These climatic needs are increasing being recognized by scientists and farmers and they have become key factors in species development and orchard management.

Common approaches developed to estimate CR and HR assume a sequential relationship between the chilling and forcing phases of dormancy (Alburquerque et al. 2008; Ruiz et al. 2007). This approach, i.e. a sequential model structure, suggests a period for chill accumulation (until fulfilling CR) followed by a heat accumulation period (until fulfilling HR) with no accumulation overlap between the chilling and forcing phases of dormancy (Ashcroft et al. 1977). Although this structure has been widely accepted, some evidence suggests a different relationship between chill and heat accumulation. On the one hand, the parallel model suggests that chill and heat can accumulate at the same time throughout winter (Landsberg 1974). On the other hand, the overlapping model assumes that the forcing phase (heat accumulation) only starts when a minimum CR is achieved, but chill can still accumulate until a certain point in time (Cannell and Smith 1983). This structure suggests that chill and heat can compensate for each other, leading to similar bloom dates (Fig. 1-3, Harrington et al. 2010; Pope et al. 2014). According to Pope et al. (2014), a model with 75% chill overlap performed better than models with 50 and 25% overlap, based on Akaike Information Criterion comparison, in explaining phenological data of almonds cv. Mission, cv. Nonpareil, and cv. Sonora under the particular conditions of California, USA. Although these results may provide a basis for the overlapping relationship between both phases of dormancy, the length of the overlapping period remains mostly unknown. Nonetheless, this approach appears to be the most biologically plausible structure for explaining the relationship between chilling and forcing phases.

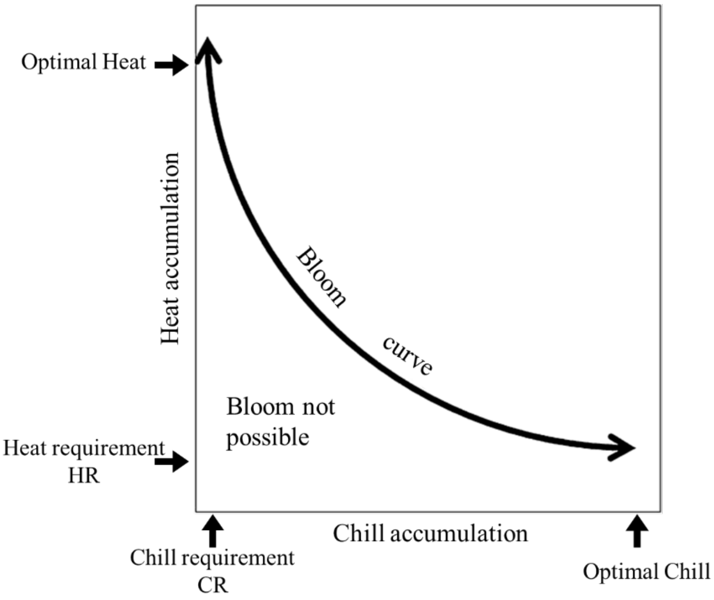

Figure 1-3. Curve representing the compensation effect suggested in the overlapping relationship between chilling and forcing phases of dormancy. After CR is fulfilled, additional chilling may reduce the need for heat and vice versa. Adapted from Pope et al. (2014) with concepts from Harrington et al. (2010)

Quantifying chill and heat accumulation

Climatic requirements (i.e. CR and HR) of fruit species are often considered in orchard planning and management (Campoy et al. 2011). Knowing CR and HR of available tree species when planning future projects helps farmers anticipate future complications regarding inadequate temperature conditions for a given species and/or variety in a given place (Luedeling 2012). To this end, scientists have developed a number of mathematical approximations for estimating chill and heat accumulation in buds. The most commonly used models in horticulture are the Chilling Hours model (first reported by Weinberger 1950), the Utah model (Richardson et al. 1974) and the Dynamic model (Erez et al. 1990; Fishman et al. 1987a, b). The Chilling Hours model suggests that all temperatures between 0 and 7.2 °C are equally effective in overcoming dormancy (Fig. 1-4 A). The Utah model assigns different chill accumulation effectiveness (from -1 to 1 Chill Unit) to different temperature ranges, according to a set of temperature thresholds (Fig. 1-4 B). This model introduced the concept of chill negation, which assumes that temperatures above 15.9 °C have a negative impact on the chill accumulation (Richardson et al. 1974). Finally, the Dynamic model suggests that chill is accumulated in a two-step process, in which cold temperatures lead to the formation of an intermediate labile compound. Once a certain amount of this compound has been accumulated, this can be permanently transformed into a Chill Portion (CP) by a process that requires relatively warm temperatures (Erez et al. 1990; Fishman et al. 1987a, b). The Dynamic model, which uses a continuous function, considers temperatures about 8 °C as most effective for overcoming dormancy (Fig. 1-4 C). Among these models, the structure of the Dynamic model has emerged as the most biologically plausible, as well as the least sensitive option from a number of model comparison studies (Luedeling et al. 2009; Zhang and Taylor 2011). Although these approximations are widely used to estimate the CR, increasing concerns have been raised due to low accuracy and low transferability of estimates across regions (Campoy et al. 2011; Luedeling 2012).

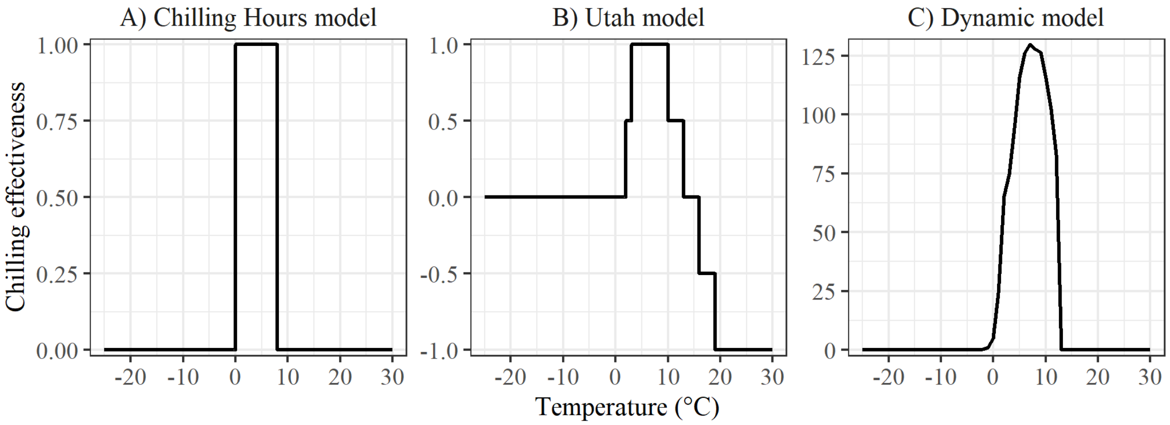

Figure 1-4. Chill effectiveness of the Chilling Hours model (A), Utah model (B), and Dynamic model (C) for a range of hourly temperatures (x-axis). The models Chilling Hours and Utah use a step function while the Dynamic model uses a continuous function. Effectiveness (y-axis) is expressed as the output unit of each model. Since the Dynamic model does not contain a simple weight function, the effectiveness curve for this model shows chill accumulation after 100 continuous hours at the specified temperature

Regarding heat quantification, scientists have developed a number of approaches focused on estimating and predicting the transition between phenological phases. Among these, the Growing Degree Hours model (Anderson et al. 1986) has emerged as the most widely used model in horticultural studies and orchard management. This model consists of a base temperature threshold of 4 °C (for fruit trees), an optimum temperature threshold of 25 °C, and a critical temperature limit (i.e. the temperature above which no appreciable growth will occur) of 36 °C (Anderson et al. 1986, Fig. 1-5). According to the authors, this model is a generalized function that can be used to describe the growth and/or development of a number of plant species and organisms associated with them.

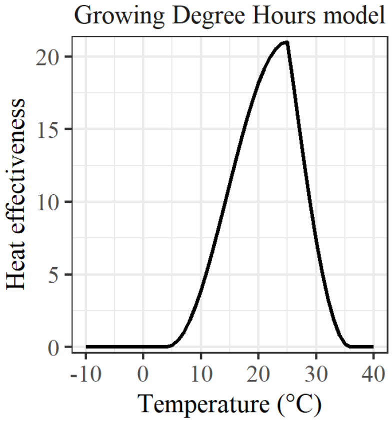

Figure 1-5. Effectiveness of the Growing Degree Hours model for a range of hourly temperatures (x-axis). Effectiveness (y-axis) is expressed as the output unit of the model

Additional efforts towards developing site-specific chill models have yielded a number of different approaches. Among these, the most relevant are the North Carolina model developed for apple trees in North Carolina, USA (Shaltout and Unrath 1983), the Positive Utah model developed for stone fruit trees in South Africa (Linsley-Noakes et al. 1994), and the Chill Days model developed for forest and fruit species in Italy (Cesaraccio et al. 2004). Other chilling sub-functions, coupled with heat sub-models, have been developed to study bloom in apple and forest trees in central and northern Europe (Chmielewski et al. 2011; Legave et al. 2013; Legave et al. 2008). However, these and the previously described models do not provide the possibility to estimate bloom dates of temperate fruit trees across zones and species, mainly because they do not account for bud and tree physiology during dormancy progression (Campoy et al. 2011; Luedeling 2012). A process-based dormancy model could improve estimates of CR and chilling availability for a wider set of climates and species.

The need for a process-based dormancy model

Current available approaches to modeling dormancy and subsequent budburst only use temperature as input, although overwhelming scientific evidence has demonstrated that environmental factors trigger and regulate major biological changes in buds as well as at tree level. This may partially explain incongruences when comparing estimates of chill requirements in different environments. For instance, chill requirement estimations by Viti et al. (2010) for apricot cv. Currot and cv. Orange Red in Italy differed by up to 20% from earlier studies by Ruiz et al. (2007) with the same cultivars in Spain. Similarly, Benmoussa et al. (2017) reported a CR of 14.4 CP for almonds cv. Ferragnes in Tunisia, whereas Ramírez et al. (2010) estimated a need of 32 CP in the Central Valley of Chile, and Egea et al. (2003) quantified a need of about 46 CP in Spain. Such incongruence between estimations might reduce the transferability of species and cultivars across cultivation zones. Although some effort has been made in this regard in the last decades (Gonzalez-Rossia et al. 2008; Sperling et al. 2019), a model considering different aspects of dormancy physiology, such as genetics, proteomics, and metabolomics, as well as the relationship between both phases of dormancy (i.e. chilling and forcing) is still missing. Renewed modeling approaches must therefore consider the physiological knowledge of dormancy gained in the last decades.

Physiological changes during dormancy establishment

Temperate trees establish dormancy in autumn in response to shortening photoperiod and cold temperatures. In poplar trees, Kozarewa et al. (2010) demonstrated that phytochromes in leaves can act as detectors of variations in the levels of red and far-red light, which trigger the downregulation of the regulatory module *CONSTANS / FLOWERING LOCUS T* (Horvath 2009). These modifications promote the down-regulation of the synthesis of gibberellins, establishing physiological conditions that allow bud formation (Lloret et al. 2018).

Together with a reduction in the synthesis of gibberellins, cold temperatures during autumn modify structures in most cells of the tree. Membrane fluidity and membrane-bound proteins act as temperature sensing systems, as falling temperatures slow down movements of proteins and lipids, making membranes more rigid (Faust et al. 1997). Similarly, dormancy establishment has been associated with blockage of cell-to-cell communication in a process that involves callose deposition in the plasmodesmata, mainly regulated by increasing levels of abscisic acid (ABA, Tylewicz et al. 2018). Apart from the key role of this hormone in interrupting intercellular communication, ABA induces the suspension of the cell cycle (Gutierrez et al. 2002). ABA has been proposed as an integral component in the signaling pathway of *DORMANCY-ASSOCIATED MADS-box* genes (*DAM*), which are key regulators of dormancy in stone fruits (Tuan et al. 2017; Yamane 2014).

The interruption of cell-to-cell communication coincides with a cessation of the transport of water and solutes at plant level. Callose plugs interrupt phloem connections (reviewed in Fadón et al. 2020), while xylem transport is diminished by a progressive reduction in transpiration due to leaf senescence and leaf fall (Améglio et al. 2005).

The energy metabolism plays a determinant role in temperate woody perennials, since such species do not have leaves in winter. During the growing season, carbohydrates are transported via the phloem from sources (i.e. mainly leaves) to sink structures (i.e. fruits, new growth units, storage organs, Smith and Samach 2013). In late autumn, storage structures such as roots and woody tissue reach a maximum level of non-structural carbohydrates (Dietze et al. 2014). This assures that active growth can resume in the next season, with early development being supported by the storage molecules.

Physiological changes during endo-dormancy

Overcoming the endo-dormancy phase requires that molecular and supramolecular mechanisms actively perceive and record cold temperatures. During this phase, continuous exposure to chilling conditions promotes callose degradation in the plasmodesmata, progressively restoring cell-to-cell communication (Rinne et al. 2011). The restoration of the symplastic pathway does not imply immediate growth resumption, but it facilitates the transition of buds into a quiescent state, in which they maintain freeze tolerance (Paul et al. 2014). However, communication at the whole tree level remains interrupted by callose plugs in the phloem and air bubbles and cavitation in the xylem.

Hormones such as ABA continue to play a key role during endo-dormancy maintenance. This has been demonstrated by external application of an ABA synthesis inhibitor (i.e. fluoridone) to dormant buds, which initiated the growth of new leaf primordia (Le Bris et al. 1999). On the contrary, gibberellin levels tend to increase while chill is accumulated in dormant buds. In poplar trees, GA4, a specific type of gibberellin, was shown to be able to replace the effect of cold temperatures in dormant buds (Rinne et al. 2011).

The expression of a group of genes, i.e. the *DAM* genes, modulates genetic aspects during this dormancy phase. These genes show high expression levels during endo-dormancy, and are progressively repressed upon exposure to chilling temperatures. Such a pattern was first demonstrated for *PpeDAM5* and *PpeDAM6* in flower buds of peach trees, but later Sasaki et al. (2011) reported the same trend for *PmeDAM4*, *PmeDAM5* and *PmeDAM6* in Japanese apricot. Similarly, the expression of proteins, micro-RNAs, and small non-coding RNAs associated with endo-dormancy maintenance changed during chill accumulation in buds of peach trees (Barakat et al. 2012).

Carbohydrates and dehydrins (a group of free-water binding proteins) contribute to enhancing cold hardiness during endo-dormancy and to maintaining plant metabolism (although at very low rates) during this apparently inactive phase. Detailed microscopic observations of starch in key tissue of the flower primordia of sweet cherry buds reveal that this molecule progressively accumulates in the ovary during exposure to chilling conditions (Fadón et al. 2018). Towards the end of endo-dormancy, carbohydrate metabolism may increase the concentration of reactive oxygen species leading to endo-dormancy release (Beauvieux et al. 2018). The dynamics of carbohydrates therefore have been hypothesized to play a key role in endo-dormancy progression.

Physiological changes during eco-dormancy

During eco-dormancy, buds have regained the capacity to grow, however growth is initially prevented by unfavorable thermal conditions. Chilling accumulated during endo-dormancy enables the symplastic communication pathway in the apical meristem, which appears to promote meristem growth (Rinne et al. 2011). This process is mediated by the formation of a group of proteins (i.e. 1,3-β-glucanases) in charge of callose degradation. Degradation of callose also helps restore sap flow and therefore communication at the tree level (reviewed in Fadón et al. 2020). This restoration allows the transport of water, nutrients and regulatory molecules between different plant organs.

During eco-dormancy, concentrations of ABA drop, whereas the levels of growth-promoting hormones such as gibberellins and auxin increase (Liu and Sherif 2019). Similarly, the expression of genes related to growth control increase when buds enter eco-dormancy. High expression levels of genes such as *SHORT VEGETATIVE PHASE (SVP)* have been found before bud break in apple (Barakat et al. 2012) and Chinese cherry (Zhu et al. 2015).

Carbohydrate metabolism during this phase has been suggested to play a multiple role. On the one hand, the degradation of starch into soluble sugars (e.g. glucose and fructose) in storage cells next to the xylem has been associated with sap flow restoration (Cochard et al. 2001). Elevated concentration of solutes in these cells might lead to increased osmotic potential, promoting water mobilization from roots to aboveground structures (Brodersen and McElrone 2013). On the other hand, increasing concentrations of soluble sugars in storage organs can be related to rapid energy supply for growing structures such as flowers (in the case of stone fruits, Kaufmann and Blanke 2017) and leaves (in the case of pome fruits, Horikoshi et al. 2017). In reproductive tissues, i.e. the ovary, starch degradation has been associated with reproductive processes such as flower development and fruit set (Alcaraz et al. 2013; Guerra and Rodrigo 2015).

Climate change and the challenges for dormancy

Most biological processes occurring during dormancy progression depend on environmental cues. Understanding the dynamics of these processes regarding temperature modifications can improve the predictive power of renewed dormancy modeling efforts. This might lead to increasing probability of success for deciduous orchards threatened by the impacts of recent and future climate change.

During recent decades, an overwhelming amount of scientific evidence has confirmed the notion that Earth is warming due to human activity (IPCC 2014). Many studies have demonstrated that global warming coincides with the recorded increase in atmospheric concentrations of anthropogenic greenhouse gases such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4, IPCC 2014). According to Allen et al. (2018), human development until 2017 induced an average warming, across land and sea surface temperature, of approximately 1 °C above pre-industrial levels (Fig. 1-6, likely between 0.8 and 1.2 °C with 66 to 100% of probability according to Mastrandrea et al. 2011).

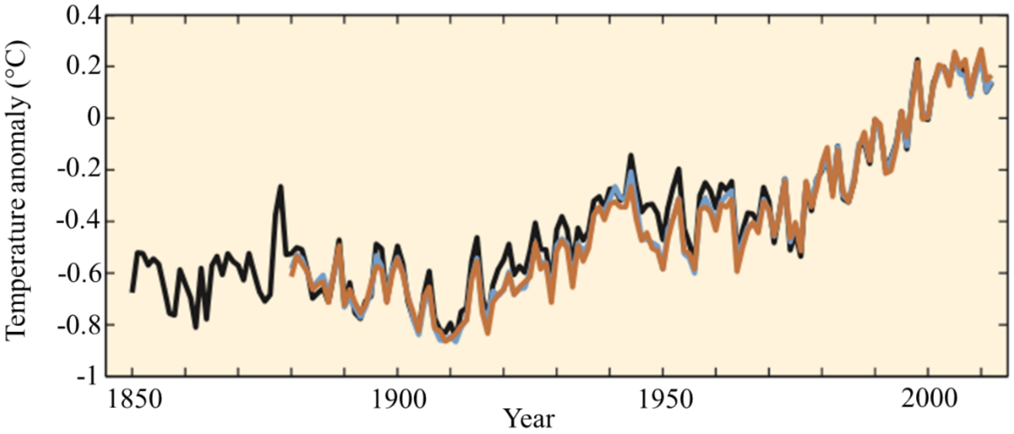

Figure 1-6. Annually and globally averaged temperature (land and ocean) anomalies relative to average temperatures of the period 1986-2005. Colors indicate different data sets. Adapted from IPCC (2014)

Together with increasing temperatures, climate change has been shown to cause modifications in precipitation regimes in several world regions. In the mid-latitude land areas of the northern hemisphere, precipitation has increased since 1901 (showing a high confidence level after 1951). At other latitudes, however, positive or negative changes are more difficult to detect due to natural variation in precipitation (estimations showing low confidence levels, IPCC 2014). Along the same lines, climate change is likely to change the frequency and intensity of many events that are currently considered unusual or extreme. According to IPCC (2014), it is very likely that the frequency of heat waves has increased in large parts of Europe, Asia and Australia since 1950. Similarly, there are likely several land regions where the number of heavy precipitation events has increased and led to increasing risk of flooding. Climate change has modified temperature regimes in several zones of the world, and it has also affected other key parameters of relevance for the cultivation of temperate fruit trees, such as precipitation regimes and the frequency of hazardous weather events.

Regarding future climatic conditions, the Intergovernmental Panel on Climate Change (IPCC) describes four Representative Concentration Pathway (RCP) scenarios. An RCP scenario represents the total additional radiative forcing (in W m^{-2}) expected by the end of the twenty-first century due to the concentration of greenhouse gasses (GHG) in the atmosphere (IPCC 2014), compared to pre-industrial conditions. These four RCPs include a stringent mitigation scenario (RCP2.6), two intermediate scenarios (RCP4.5 and RCP6.0), and one scenario with very high GHG concentrations (RCP8.5, IPCC 2014). According to IPCC (2014), scenarios without additional efforts to constrain emissions (i.e. baseline scenarios) will lead to pathways ranging between RCP6.0 and RCP8.5. On the other hand, RCP2.6 is expected to limit global warming to below 2 °C compared to pre-industrial temperatures. The expected increase of global mean surface temperature by the end of the century (period 2081-2100) relative to 1986-2005 is likely to range between 0.3 and 1.7 °C under RCP2.6, between 1.1 and 2.6 °C under RCP4.5, between 1.4 and 3.1 °C under RCP6.0, and between 2.6 and 4.8 °C under RCP8.5 (IPCC 2014). Regardless of the RCP scenario, considerable increments in temperature, and possibly changes in other climatic parameters, are likely to occur in the medium and long term in many regions, including some of the world's most important production zones of deciduous tree fruit.

A number of studies have pointed out the possible impacts of climate change on the availability of winter chill, a major factor for determining which temperate species can be cultivated where. In 2008, Baldocchi and Wong (2008) performed one of the first regional analyses regarding the impacts of global warming on the number of Chilling Hours (CH) in California, USA. Results showed that observed chill decreased by between 50 and 260 CH per decade, and future estimations projected a decrease by about 40 CH per decade for the period between 1950 – 2100 (Baldocchi and Wong 2008). A few years later, Luedeling et al. (2011) estimated the impacts of climate change on winter chill availability at a global scale under the climatic scenarios B1, A1B and A2. The authors concluded that Mediterranean-like areas, such as the Central Valley of California, the Central Valley of Chile and the Cape region in South Africa, among others, which represent a major zone for the cultivation of temperate fruit trees, are among the most vulnerable regions regarding winter chill accumulation. In contrast, temperate climate regions may benefit from global warming, and some may even present more winter chill in the future. In these regions global warming is likely to reduce the frequency of extremely low temperatures (i.e. < 0 °C), which do not contribute to overcoming tree dormancy according to the current knowledge. Newer local studies performed in Spain (Rodríguez et al. 2019), Australia (Darbyshire et al. 2016), and Germany (Chmielewski et al. 2012) have confirmed the trend in chill accumulation observed and projected by Luedeling et al. (2011). In this regard, the use of modern methods, applied in regional or local assessments, might help improve the accuracy of future winter chill estimations as well as support farmers when selecting species and varieties in future orchard development.

Insufficient winter chill in temperate fruit orchards may affect tree orchard productivity and farm sustainability. In such orchards, the number of flowers at the beginning of the season is a key factor defining the number of fruits and therefore total yield. According to Lavee and May (1997) and Erez (2000), lack of chilling during bud dormancy can cause dispersed and irregular bud break, reduce the percentage of bud break, and in severe cases, lead to bud abortion (Fig. 1-7). In the context of climate change, lack of chilling is a situation likely to be frequently observed in several of the most important regions for nut and fruit trees. Although orchard management measures, such as the application of external dormancy breaking agents (i.e. hydrogen cyanamide – HC), may reduce the negative influences of warm winters (Perez et al. 2009), such techniques are constantly under criticism due to negative environmental side effects. Thus, future advances and development of new growing techniques must rely on understanding chill accumulation and dormancy release processes to cater for current and future environmental needs.

Figure 1-7. Three-year-old apple tree exposed to warm winter conditions (i.e. about 35 Chill Portions). Note the irregular budburst as well as the absence of growth in apical branches

Research objectives

Major challenges in dormancy research are associated with characterizing key processes occurring at bud or tree level during winter. Improving the understanding of these processes is likely to help identify biological drivers of dormancy establishment and dormancy release, which may serve as biological proxies for developing new modeling approaches. Dormancy models, based on a biologically plausible structure, are hypothesized to improve estimations of species' chill needs as well as facilitating accurate chill projections in the context of climate change. In this regard, my first aim was to explore the dynamics and role of the carbohydrate metabolism in the woody tissue of sweet cherry branches during the progression of dormancy in relation to chill accumulation (chapter 2). In brief, the concentration of hexoses (glucose + fructose) and starch was analyzed in one-year-old twigs exposed to ten chill levels under field conditions. These concentrations were then used as predictors to model the moment of budburst through logistic regression analysis. My co-authors and I hypothesized that carbohydrates in woody tissue are key metabolites during dormancy and can serve as good predictors for forecasting budburst dates in sweet cherry trees.

While process-based dormancy models would be desirable for assessing climate change impacts on the dormancy of temperate fruit trees, considerable efforts would be needed to develop such a model and validate it across the climatic range where these species are cultivated. On the other hand, climate change and its impacts are already changing the cultivation of deciduous fruit trees, with negative impacts in some of the most important regions of the world where these species are cultivated. In this regard, updated assessments of climate change impacts on the cultivation of deciduous species with the current available tools might help farmers and other stakeholders in the decision making process. Estimating the future availability of winter chill, a major determinant for species selection and orchard design, might support orchard planning and management. The second aim of this doctorate was to estimate the potential impacts of climate change on relevant agricultural climate-related metrics (i.e. winter chill availability, and spring frost risk) in Mediterranean climate areas such as the major agricultural zones of Chile (chapter 3). Future levels of winter chill as well as spring frost risk were projected by mid- and end-century using an ensemble of climate models and future climate scenarios.

Quantifying seasonal winter chill availability is a key step in the management of deciduous fruit orchards. A number of farm management measures, especially in warm winter areas, depends on achieving a certain level of cold exposure during dormancy. However, estimating chill accumulation in buds is a difficult task, since these organs do not visibly change during this process. Various mathematical approximations have been developed to address chill accumulation in temperate fruit and forest trees. Nonetheless, these models are difficult to transfer across regions, and chill estimations often differ widely. My third aim was to compare available options for winter chill quantification in a global assessment across relevant temperate fruit production regions in a planetary warming context (chapter 4). Using the methodology presented in chapter 3, I compared the outputs of 13 horticultural and forest chill models by mid- and end-century in agricultural locations of Chile, Tunisia and Germany.

Climate change and its impacts have already changed many of the most relevant areas for deciduous fruit production around the world. These systems are affected by increasing temperatures due to global warming but also by hazardous climatic events such as floods, rain during sensitive phases of fruit development and long periods of drought. My fourth aim was to assess additional effects of climate change (i.e. unusual drought seasons) on the dormancy stage and subsequent bloom of deciduous fruit orchards through a case study in Germany (chapter 5). In brief, two apple orchard blocks (irrigated during summer versus non-irrigated) were compared by evaluating the morphology, physiology and phenology of apical buds on one-year-old shoots.

Besides the specific objectives I set for this thesis, I contributed to a number of studies related to dormancy and the cultivation of temperate fruit trees (abstracts are reproduced in chapter 6). In collaboration with Fadón et al. (2020), I reviewed the current literature on the dormancy phase of deciduous forest and fruit trees. In del Barrio et al. (2020) and Buerkert et al. (2020), we estimated future chill levels and other agricultural metrics in Northern Patagonia, Argentina and mountain oases in Oman, respectively. Finally, in Valdebenito et al. (2020), we studied the effect of position inside the canopy on branch development and the relationship between parent wood and new shoots in temperate fruit trees. Contributing to these studies helped broaden the knowledge generated by the specific objectives of this thesis and establish a collaborative network for future dormancy-related studies.

References

Alburquerque N, Garcia-Montiel F, Carrillo A, Burgos L (2008) Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. Environ Exp Bot 64 (2):162-170. doi:10.1016/j.envexpbot.2008.01.003

Alcaraz ML, Hormaza JI, Rodrigo J (2013) Pistil starch reserves at anthesis correlate with final flower fate in avocado (*Persea americana*). PLoS One 8 (10):12. doi:10.1371/journal.pone.0078467

Allen MR, Dube OP, Solecki W, Aragón-Durand F, Cramer W, Humphreys S, Kainuma M, Kala J, Mahowald NM, Mulugetta Y, Perez R, Wairiu M, Zickfeld K (2018) Framing and context. In: Masson-Delmotte V, Zhai P, Pörtner H-O et al. (eds) Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty

Améglio T, Alves G, Decourteix M, M. Poirier M, Bonhome M, Guilliot A, Sakr S, Brunel N, Petel G, Rageau R, Cochard H, Julien JLJ, Lacointe A Winter biology in walnut tree: Freezing tolerance by cold acclimation and embolism repair. In: Malvolti ME, Avanzato D (eds) Fifth International Walnut Symposium, 2005. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp 241-249. doi:10.17660/ActaHortic.2005.705.29

Anderson JL, Richardson EA, Kesner CD (1986) Validation of chill unit and flower bud phenology models for 'Montmorency' sour cherry. Acta Horticulturae (ISHS) 184:71-78. doi:10.17660/ActaHortic.1986.184.7

Ashcroft GL, Richardson EA, Seeley SD (1977) A statistical method of determining chill unit and growing degree hour requirements for deciduous fruit trees. HortScience 12 (4):347-248

Baldocchi D, Wong S (2008) Accumulated winter chill is decreasing in the fruit growing regions of California. Clim Change 87:S153-S166. doi:10.1007/s10584-007-9367-8

Barakat A, Sriram A, Park J, Zhebentyayeva T, Main D, Abbott A (2012) Genome wide identification of chilling responsive microRNAs in *Prunus persica*. BMC Genomics 13:11. doi:10.1186/1471-2164-13-481

Beauvieux R, Wenden B, Dirlewanger E (2018) Bud dormancy in perennial fruit tree species: A pivotal role for oxidative cues. Front Plant Sci 9:13. doi:10.3389/fpls.2018.00657

Benmoussa H, Ghrab M, Ben Mimoun M, Luedeling E (2017) Chilling and heat requirements for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a warm Mediterranean location based on 30 years of phenology records. Agric For Meteorol 239:34-46. doi:10.1016/j.agrformet.2017.02.030

Brodersen CR, McElrone AJ (2013) Maintenance of xylem network transport capacity: A review of embolism repair in vascular plants. Front Plant Sci 4:11. doi:10.3389/fpls.2013.00108

Buerkert A, Fernandez E, Tietjen B, Luedeling E (2020) Revisiting climate change effects on winter chill in mountain oases of northern Oman. Clim Change 162:1399-1417. doi:10.1007/s10584-020-02862-8

Campoy JA, Ruiz D, Egea J (2011) Dormancy in temperate fruit trees in a global warming context: A review. Sci Hortic 130 (2):357-372. doi:10.1016/j.scienta.2011.07.011

Cannell MGR, Smith RI (1983) Thermal Time, Chill Days and Prediction of Budburst in *Picea sitchensis*. J Appl Ecol 20 (3):951-963. doi:10.2307/2403139

Cesaraccio C, Spano D, Snyder RL, Duce P (2004) Chilling and forcing model to predict bud-burst of crop and forest species. Agric For Meteorol 126 (1-2):1-13. doi:10.1016/j.agrformet.2004.03.002

Chmielewski FM, Blumel K, Henniges Y, Blanke M, Weber RWS, Zoth M (2011) Phenological models for the beginning of apple blossom in Germany. Meteorol Z 20 (5):487-496. doi:10.1127/0941-2948/2011/0258

Chmielewski FM, Blumel K, Palesova I (2012) Climate change and shifts in dormancy release for deciduous fruit crops in Germany. Clim Res 54 (3):209-219. doi:10.3354/cr01115

Cochard H, Lemoine D, Ameglio T, Granier A (2001) Mechanisms of xylem recovery from winter embolism in *Fagus sylvatica*. Tree Physiol 21 (1):27-33. doi:10.1093/treephys/21.1.27

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. Plant Cell Environ 35 (10):1707-1728. doi:10.1111/j.1365-3040.2012.02552.x

Darbyshire R, Measham P, Goodwin I (2016) A crop and cultivar-specific approach to assess future winter chill risk for fruit and nut trees. Clim Change 137 (3-4):541-556. doi:10.1007/s10584-016-1692-3 del Barrio R, Fernandez E, Brendel AS, Whitney C, Campoy JA, Luedeling E (2020) Climate change impacts on agriculture's southern frontier – Perspectives for farming in North Patagonia. Int J Climatol *in press*:1-17. doi:10.1002/joc.6649

Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R (2014) Nonstructural carbon in woody plants. In: Merchant SS (ed) Annual Review of Plant Biology, Vol 65. Annual Review of Plant Biology. Annual Reviews, Palo Alto, pp 667-687. doi:10.1146/annurev-arplant-050213-040054

Egea J, Ortega E, Martinez-Gomez P, Dicenta F (2003) Chilling and heat requirements of almond cultivars for flowering. Environ Exp Bot 50 (1):79-85. doi:10.1016/s0098-8472(03)00002-9

Erez A (2000) Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics. In: Erez A (ed) Temperate Fruit Crops in Warm Climates. Springer Netherlands, Dordrecht, pp 17-48. doi:10.1007/978-94-017-3215-4_2

Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. Acta Hort 276:165-174

Fadón E, Fernandez E, Behn H, Luedeling E (2020) A conceptual framework for winter dormancy in deciduous trees. Agronomy 10 (2):241. doi:10.3390/agronomy10020241

Fadón E, Herrero M, Rodrigo J (2018) Dormant flower buds actively accumulate starch over winter in sweet cherry. Front Plant Sci 9:10. doi:10.3389/fpls.2018.00171

FAOSTAT (2017) Rankings: Countries by commodity. http://www.fao.org/faostat/en/#rankings/countries_by_commodity_exports. Accessed 18-07-2018

FAOSTAT (2020) Food and agriculture data. http://www.fao.org/faostat/en/#data. Accessed 05-10-2020

Faust M, Erez A, Rowland LJ, Wang SY, Norman HA (1997) Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance, and release. HortScience 32 (4):623-629

Fishman S, Erez A, Couvillon GA (1987a) The temperature-dependence of dormancy breaking in plants - Computer-simulation of processes studied under controlled temperatures. J Theor Biol 126 (3):309-321. doi:10.1016/s0022-5193(87)80237-0

Fishman S, Erez A, Couvillon GA (1987b) The temperature-dependence of dormancy breaking in plants - Mathematical-analysis of a 2-step model involving a cooperative transition. J Theor Biol 124 (4):473-483. doi:10.1016/s0022-5193(87)80221-7

Gonzalez-Rossia D, Reig C, Dovis V, Gariglio N, Agusti M (2008) Changes on carbohydrates and nitrogen content in the bark tissues induced by artificial chilling and its relationship with dormancy bud break in *Prunus* sp. Sci Hortic 118 (4):275-281. doi:10.1016/j.scienta.2008.06.011

Guerra ME, Rodrigo J (2015) Japanese plum pollination: A review. Sci Hortic 197:674-686. doi:10.1016/j.scienta.2015.10.032

Gutierrez C, Ramirez-Parra E, Castellano MM, del Pozo JC (2002) G(1) to S transition: More than a cell cycle engine switch. Curr Opin Plant Biol 5 (6):480-486. doi:10.1016/s1369-5266(02)00301-1

Harrington CA, Gould PJ, St Clair JB (2010) Modeling the effects of winter environment on dormancy release of Douglas-fir. For Ecol Manage 259 (4):798-808. doi:10.1016/j.foreco.2009.06.018

Horikoshi HM, Sekozawa Y, Sugaya S (2017) Inhibition of carbohydrate metabolism by thermal fluctuations during endodormancy lead to negative impacts on bud burst and incidence of floral necrosis in 'Housur' Japanese pear flower buds. Sci Hortic 224:324-331. doi:10.1016/j.scienta.2017.06.018

Horvath D (2009) Common mechanisms regulate flowering and dormancy. Plant Sci 177 (6):523-531. doi:10.1016/j.plantsci.2009.09.002

IPCC (2014) Climate Change 2014: Synthesis report. Contributions of working groups I, II and III to the fifth assesment report of the Intergovernmental Panel on Climate Change IPCC, Geneva, Switzerland

Kaufmann H, Blanke M (2017) Changes in carbohydrate levels and relative water content (RWC) to distinguish dormancy phases in sweet cherry. J Plant Physiol 218:1-5. doi:10.1016/j.jplph.2017.07.004

Kozarewa I, Ibáñez C, Johansson M, Ögren E, Mozley D, Nylander E, Chono M, Moritz T, Eriksson ME (2010) Alteration of PHYA expression change circadian rhythms and timing of bud set in *Populus*. Plant Mol Biol 73 (1-2):143-156. doi:10.1007/s11103-010-9619-2

Landsberg JJ (1974) Apple fruit bud development and growth - Analysis and an empirical model. Ann Bot 38 (158):1013-1023. doi:10.1093/oxfordjournals.aob.a084891

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo, para-, and ecodormancy: Physiological terminology and classification for dormancy research. HortScience 22:371-377

Lavee S, May P (1997) Dormancy of grapevine buds - Facts and speculation. Aust J Grape Wine R 3 (1):31-46. doi:10.1111/j.1755-0238.1997.tb00114.x

Le Bris M, Michaux-Ferriere N, Jacob Y, Poupet A, Barthe P, Guigonis JM, Le Page-Degivry MT (1999) Regulation of bud dormancy by manipulation of ABA in isolated buds of *Rosa hybrida* cultured in vitro. Aust J Plant Physiol 26 (3):273-281. doi:10.1071/pp98133

Legave JM, Blanke M, Christen D, Giovannini D, Mathieu V, Oger R (2013) A comprehensive overview of the spatial and temporal variability of apple bud dormancy release and blooming phenology in Western Europe. Int J Biometeorol 57 (2):317-331. doi:10.1007/s00484-012-0551-9

Legave JM, Farrera I, Almeras T, Calleja M (2008) Selecting models of apple flowering time and understanding how global warming has had an impact on this trait. J Horticult Sci Biotechnol 83 (1):76-84. doi:10.1080/14620316.2008.11512350

Linsley-Noakes G, Allan P, Matthee G (1994) Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J S Afr Soc Hort Sci 4:13-15

Liu JY, Sherif SM (2019) Hormonal orchestration of bud dormancy cycle in deciduous woody perennials. Front Plant Sci 10:21. doi:10.3389/fpls.2019.01136

Lloret A, Badenes ML, Rios G (2018) Modulation of dormancy and growth responses in reproductive buds of temperate trees. Front Plant Sci 9:12. doi:10.3389/fpls.2018.01368

Luedeling E (2012) Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci Hortic 144:218-229. doi:10.1016/j.scienta.2012.07.011

Luedeling E, Girvetz EH, Semenov MA, Brown PH (2011) Climate change affects winter chill for temperate fruit and nut trees. PLoS One 6 (5):13. doi:10.1371/journal.pone.0020155

Luedeling E, Zhang MH, Luedeling V, Girvetz EH (2009) Sensitivity of winter chill models for fruit and nut trees to climatic changes expected in California's Central Valley. Agric Ecosyst Environ 133 (1-2):23-31. doi:10.1016/j.agee.2009.04.016

Mastrandrea MD, Mach KJ, Plattner G-K, Edenhofer O, Stocker TF, Field CB, Ebi KL, Matschoss PR (2011) The IPCC AR5 guidance note on consistent treatment of uncertainties: A common approach across the working groups. Clim Change 108 (4):675. doi:10.1007/s10584-011-0178-6

ODEPA (2017) Chilean agriculture overview. Office of Agricultural Studies and Policies, Santiago, Chile

Paul LK, Rinne PLH, van der Schoot C (2014) Shoot meristems of deciduous woody perennials: Self-organization and morphogenetic transitions. Curr Opin Plant Biol 17:86-95. doi:10.1016/j.pbi.2013.11.009

Perez FJ, Vergara R, Or E (2009) On the mechanism of dormancy release in grapevine buds: A comparative study between hydrogen cyanamide and sodium azide. Plant Growth Regul 59 (2):145-152. doi:10.1007/s10725-009-9397-5

Pope KS, Da Silva D, Brown PH, DeJong TM (2014) A biologically based approach to modeling spring phenology in temperate deciduous trees. Agric For Meteorol 198:15-23. doi:10.1016/j.agrformet.2014.07.009

Ramírez L, Sagredo KX, Reginato GH (2010) Prediction models for chilling and heat requirements to estimate full bloom of almond cultivars in the Central Valley of Chile. Acta Horticulturae (ISHS) 872:107-112. doi:10.17660/ActaHortic.2010.872.12

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 1:331-332

Rinne PLH, Welling A, Vahala J, Ripel L, Ruonala R, Kangasjarvi J, van der Schoot C (2011) Chilling of dormant buds hyperinduces FLOWERING LOCUS T and recruits GA-inducible 1,3-beta-glucanases to reopen signal conduits and release dormancy in *Populus*. Plant Cell 23 (1):130-146. doi:10.1105/tpc.110.081307

Rodríguez A, Pérez-López D, Sánchez E, Centeno A, Gómara I, Dosio A, Ruiz-Ramos M (2019) Chilling accumulation in fruit trees in Spain under climate change. Nat Hazards Earth Syst Sci 19 (5):1087-1103. doi:10.5194/nhess-19-1087-2019

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12 (5):217-223. doi:10.1016/j.tplants.2007.03.012

Ruiz D, Campoy JA, Egea J (2007) Chilling and heat requirements of apricot cultivars for flowering. Environ Exp Bot 61 (3):254-263. doi:10.1016/j.envexpbot.2007.06.008

Sasaki R, Yamane H, Ooka T, Jotatsu H, Kitamura Y, Akagi T, Tao R (2011) Functional and expressional analyses of PmDAM genes associated with endodormancy in Japanese apricot. Plant Physiol 157 (1):485-497. doi:10.1104/pp.111.181982

Shaltout AD, Unrath CR (1983) Rest completion prediction model for 'Starkrimson Delicious' apples. J Am Soc Hort Sci 108 (6):957-961

Singh RK, Maurya JP, Azeez A, Miskolczi P, Tylewicz S, Stojkovič K, Delhomme N, Busov V, Bhalerao RP (2018) A genetic network mediating the control of bud break in hybrid aspen. Nat Commun 9 (1):4173. doi:10.1038/s41467-018-06696-y

Singh RK, Miskolczi P, Maurya JP, Bhalerao RP (2019) A tree ortholog of SHORT VEGETATIVE PHASE floral repressor mediates photoperiodic control of bud dormancy. Curr Biol 29 (1):128-133. doi:10.1016/j.cub.2018.11.006

Smith HM, Samach A (2013) Constraints to obtaining consistent annual yields in perennial tree crops. I: Heavy fruit load dominates over vegetative growth. Plant Sci 207:158-167. doi:10.1016/j.plantsci.2013.02.014

Sperling O, Kamai T, Tixier A, Davidson A, Jarvis-Shean K, Raveh E, DeJong TM, Zwieniecki MA (2019) Predicting bloom dates by temperature mediated kinetics of carbohydrate metabolism in deciduous trees. Agric For Meteorol 276:9. doi:10.1016/j.agrformet.2019.107643

Tuan PA, Bai S, Saito T, Ito A, Moriguchi T (2017) Dormancy-Associated MADS-Box (DAM) and the abscisic acid pathway regulate pear endodormancy through a feedback mechanism. Plant and Cell Physiology 58 (8):1378-1390. doi:10.1093/pcp/pcx074

Tylewicz S, Petterle A, Marttila S, Miskolczi P, Azeez A, Singh RK, Immanen J, Mähler N, Hvidsten TR, Eklund DM, Bowman JL, Helariutta Y, Bhalerao RP (2018) Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. Science 360 (6385):212-215. doi:10.1126/science.aan8576

Valdebenito D, Laca EA, Fernandez E, Saa S (2020) A network of shoots: Effects of ontogeny and light availability on growth units in Chandler walnuts. Trees-Struct Funct 34 (1):177-188. doi:10.1007/s00468-019-01909-3

Vegis A (1964) Dormancy in higher plants. Annual Review of Plant Physiology 15:185-224. doi:10.1146/annurev.pp.15.060164.001153

Viti R, Andreini L, Ruiz D, Egea J, Bartolini S, Iacona C, Campoy JA (2010) Effect of climatic conditions on the overcoming of dormancy in apricot flower buds in two Mediterranean areas: Murcia (Spain) and Tuscany (Italy). Sci Hortic 124 (2):217-224. doi:10.1016/j.scienta.2010.01.001

Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56:122-128

Yamane H (2014) Regulation of bud dormancy and bud break in japanese apricot (*Prunus mume* Siebold & Zucc.) and peach [*Prunus persica* (L.) Batsch]: A summary of recent studies. J Jpn Soc Hortic Sci 83 (3):187-202. doi:10.2503/jjshs1.CH-Rev4

Zhang JL, Taylor C (2011) The Dynamic Model provides the best description of the chill process on 'Sirora' pistachio trees in Australia. HortScience 46 (3):420-425

Zhu YY, Li YQ, Xin DD, Chen WR, Shao X, Wang Y, Guo WD (2015) RNA-Seq-based transcriptome analysis of dormant flower buds of Chinese cherry (*Prunus pseudocerasus*). Gene 555 (2):362-376. doi:10.1016/j.gene.2014.11.032

Chapter 2

Starch and hexoses concentrations as physiological markers in dormancy progression of sweet cherry twigs

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Introduction

Many trees from temperate and boreal climates display a lack of growth during winter even if conditions are favorable. This process, known as dormancy, allows trees to stay alive for long periods despite adverse environmental conditions (Faust et al. 1997; Fuchigami and Wisniewski 1997; Lang 1987). Several factors have been related with the start, progression and end of dormancy. Many studies have demonstrated the importance of photoperiod (Ibáñez et al. 2010; Kozarewa et al. 2010), but the most accepted driver of dormancy is temperature (Alburquerque et al. 2008; Benmoussa et al. 2017; Cooke et al. 2012; Egea et al. 2003; Guo et al. 2015). Based on the temperature requirement during dormancy, Lang et al. (1987) proposed a division of the dormant period into endo- and eco-dormancy. While endo-dormancy is broken by low temperatures (Faust et al. 1997), eco-dormancy (i.e. the state before budburst) is mainly modulated by heat (Harrington et al. 2010; Luedeling 2012). The requirements in each phase can be expressed as chill requirement (CR) and heat requirement (HR) for endo- and eco-dormancy, respectively (Faust et al. 1997; Luedeling 2012). While HR has been easily quantifiable through models such as Growing Degree Days (GDD) or Growing Degree Hours (GDH, Anderson et al. 1986), the models to estimate CR and monitoring chill accumulation are continuously under discussion due to their poor interchangeability among climates and the lack of physiological knowledge involved in their development (Luedeling 2012; Luedeling and Brown 2011).

During the endo-dormancy period, low temperatures trigger several structural and biochemical changes at the cellular level that are related to frost protection (Faust et al. 1997). Endo-dormancy release, which may coincide with an increase of external temperatures in some regions and years, has been studied in terms of changes in the degree of unsaturation of plasma membrane lipids (Wang and Faust 1988a), changes in sucrose transporters, H+/ATPase channels and calcium channels (Beauvieux et al. 2018; Portrat et al. 1995) and changes in symplasmic pathways (Rinne and van der Schoot 2003). Regarding biochemical changes, several metabolites have been reviewed and proposed as bioindicators in the past (Beauvieux et al. 2018; Hillmann et al. 2016). For instance, increase in the production of reactive oxygen species (ROS) due to an increase of lipid and carbohydrate catabolism has been associated with the transition from endo- to eco-dormancy (Beauvieux et al. 2018). Moreover, recent studies have reported a strong relationship between temperature and carbohydrate metabolism and movement dynamics, which impacts on the timing and rate of budburst in walnuts (Tixier et al. 2017a) and pears (Horikoshi et al. 2017).

Thus, the release of dormancy in vegetative tissues has been related with an increase in oxidative and respiratory stress at the cellular level. In seed germination, for example, reactive nitrogen species (RNS) have an important role as signal molecules (Arc et al. 2013). In deciduous trees, nitric oxide (NO) has been related with heat and other stresses, which do not involve cold (Domingos et al. 2015; Parankusam et al. 2017). The role of ROS such as H_2O_2 as signal molecules during endo-dormancy release has been widely studied and reviewed (Beauvieux et al. 2018; Hussain et al. 2015; Perez and Burgos 2004). In fact, an experiment by Perez and Lira (2005) in grapevines showed that dormancy-breaking compounds, such as hydrogen cyanamide (HC), promoted dormancy release by inducing an increase in the concentration of H_2O_2 in dormant buds. During dormancy release, these compounds (ROS) are produced naturally via catabolism of glucose at a reduced concentration of oxygen at the cellular level (Beauvieux et al. 2018; Perez et al. 2009). Glucose is obtained via catalysis of starch and/or sucrose, as well as through gluconeogenesis from lipid degradation processes (Beauvieux et al. 2018). Thus, hexoses that together with sorbitol and sucrose are the main sugars in the bark of *Prunus* spp. during dormancy release and cold acclimation (Gonzalez-Rossia et al. 2008; Yu et al. 2017) seem to be key metabolites during the late phase of endo-dormancy.

Chilling requirements are commonly estimated by exposing lateral branches or shoots to different cold conditions followed by a forcing phase in which laterals reach a certain percentage of budburst (Alburquerque et al. 2008; Egea et al. 2003; Ruiz et al. 2007). In this methodology and in others, such as Partial Least Squares regression (Benmoussa et al. 2017; Luedeling and Gassner 2012), the CR is generally expressed in terms of Chilling Hours (according to the Chilling Hours model, Bennett 1949; Weinberger 1950), Chill Units (according to the Utah Model, Richardson et al. 1974) or Chill Portions (CP according to the Dynamic Model, Erez et al. 1990; Fishman et al. 1987a, b). However, the estimated CR is rarely the same across different climates. Specifically, Benmoussa et al. (2017) highlighted that their estimations of CR in almond were at least 22% lower than values reported in earlier studies, mainly due to the inaccuracy of models used for monitoring chill accumulation (Campoy et al. 2011; Luedeling 2012; Luedeling and Brown 2011). On the other hand, an interaction may exist between specific climatic conditions and species or varieties, which may affect chill accumulation (Benmoussa et al. 2017; Campoy et al. 2012; Tixier et al. 2017a). Nonetheless, current chilling models are incapable of considering specific relationships between climate and species. Thus, the main reason why the models are not consistent across environmental conditions may lie in the models' failure to take into account the physiology of dormant buds (Luedeling 2012).

Lack of chilling can lead to bud abortion, low budburst percentages and dispersed budburst, causing an important reduction in yields of nut and fruit trees (Erez 2000; Lavee and May 1997). Therefore, CR considerations can become critical for deciduous trees growing in Mediterranean climates, where winter chill losses are projected as a consequence of climate change (Luedeling 2012; Luedeling and Brown 2011; Luedeling et al. 2009b). Luedeling et al. (2009b) predicted at least 19% less winter chill by 2050 for the Central Valley of California. Projections are even more alarming for regions surrounding the Mediterranean Sea, where winter chill may be reduced by 40 CP by the end of the twenty-first century (Luedeling et al. 2011). In this scenario, the current models are becoming increasingly inaccurate with a weak transferability among climates (Luedeling and Brown 2011). Here, we propose to approach CR and subsequent budburst as a function of carbohydrate dynamics recorded during dormancy progression to improve our understanding on physiological markers related to endo-dormancy maintenance and release. We monitored changes in starch and glucose + fructose concentrations obtained from sweet cherry twigs exposed to different amounts of chilling and used a logistic regression analysis to model the budburst moment.

Materials and methods

Site, plant material, forcing conditions and experimental design

This research is part of a larger experiment focused on modeling budburst as a continuous, sequential, and time-dependent process that is particular for each bud in a shoot. Samples were taken in a commercial mature sweet cherry (*Prunus avium* L.) orchard with trees between 5 and 7 years old grafted onto Colt rootstock. The orchard is located in Río Claro, Región del Maule, Chile, which is a major sweet cherry production region. In the orchard, six trees of the varieties Bing, Kordia, Lapins, Rainier, Regina, Santina, Skeena, and Sweetheart were used as twig donors (i.e. 48 trees in total). A total of 20 one-year-old branches were tagged in each tree between 1.5 and 3 m above the ground, on May 17, 2017. After tagging, two twigs per tree were collected on ten occasions, according to chilling received in the field (recorded as Chill Portions—CP). Sample dates and chill levels recorded were: May 17th (16 CP), May 29th (26 CP), June 8th (32 CP), June 14th (38 CP), June 21st (42 CP), June 28th (48 CP), July 5th (52 CP), July 11th (58 CP), July 19th (63 CP), and July 26th (70 CP). All selected twigs were between 30 and 50 cm in length and 4–6 mm in diameter. Collected twigs were transported to the Deciduous Fruit Tree Laboratory at the Escuela de Agronomía of the Pontificia Universidad Católica de Valparaíso. Upon arrival in the lab, twigs were disinfected with a solution of sodium hypochlorite (0.5% v/v) and cut at the bottom end while submerged in distilled water to avoid embolism formation. Twigs were then placed in containers with 250 mL of a sucrose solution (5% w/v) and transferred to a forcing chamber with environmental conditions of 21 ± 0.71 °C, 65% relative humidity, 16/8 h photoperiod, 50 μmol m^{-2} s^{-1} metal halide cold white light. Temperature regimes in the orchard and inside the growing chamber during the duration of the experiment are shown in Fig. 2-1.

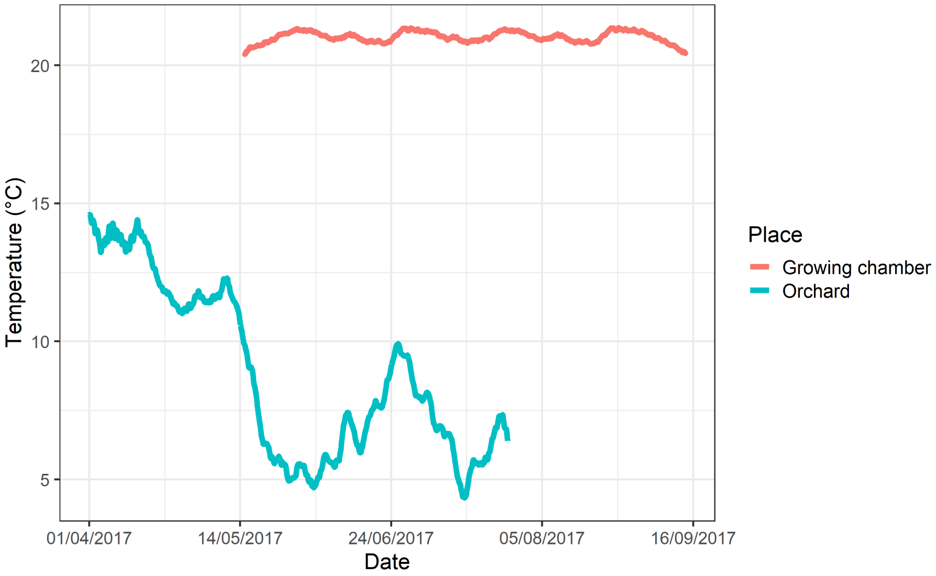

Figure 2-1. Running mean of hourly temperatures (computed over a period of 10 days) from the orchard (solid green line) and from the growing chamber (solid red line). Data for the orchard were obtained from the meteorological station of San Pedro, Molina, located 21 km away from the field

Budburst was recorded based on 960 twigs following the procedure described by Ruiz et al. (2007) and Campoy et al. (2013) with some modifications. In brief, we recorded budburst after 15 days (approx. 7,286 GDH) and 21 days (approx. 10,100 GDH) under forcing conditions. In this study, we used data collected at 21 days due to the low response (lower than 50%) in most of the varieties at 15 days (Fig. 2-2). As reported for apples by Naor et al. (2003), we used branches containing only vegetative buds. We defined budburst according to a binary scale considering 0 when less than 50% of buds in a twig were in state BBCH 08 (green tip) or in a prior state, and 1 when 50% of buds in the twig were in state BBCH 08 (green tip) or in a more advanced state.

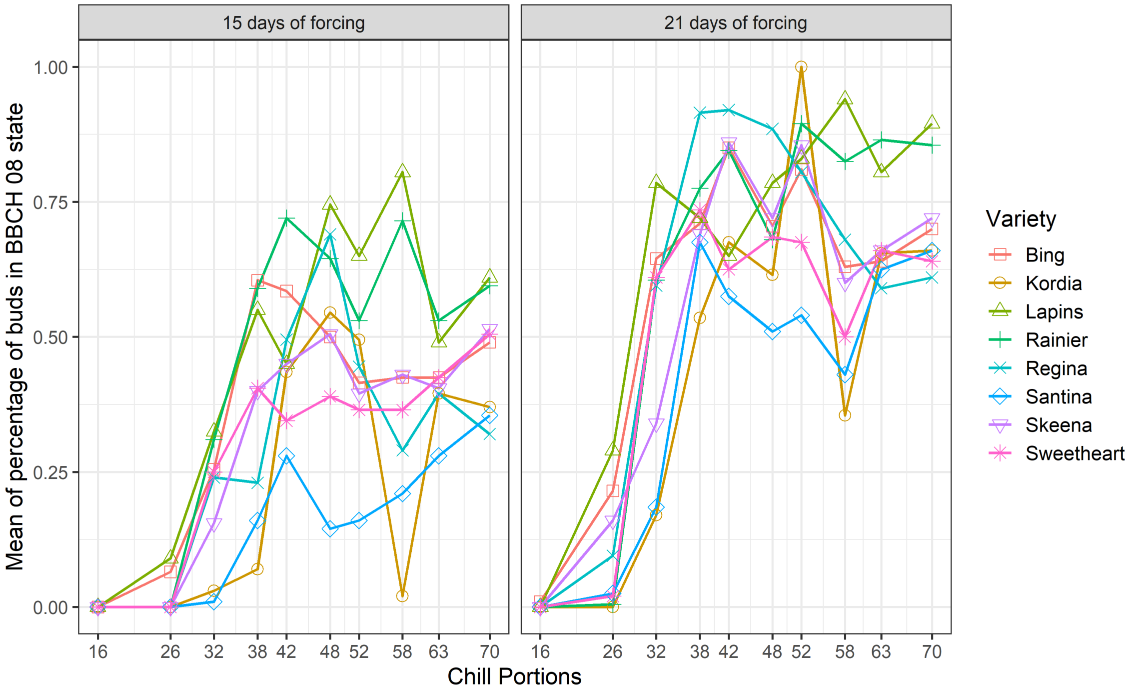

Figure 2-2. Budburst response of twigs (as the mean percentage of buds in BBCH 08 state) from eight sweet cherry varieties (Bing, Kordia, Lapins, Rainier, Regina, Santina, Skeena and Sweetheart) at different chill levels expressed as Chill Portions received in the orchard after two periods (15 and 21 days) in forcing conditions

The experimental design was a randomized complete block with a split plot factorial structure. The main plot was the date of twig harvest, which is analogous to chill received in the field, while the sub-plot was the variety. Six blocks were used with two sub-samples in the sub-plot (i.e. two shoots per sampling date, per variety replicate).

Analysis of hexoses and starch

On each sampling date, a sub-sample of two twig portions was collected for each variety. These portions of 2 cm in length were taken from the internode zone of the twigs. Upon arrival in the lab, sub-samples were stored at − 80 °C until carbohydrate analysis. Once all the samples were collected, they were ground and freeze-dried (Biobase BK-FD10S) in the lab. The extraction and quantification of hexoses (glucose + fructose) and starch was performed according to Fernandez et al. (2018) and Sperling et al. (2017) with modifications. In brief, lyophilized tissue samples of ~ 40 mg were mixed with 1 mL of ultrapure water and incubated at 70 °C for 15 min (Witeg, WiseBath WB, Germany). Samples were then centrifuged for 10 min at 21,000 *g* (Labnet Prism C2500, USA) and washed twice with 1 mL of ultrapure water. The supernatants (~ 3 mL) were kept for the subsequent analysis of hexoses, while the pellet was incubated at 100 °C for 10 min. After 10 min at room temperature, we mixed the pellet with 500 µL of acetate (pH 5.5), 100 µL of alpha amylase (7 units mL^{-1}, Sigma-Aldrich) and 100 µL of amyloglucosidase (70 units mL^{-1}, Sigma-Aldrich). This mix was incubated at 37 °C for 4 h for full starch digestion. After centrifuging for 5 min at 15,800 *g*, 150 µL of both hexoses and starch supernatant was mixed with 150 µL of DNS (3,5-dinitrosalicylic acid, Sigma-Aldrich). This mix was incubated for 15 min at 100 °C. After incubation, we added 700 µL of ultrapure water plus 50 µL of Rochelle salt. Carbohydrate levels were quantified using a spectrophotometer (Biobase BK-UV1800) at 550 nm with a glucose standard. The result of hexoses quantification was adjusted according to the dilution during washing of the pellet. For each sample, we also calculated the ratio between the concentration of hexoses (glucose + fructose) and the concentration of starch.

Statistical analysis

The concentration of hexoses and the concentration of starch, as well as the ratio between both variables were modeled via a generalized linear mixed model framework using gamma (log link function) with the lme4 package (Bates et al. 2015) in R (R Core Team 2017). Repetition of subsamples was introduced as random effect in the models, while Chill Portions and Variety were introduced as fixed effects individually and as interaction for both response variables. Significance of the effect of each factor was determined with a critical p value of 0.05. Models were selected by comparing AIC scores (Akaike Information Criterion). Post hoc estimates of mean separations were conducted using contrast of least squares means of variables included as non-random effects via the emmeans package (Lenth 2018) using a critical p value of 0.05. All selected outputs were plotted using the ggplot2 package (Wickham 2016).

In line with previous studies in trees using regression analysis (Charrier et al. 2013; Gonzalez-Rossia et al. 2008; Hein and Weiskittel 2010), we correlated budburst with the concentration of hexoses and starch, as well as the variety. Thus, in this experiment, four linear regression models were evaluated to correlate budburst with the concentrations of both hexoses and starch, as well as variety. These models were (Eqs. 2-1–2-4):

$$y = a + b[hexoses] + c[starch] + d[variety] + \epsilon, \tag{2-1}$$

$$y = a + b[hexoses] + c[starch] + \epsilon, \tag{2-2}$$

$$y = a + b[hexoses] + c[starch] + d[hexoses \times starch] + \epsilon, \tag{2-3}$$

$$y = a + b[ratio\ hexoses\colon starch] + \epsilon \tag{2-4}$$

In Eqs. (2-1)–(2-4), y represents an observation of budburst (0 or 1), a represents the intercept of each model, b, c and d are parameters for each variable included in the model and ϵ represents the error. The relationship between a binary variable such as budburst (0 or no and 1 or yes) and continuous variables such as the concentration of hexoses and starch or a categorical variable such as variety can be modeled via a link function, such as a logistic function (see Eq. 2-5), where $p(x)$ denotes the probability of x being 1. Specifically, this logistic regression describes the probability of budburst in a twig (according to the budburst definition in section Site, plant material, forcing conditions and experimental design of Materials and methods), as a function of the concentration of hexoses, the concentration of starch, and the variety. In Eq. (2-6), the probability of x occurring ($p(x)$) is expressed by the regression equation ($\alpha + \beta x$) defined for each model in Eqs. (2-1)–(2-4). In the case of categorical variables (i.e. the variety factor in this study) included as predictors in a binary model, the variable takes value 1 if the model is used for the respective category and 0 for all other categories.

$$logit[p(x)] = \ln\left[\frac{p(x)}{1-p(x)}\right] = \alpha + \beta x \tag{2-5}$$

$$p(x) = \frac{1}{1+Exp(-[\alpha+\beta x])} \tag{2-6}$$

All evaluated models were built via the *glm*() function in R (R Core Team 2017). Confidence intervals (95%) for all model coefficients were extracted with the function *confint.default*() in R. Residuals of the model were plotted to evaluate the model fit graphically. The goodness of fit was evaluated with the receiver-operating characteristic (ROC) curve and by calculating the area under the curve (AUC, Hein and Weiskittel 2010). The ROC curve plot displays the relationship between the true-positive rate (y axes) and the false-positive rate (x axes). An ROC graph is a useful technique for visualizing the performance of binary models (Hein and Weiskittel 2010). As AUC increases above 0.5 (represented by a line with true positive rate = false positive rate) the prediction error of the model decreases. Hoesmer and Lemeshow (2005) suggest that AUC values between 0.7 and 0.8 indicate acceptable discrimination, while AUC values above 0.8 signify excellent discrimination in models' comparison.

Results

Effect of chill accumulation and variety on starch concentration

In this experiment, the amount of chilling received in the orchard (CP) and the variety had significant effects on the concentration of starch in woody tissue of sweet cherry twigs (Fig. 2-3). Specifically, the varieties Kordia, Lapins, Rainier, Regina, Skeena and Sweetheart showed significantly higher starch concentrations at 16 CP compared to 70 CP, while Bing showed the same behavior between 26 CP and 70 CP (Fig. 2-3). Only one of the eight varieties showed the same starch concentration at the beginning and at the end of the period. However, for this variety (i.e. Santina), we observed a significant difference between 26 CP and 52 CP (Fig. 2-3). Between 16 CP and 70 CP, Bing, Kordia, Lapins, Rainier, Regina, Santina, Skeena and Sweetheart showed average reductions of 29%, 51%, 40%, 46%, 57%, 26%, 47% and 55%, respectively, in the amount of starch (mg) per gram of woody tissue. Regarding the comparison between varieties at the same sampling time, at 16 CP only starch concentrations for Santina and Rainier were significantly different, showing 28.49 ± 2.98 mg g^{-1} and 46.75 ± 4.89 mg g^{-1} of starch, respectively ($p < 0.05$). At the end of the experiment (63 and 70 CP), all varieties showed statistically similar concentrations of starch (Fig. 2-3).

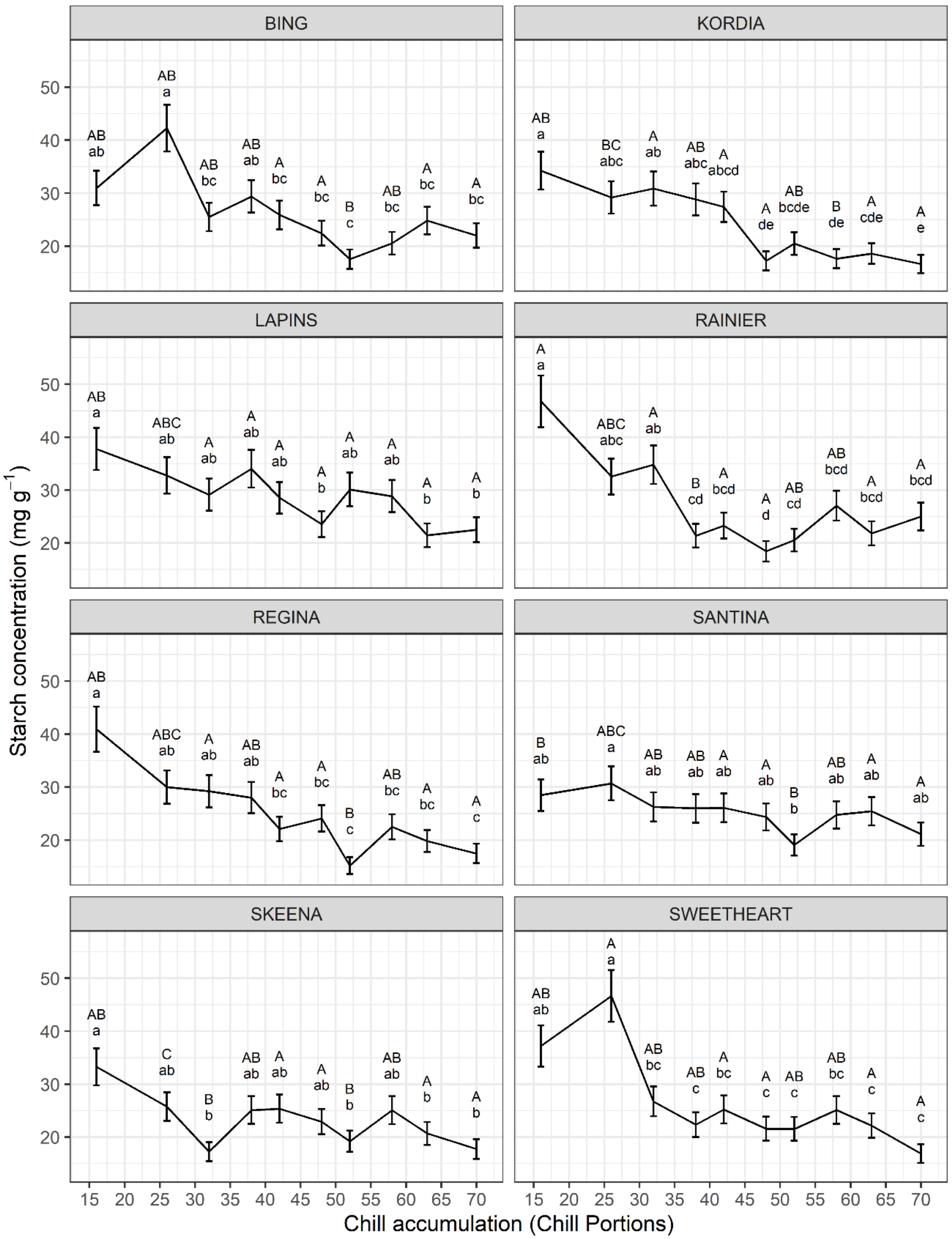

Figure 2-3. Starch concentration (mg g^{-1}) in woody samples of eight sweet cherry varieties (Bing, Kordia, Lapins, Rainier, Regina, Santina, Skeena and Sweetheart) at different chill levels expressed as Chill Portions received in the orchard. The solid line represents the mean and bars represent the standard error. n = 160 (20 per variety). Different lowercase letters in the same panel of each variety denote significant differences between chill accumulation rates (HSD-Tukey $\alpha = 0.05$), while different uppercase letters across panels at the same chill accumulation rate denote significant differences between varieties (HSD-Tukey $\alpha = 0.05$)

Effect of chill accumulation and variety on the concentration of hexoses

The amount of glucose + fructose per gram of woody tissue was significantly affected by the chilling received in the orchard and the variety (Fig. 2-4). Kordia, Lapins, Rainier, Regina, Skeena and Sweetheart showed significantly lower concentrations of hexoses at the beginning of the experiment (16 CP) than at the end (Fig. 2-4). The same trend was observed between 26 CP and 70 CP for Santina and between 32 CP and 63 CP for Bing (Fig. 2-4). These changes in the concentrations of hexoses represent average increases of 79%, 38%, 38%, 91%, 65%, 47%, 75% and 37% for Kordia, Lapins, Rainier, Regina, Skeena, Sweetheart, Santina and Bing, respectively. At 16 CP, Bing and Santina showed the highest concentrations of hexoses compared to other varieties (37.72 ± 2.71 mg g^{-1} and 38.21 ± 2.75 mg g^{-1}, respectively), while Kordia, Lapins, Rainier and Regina showed the lowest concentration of these sugars with values of 21.51 ± 1.55 mg g^{-1}, 21.38 ± 1.54 mg g^{-1}, 22.81 ± 1.64 mg g^{-1} and 21.06 ± 1.52 mg g^{-1}, respectively. At the end of the experiment (70 CP), Santina, Skeena and Sweetheart displayed the highest concentrations of hexoses, while Lapins and Rainier showed the lowest values for these carbohydrates (Fig. 2-4).

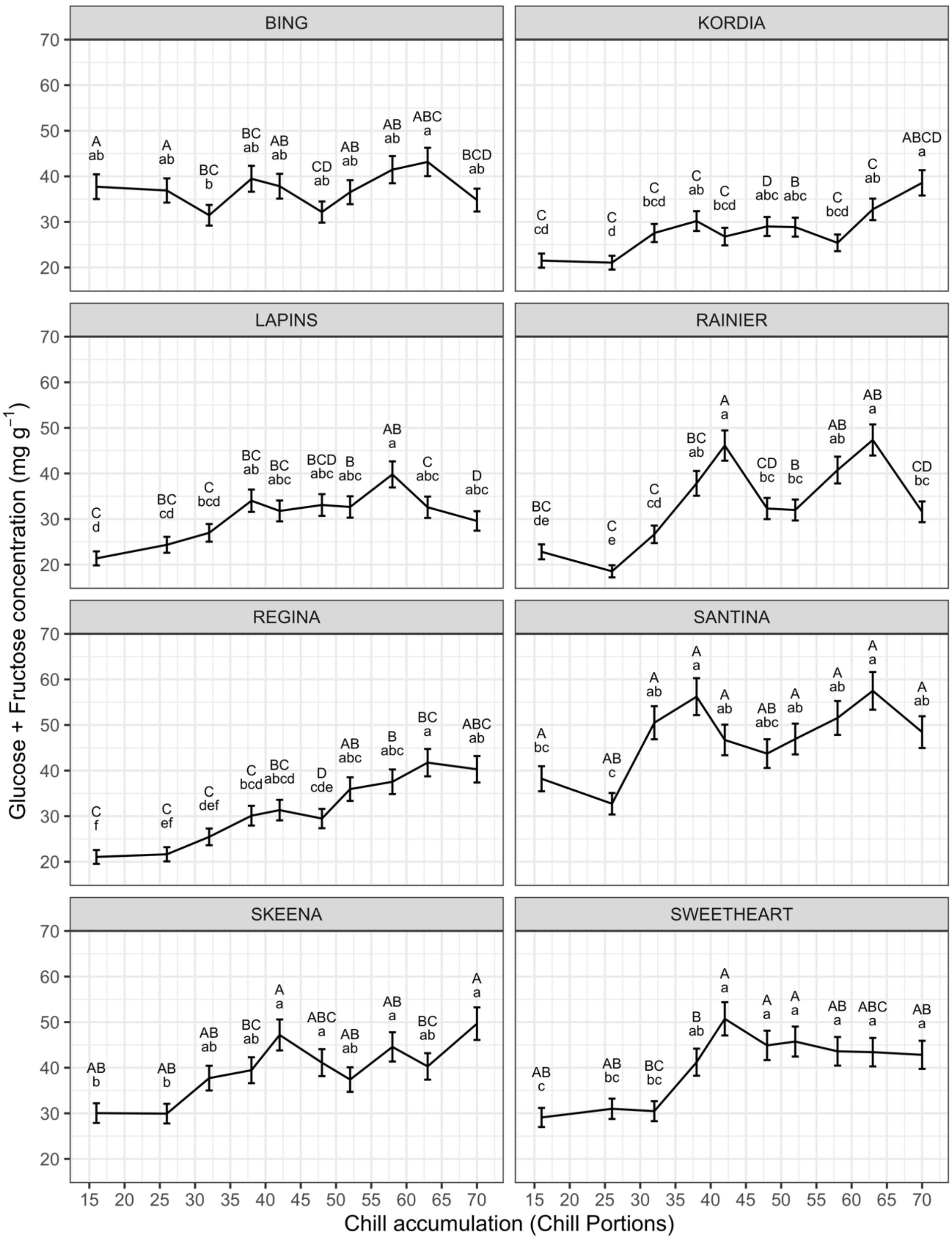

Figure 2-4. Concentration of hexoses (mg g^{-1}) in woody samples of eight sweet cherry varieties (Bing, Kordia, Lapins, Rainier, Regina, Santina, Skeena and Sweetheart) at different chill levels expressed as Chill Portions received in the orchard. The solid line represents the mean and bars represent the standard error. n = 160 (20 per variety). Different lowercase letters in the same panel of each variety denote significant differences between chill accumulation rates (HSD-Tukey α = 0.05), while different uppercase letters across panels at the same chill accumulation rate denote significant differences between varieties (HSD-Tukey α = 0.05)

Ratio between hexoses and starch

The ratio between the concentrations of glucose + fructose and starch was significantly affected by the rate of chill accumulation and the variety (Fig. 2-5). Bing and Santina showed ratios above 1 at the minimum rate of chill accumulation evaluated, while the remaining varieties displayed ratios below 1 at the same rate. This difference was significant among the group composed of Bing and Santina and the group composed of Kordia, Lapins, Rainier and Regina (Fig. 2-5). Regarding the effect of the rate of chill accumulation, 6 of the 8 varieties showed significant differences in the ratio between 16 and 70 CP (Fig. 2-5). Moreover, in these varieties differences appeared earlier than 70 CP and remained until the end of the experiment. Specifically, using the ratio observed at 16 CP as baseline, Rainier, Regina and Sweetheart showed significant differences since 38 CP, while Lapins and Skeena showed significant differences since 42 CP and Kordia since 48 CP. In the case of Santina, the difference was significant between 26 CP and between 52 CP and 70 CP (Fig. 2-5). Finally, the ratio between carbohydrates for Bing differed between 26 CP and since 52 CP.

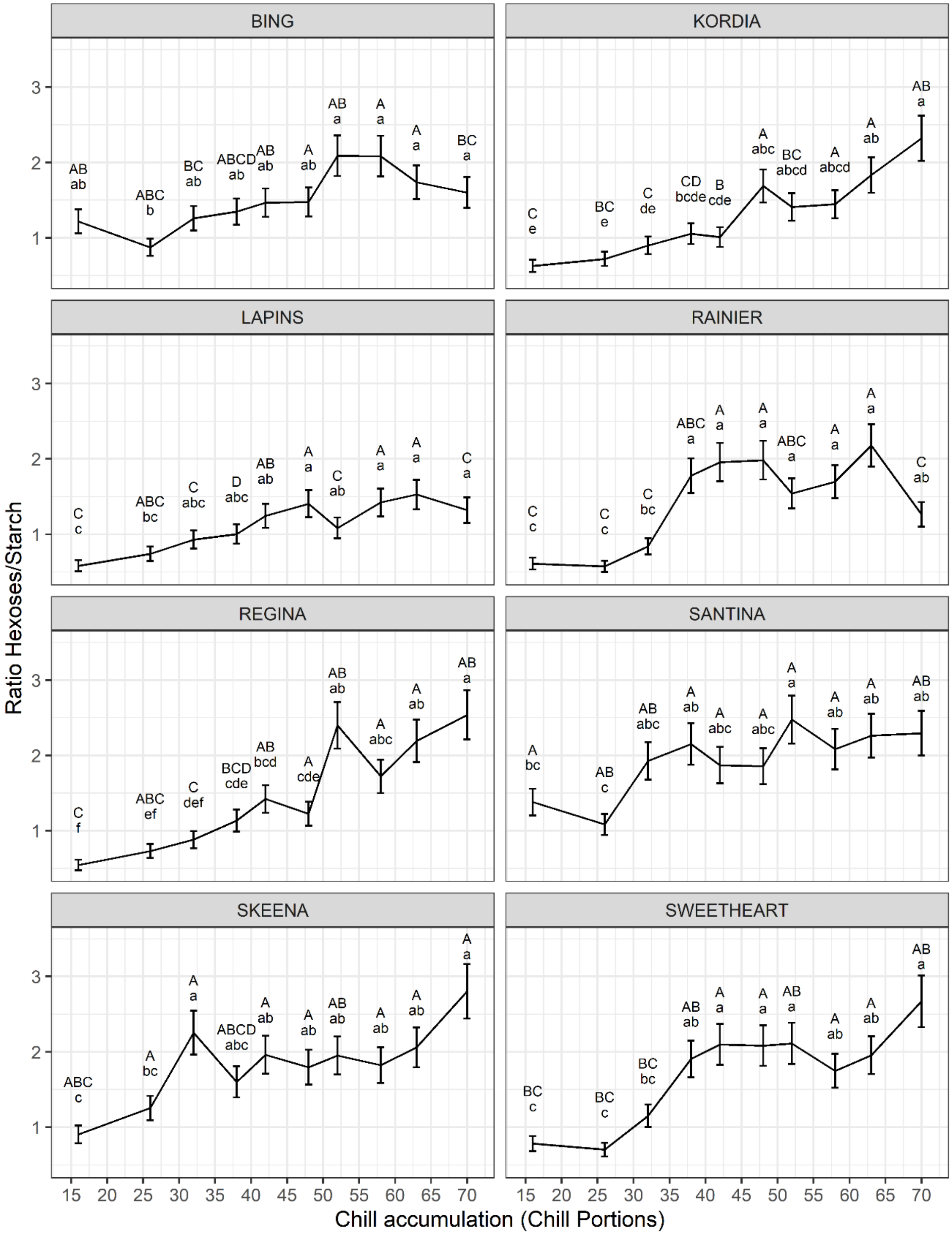

Figure 2-5. Ratio of the concentration of hexoses and starch at different chill levels expressed as Chill Portions received in the orchard for eight sweet cherry varieties (Bing, Kordia, Lapins, Rainier, Regina, Santina, Skeena and Sweetheart). The solid line represents the mean and bars represent the standard error. *n* = 160 (20 per variety). Different lowercase letters in the same panel of each variety denote significant differences between chill accumulation rates (HSD-Tukey *α* = 0.05), while different uppercase letters across panels at the same chill accumulation rate denote significant differences between varieties (HSD-Tukey *α* = 0.05)

Logistic regression analysis to estimate the probability of budburst

The best model was selected comparing ROC curves and according to the AUC values for each model (Fig. 2-6). The AUC values for the evaluated models were 0.9046 for the model in Eq. (2-1), 0.7866 for the model in Eq. (2-2), 0.5354 for the model in Eq. (2-3) and 0.7906 for the model in Eq. (2-4).

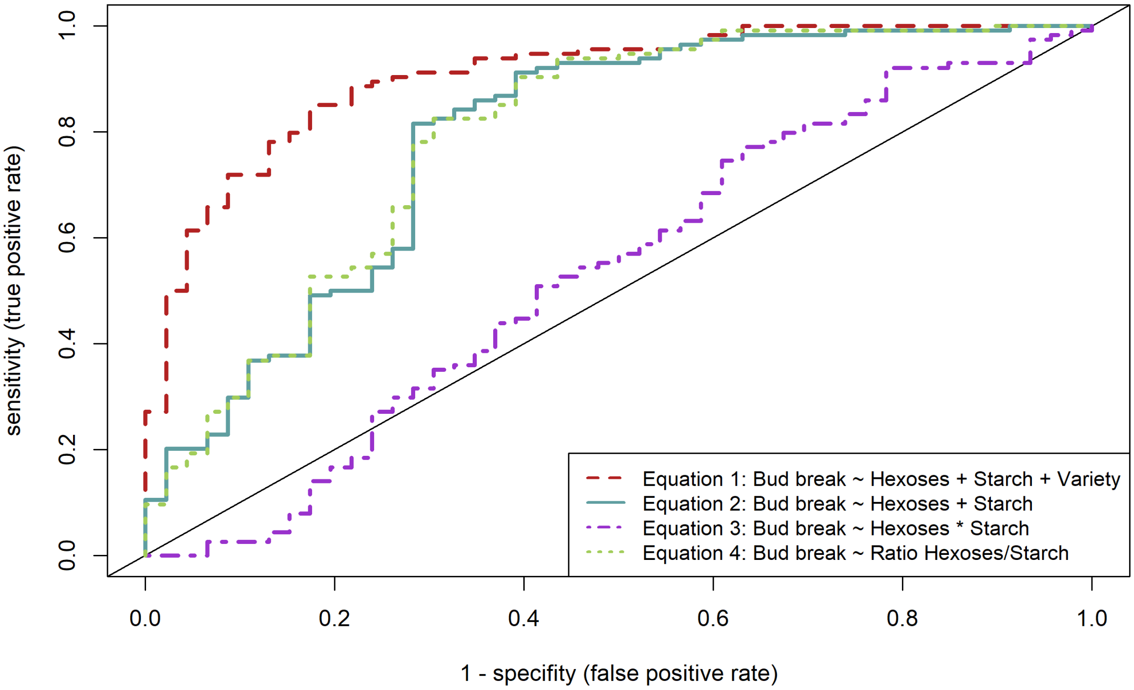

Figure 2-6. Receiver-operating characteristic (ROC) curve of the four evaluated models of logistic regression to estimate the probability of budburst. The diagonal line represents chance performance with an AUC = 0.5. In Eq. (3), the symbol asterisk (*) represents the interaction between factors

Thus, the best model to estimate the probability of budburst in a twig is shown in the following equation:

$$y = -2.5386 + 0.2252[hexoses] - 0.1493[starch] - 0.2147[Kordia] + 2.0081[Lapins] + 2.1844[Rainier] + 1.9534[Regina] - 4.1352[Santina] - 1.6011[Skeena] - 0.3616[Sweetheart] \tag{2-7}$$

In Eq. 2-7, for example, the probability of bud break in the variety Kordia is given by both concentrations of hexoses and starch and by the parameter estimated for this variety, while the values for the other varieties are set to 0.

In this regression analysis, using the variety Bing as reference for the variety variable, the variables hexoses concentration ($p > 0.0001$), starch concentration ($p > 0.001$) and the varieties Santina ($p > 0.001$) and Lapins (p

= 0.0465) had a significant effect on the probability of budburst. The coefficients fitted by the model were − 0.1493 for the starch concentration and 0.2252 for the hexoses concentration. On the other hand, Kordia, Santina, Skeena and Sweetheart showed negative coefficients, while in Lapins, Rainier and Regina positive values were observed (Table 2-1). The 95% confidence intervals for all model parameters are presented in Table 2-1.

Table 2-1. Model coefficients fitted by the model and confidence intervals (95%) of each coefficient

Coefficients	2.5% CI	Fitted by model	97.5% CI
a (intercept)	-7.0155	-2.5386	1.7797
b (hexoses)	0.1323	0.2252	0.3354
c (starch)	-0.2336	-0.1493	-0.0756
d (Kordia)	-2.1455	-0.2147	1.6071
d (Lapins)	0.0614	2.0081	4.0894
d (Rainier)	-0.0730	2.1844	4.6712
d (Regina)	-0.1766	1.9534	4.2163
d (Santina)	-6.4313	-4.1352	-2.1270
d (Skeena)	-3.5120	-1.6011	0.1992
d (Sweetheart)	-2.4521	-0.3616	1.7378

We created a classification table using the observed classifications of budburst in each sample according to a cut point of 0.5 (assuming budburst when 50% of buds in a shoot were in stage BBCH 08 or later) and the predicted classification of budburst fitted by the model (Table 2-2). In general, validating this model with the same dataset, the accuracy in the classification was 85%. Specifically, in 10% of the cases, the model assigned a value greater than 0.5 for the probability of budburst when the observation of budburst was lower than 0.5 (false positive). On the other hand, 5% of the time the model assigned a value lower than 0.5 for the probability of budburst when the observed value was greater than 0.5 (false negative, Table 2-2).

Table 2-2. Classification table of the logistic regression model comparing predicted classifications by the model and observed classifications in the experiment according to a cut point of 0.5

		Predicted by the model	
		Negative (0 = not budburst)	Positive (1 = budburst)
Observed in the experiment	Negative (0 = not budburst)	30/160 (18.75%)	16/160 (10.00%)
	Positive (1 = budburst)	8/160 (5.00%)	106/160 (66.25%)

According to the specific probabilities of budburst assigned by the model in each variety at the budburst moment, the varieties Santina, Skeena, Rainier, Lapins, Regina, Kordia and Bing showed the highest probabilities of budburst (up to 0.5) when budburst was recorded after the forcing period (Table 2-3). On the other hand, the variety Sweetheart showed the lowest rate of predicted probability (i.e. 0.49).

Table 2-3. Predicted probability of budburst at the time of recorded budburst, concentrations of starch and hexoses, and hexoses/starch ratio for each variety

Variety	Predicted probability[a]	Concentration of hexoses (mg g^{-1})	Concentration of starch (mg g^{-1})	Concentration of hexoses/starch ratio
Bing	0.67	36.95	42.27	0.87
Kordia	0.64	30.17	28.80	1.05
Lapins	0.77	27.05	29.12	0.93
Rainier	0.87	26.09	24.11	1.08
Regina	0.71	25.49	29.22	0.88
Santina	0.76	50.51	26.26	1.92
Skeena	0.88	28.64	18.29	1.57
Sweetheart	0.49	30.48	26.73	1.15

[a] Probabilities were estimated with the parameters fitted by the model and the average concentrations of starch and hexoses in woody tissue of sweet cherry

Discussion

Our results provide evidence that carbohydrate metabolism is closely related with endo-dormancy maintenance and release, supporting a number of earlier studies (Bonhomme et al. 2005; Charrier et al. 2013; Elle and Sauter 2000; Gonzalez-Rossia et al. 2008; Hamman et al. 1996; Hillmann et al. 2016; Horikoshi et al. 2017; Ito et al. 2012; Kaufmann and Blanke 2017; Keller and Loescher 1989; Morin et al. 2007; Sivaci 2006; Wang and Faust 1988b). Based on this finding, we used the variety and the concentration of starch and hexoses in sweet cherry twigs as predictors to estimate the probability of budburst using a logistic regression analysis.

Starch, which is stored mainly in twigs, branches and roots, is the main carbon storage polymer in vascular plants (Bahaji et al. 2014) and its degradation into soluble sugars provides energy for many plant processes. Specifically in deciduous trees, the initial growth at the beginning of a new season depends on starch (Guerra and Rodrigo 2015; Klein et al. 2016; Rodrigo et al. 2000), until leaves reach maturity and become source organs that supply other plant structures (Marchi et al. 2008; Naschitz et al. 2010). On the other hand, starch degradation and, therefore, the increase in concentrations of soluble sugars (e.g. hexoses, sucrose, raffinose, among others) during winter (Hallowell 1980) has been associated with frost tolerance, afforded by a reduction in the content of free water at the cellular level (Dietze et al. 2014; Faust et al. 1997; Vitra et al. 2017; Yu et al. 2017). In accordance with previous studies in *Prunus* species (Bonhomme et al. 2005; Gonzalez-Rossia et al. 2008; Kaufmann and Blanke 2017; Keller and Loescher 1989), pear (Horikoshi et al. 2017; Ito et al. 2012), apple (Sivaci 2006), walnut (Charrier et al. 2013), grape (Hamman et al. 1996), oak (Morin et al. 2007), poplar (Elle and Sauter 2000) and deciduous fruit trees in general (Hillmann et al. 2016; Wang and Faust 1988b) starch concentration in twigs decreased as chill accumulated in the orchard, while concentrations of hexoses (glucose + fructose) increased. Our results differ from results obtained in sweet cherry by Fadón et al. (2018) and in walnut by Farokhzad et al. (2018), who showed that starch concentration increased in ovary cells during dormancy progression. Specifically, Fadón et al. (2018) highlighted that the highest concentration of starch in flower buds of sweet cherry was recorded in mid-winter. However, this apparent discrepancy may be explained by Fadón et al. (2018) focusing on buds, where the physiological function of starch is not predominantly storage (Bahaji et al. 2014), and by them not including soluble sugar analysis for comparison. It is possible that during mid-winter and later, buds act as sinks of soluble sugars provided by starch degradation in shoots and then store (in short term) these sugars as starch (Bahaji et al. 2014) to support future ovary development (Rodrigo et al. 2000).

The relationship between concentrations of starch and hexoses was also identified by the logistic regression analysis performed in this study, through the coefficient value of each estimated parameter. The negative coefficient assigned for the starch concentration (− 0.1493) highlights that this molecule is closely related to the endo-dormancy stage (Gonzalez-Rossia et al. 2008; Kaufmann and Blanke 2017). This finding is comparable with results obtained by Farokhzad et al. (2018), who reported that genotypes of late budburst showed the highest concentrations of starch compared to early budburst genotypes. In contrast, the positive coefficient value describing the pool of hexoses is associated mainly with the eco-dormancy stage, where the bud can burst once its CR has been met (Lang 1987; Lang et al. 1987). Regarding different coefficient values in the varieties assigned by the proposed model, these may confirm that CR is an intrinsic feature of each species and variety (Campoy et al. 2011; Saure 2011). For instance, according to Erez (2000) the variety Lapins has a lower CR than Rainier (35 CP versus 45 CP), which matches the difference observed in this experiment through the coefficient assigned by the model (2.0081 in Lapins versus 2.1844 in Rainier).

Winter chill is expected to decrease considerably in the future in many regions because of climate change (Luedeling 2012; Luedeling et al. 2011), and the Mediterranean climate regions are facing particular risks of chill shortfalls (Benmoussa et al. 2018; Luedeling et al. 2009a). In these regions, several agricultural practices based on CR information are applied in fruit and nut tree orchards to obtain acceptable yields. Among these, the application of dormancy breakers, such as hydrogen cyanamide, has become indispensable in many climates with warm winters (Perez et al. 2009).

Impacts of increasing winter temperatures on carbohydrate metabolism have been reported in the past by Tixier et al. (2017a), Sperling et al. (2017), Horikoshi et al. (2017) and others. Specifically, Tixier et al. (2017a) showed that painting walnut branches after the CR has been met reduces the temperature of their stems by 1–2 °C, increasing the level of starch in woody tissue and delaying their budburst timing by several days. These results suggest that the carbohydrate metabolism in stems acts as an important factor during dormancy release (Tixier et al. 2017b) and might support the hypothesis regarding the interaction between regional climate conditions and the way in which plants accumulate chill. This is especially relevant in climates where the chill requirements reported in previous studies differ from those observed in situ (Benmoussa et al. 2017), and where the chilling models used for monitoring chill accumulation are consistently failing (Luedeling and Brown 2011).

The regression analysis presented here specifically models the probability that at least 50% of a twig's buds reach phenological stage BBCH 08 as a function of the variety and the concentration of carbohydrates. This means that using a cut point of 0.5 (Alburquerque et al. 2008; Campoy et al. 2013; Ruiz et al. 2007), the probability of budburst assigned by the model might represent the probability of having fulfilled the CR. Nonetheless, the arbitrary use of different cut points to evaluate budburst is still a controversial aspect which needs further research and standardizing.

Regression analysis in dormancy has been performed in the past by Gonzalez-Rossia et al. (2008), who correlated budburst percentage with the concentration of ammonium in excised shoots of four *Prunus* species through polynomial regression. However, no further analyses of this nature have been reported so far. In our study, comparing (with a cut point of 0.5) the observed budburst classifications with predicted CR fulfillment classifications, the logistic regression showed 85% accuracy (10% false positives and 5% false negatives). However, we also observed differences among varieties in the predicted probabilities of reaching the CR at budburst. Specifically, the variety Sweetheart showed the lowest probability of having reached its CR at the moment of budburst, while the remaining varieties showed higher values (up to 0.5). These differences across varieties may be explained by the logistic regression using only the concentrations of hexoses and starch and the variety as predictors in a small dataset with one species and one location, while dormancy progression may also induce changes in other metabolites, such as ammonium (Gonzalez-Rossia et al. 2008), hydrogen peroxide (Beauvieux et al. 2018), and amino-acids (Gotz et al. 2014), among others (Hillmann et al. 2016). Carbohydrate metabolism has been linked with multiple processes which can occur simultaneously. Specifically, dormancy progression may coincide with cold acclimation in autumn, and dormancy release with growth resumption in spring, among others (Gonzalez-Rossia et al. 2008; Yu et al. 2017). These co-occurring processes are closely related, making it difficult to decipher whether carbohydrate metabolism is an exclusive pathway for each process or a common response in plant physiology. Further research is needed to elucidate this question.

Budburst has been proposed to be an intrinsic characteristic of a bud (Rohde and Bhalerao 2007), and, therefore, its interaction with other structures needs further research. Finally, it must be noted that physiological markers might vary across years. Here, we have used physiological markers which are closely related to the interaction between genotype and environment (Tixier et al. 2017a) and may possibly affect chill accumulation. However, further research should include other physiological factors that also affect the CR (Beauvieux et al. 2018; Rios et al. 2014; Zheng et al. 2018), considering different locations, years and deciduous trees, in an effort to deepen and validate our results.

Conclusions

The results obtained in this experiment suggest that carbohydrate metabolism is closely related with endo-dormancy maintenance and release. Starch is catabolized in reserve zones to yield hexoses, probably to increase frost protection and to provide energy for the resumption of growth. The logistic regression analysis showed that varieties used in this study differed according to their probabilities of reaching the CR when budburst was recorded. This analysis is novel in the use of carbohydrates and varieties to predict the probability of budburst and, therefore, having reached CR. Additional markers, such as hormone levels, water content and morphological changes, among others, that are also involved in dormancy maintenance and release should be addressed in further research. Despite the limitations of this work, the results obtained in this study might improve our understanding on dormancy of deciduous trees, which is being increasingly affected in Mediterranean regions by climate change.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

Author contributions

SS designed the experiment and directed the data collection, LA and DF established the experiment and collected the data and EF performed the analyses. EF, IC, EL and SS wrote the manuscript.

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References

Alburquerque N, Garcia-Montiel F, Carrillo A, Burgos L (2008) Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. Environ Exp Bot 64 (2):162-170. doi:10.1016/j.envexpbot.2008.01.003

Anderson JL, Richardson EA, Kesner CD (1986) Validation of chill unit and flower bud phenology models for 'Montmorency' sour cherry. Acta Horticulturae (ISHS) 184:71-78. doi:10.17660/ActaHortic.1986.184.7

Arc E, Sechet J, Corbineau F, Rajjou L, Marion-Poll A (2013) ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. Front Plant Sci 4:19. doi:10.3389/fpls.2013.00063

Bahaji A, Li J, Sanchez-Lopez AM, Baroja-Fernandez E, Munoz FJ, Ovecka M, Almagro G, Montero M, Ezquer I, Etxeberria E, Pozueta-Romero J (2014) Starch biosynthesis, its regulation and biotechnological approaches to improve crop yields. Biotechnol Adv 32 (1):87-106. doi:10.1016/j.biotechadv.2013.06.006

Bates DM, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models using lme4. J Stat Softw 67 (1):1-48. doi:10.18637/jss.v067.i01

Beauvieux R, Wenden B, Dirlewanger E (2018) Bud dormancy in perennial fruit tree species: A pivotal role for oxidative cues. Front Plant Sci 9:13. doi:10.3389/fpls.2018.00657

Benmoussa H, Ben Mimoun M, Ghrab M, Luedeling E (2018) Climate change threatens central Tunisian nut orchards. Int J Biometeorol 62 (12):2245-2255. doi:10.1007/s00484-018-1628-x

Benmoussa H, Ghrab M, Ben Mimoun M, Luedeling E (2017) Chilling and heat requirements for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a warm Mediterranean location based on 30 years of phenology records. Agric For Meteorol 239:34-46. doi:10.1016/j.agrformet.2017.02.030

Bennett JP (1949) Temperature and bud rest period. Calif Agric 3 (11):9-12

Bonhomme M, Rageau R, Lacointe A, Gendraud M (2005) Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batch). Sci Hortic 105 (2):223-240. doi:10.1016/j.scienta.2005.01.015

Campoy JA, Ruiz D, Allderman L, Cook N, Egea J (2012) The fulfilment of chilling requirements and the adaptation of apricot (*Prunus armeniaca* L.) in warm winter climates: An approach in Murcia (Spain) and the Western Cape (South Africa). Eur J Agron 37 (1):43-55. doi:10.1016/j.eja.2011.10.004

Campoy JA, Ruiz D, Egea J (2011) Dormancy in temperate fruit trees in a global warming context: A review. Sci Hortic 130 (2):357-372. doi:10.1016/j.scienta.2011.07.011

Campoy JA, Ruiz D, Nortes MD, Egea J (2013) Temperature efficiency for dormancy release in apricot varies when applied at different amounts of chill accumulation. Plant Biol 15:28-35. doi:10.1111/j.1438-8677.2012.00636.x

Charrier G, Poirier M, Bonhomme M, Lacointe A, Ameglio T (2013) Frost hardiness in walnut trees (*Juglans regia* L.): How to link physiology and modelling? Tree Physiol 33 (11):1229-1241. doi:10.1093/treephys/tpt090

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. Plant Cell Environ 35 (10):1707-1728. doi:10.1111/j.1365-3040.2012.02552.x

Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R (2014) Nonstructural carbon in woody plants. In: Merchant SS (ed) Annual Review of Plant Biology, Vol 65. Annual Review of Plant Biology. Annual Reviews, Palo Alto, pp 667-687. doi:10.1146/annurev-arplant-050213-040054

Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: A multitasked signaling gas in plants. Mol Plant 8 (4):506-520. doi:10.1016/j.molp.2014.12.010

Egea J, Ortega E, Martinez-Gomez P, Dicenta F (2003) Chilling and heat requirements of almond cultivars for flowering. Environ Exp Bot 50 (1):79-85. doi:10.1016/s0098-8472(03)00002-9

Elle D, Sauter JJ (2000) Seasonal changes of activity of a starch granule bound endoamylase and of a starch phosphorylase in poplar wood (*Populus* x *canadensis* Moench < robusta >) and their possible regulation by temperature and phytohormones. J Plant Physiol 156 (5-6):731-740. doi:10.1016/s0176-1617(00)80239-4

Erez A (2000) Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics. In: Erez A (ed) Temperate Fruit Crops in Warm Climates. Springer Netherlands, Dordrecht, pp 17-48. doi:10.1007/978-94-017-3215-4_2

Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. Acta Hort 276:165-174

Fadón E, Herrero M, Rodrigo J (2018) Dormant flower buds actively accumulate starch over winter in sweet cherry. Front Plant Sci 9:10. doi:10.3389/fpls.2018.00171

Farokhzad A, Nobakht S, Alahveran A, Sarkhosh A, Mohseniazar M (2018) Biochemical changes in terminal buds of three different walnut (*Juglans regia* L.) genotypes during dormancy break. Biochem Syst Ecol 76:52-57. doi:10.1016/j.bse.2017.12.002

Faust M, Erez A, Rowland LJ, Wang SY, Norman HA (1997) Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance, and release. HortScience 32 (4):623-629

Fernandez E, Baird G, Farias D, Oyanedel E, Olaeta JA, Brown P, Zwieniecki M, Tixier A, Saa S (2018) Fruit load in almond spurs define starch and total soluble carbohydrate concentration and therefore their survival and bloom probabilities in the next season. Sci Hortic 237:269-276. doi:10.1016/j.scienta.2018.04.030

Fishman S, Erez A, Couvillon GA (1987a) The temperature-dependence of dormancy breaking in plants - Computer-simulation of processes studied under controlled temperatures. J Theor Biol 126 (3):309-321. doi:10.1016/s0022-5193(87)80237-0

Fishman S, Erez A, Couvillon GA (1987b) The temperature-dependence of dormancy breaking in plants - Mathematical-analysis of a 2-step model involving a cooperative transition. J Theor Biol 124 (4):473-483. doi:10.1016/s0022-5193(87)80221-7

Fuchigami LH, Wisniewski M (1997) Quantifying bud dormancy: Physiological approaches. HortScience 32 (4):618-623

Gonzalez-Rossia D, Reig C, Dovis V, Gariglio N, Agusti M (2008) Changes on carbohydrates and nitrogen content in the bark tissues induced by artificial chilling and its relationship with dormancy bud break in *Prunus* sp. Sci Hortic 118 (4):275-281. doi:10.1016/j.scienta.2008.06.011

Gotz KP, Chmielewski FM, Homann T, Huschek G, Matzneller P, Rawel HM (2014) Seasonal changes of physiological parameters in sweet cherry (*Prunus avium* L.) buds. Sci Hortic 172:183-190. doi:10.1016/j.scienta.2014.04.012

Guerra ME, Rodrigo J (2015) Japanese plum pollination: A review. Sci Hortic 197:674-686. doi:10.1016/j.scienta.2015.10.032

Guo L, Dai JH, Wang MC, Xu JC, Luedeling E (2015) Responses of spring phenology in temperate zone trees to climate warming: A case study of apricot flowering in China. Agric For Meteorol 201:1-7. doi:10.1016/j.agrformet.2014.10.016

Hallowell ER (1980) Cold and freezer storage manual. AVI Pub. Co

Hamman RA, Dami IE, Walsh TM, Stushnoff C (1996) Seasonal carbohydrate changes and cold hardiness of 'Chardonnay' and 'Riesling' grapevines. Am J Enol Vitic 47 (1):31-36

Harrington CA, Gould PJ, St Clair JB (2010) Modeling the effects of winter environment on dormancy release of Douglas-fir. For Ecol Manage 259 (4):798-808. doi:10.1016/j.foreco.2009.06.018

Hein S, Weiskittel AR (2010) Cutpoint analysis for models with binary outcomes: A case study on branch mortality. Eur J For Res 129 (4):585-590. doi:10.1007/s10342-010-0358-3

Hillmann L, Kaufmann H, Blanke M (2016) Bioindicators for the dormancy status of fruit trees. Erwerbs-Obstbau 58 (3):141-157. doi:10.1007/s10341-016-0284-8

Hoesmer D, Lemeshow S (2005) Multiple logistic regression. In: Shewhart WA, Wilks SS, Hosmer DW, Lemeshow S (eds) Applied Logistic Regression. doi:10.1002/0471722146.ch2

Horikoshi HM, Sekozawa Y, Sugaya S (2017) Inhibition of carbohydrate metabolism by thermal fluctuations during endodormancy lead to negative impacts on bud burst and incidence of floral necrosis in 'Housur' Japanese pear flower buds. Sci Hortic 224:324-331. doi:10.1016/j.scienta.2017.06.018

Hussain S, Niu Q, Yang F, Hussain N, Teng Y (2015) The possible role of chilling in floral and vegetative bud dormancy release in *Pyrus pyrifolia*. Biol Plant 59 (4):726-734. doi:10.1007/s10535-015-0547-5

Ibáñez C, Kozarewa I, Johansson M, Ögren E, Rohde A, Eriksson ME (2010) Circadian clock components regulate entry and affect exit of seasonal dormancy as well as winter hardiness in populus trees. Plant Physiol 153 (4):1823-1833. doi:10.1104/pp.110.158220

Ito A, Sakamoto D, Moriguchi T (2012) Carbohydrate metabolism and its possible roles in endodormancy transition in Japanese pear. Sci Hortic 144:187-194. doi:10.1016/j.scienta.2012.07.009

Kaufmann H, Blanke M (2017) Changes in carbohydrate levels and relative water content (RWC) to distinguish dormancy phases in sweet cherry. J Plant Physiol 218:1-5. doi:10.1016/j.jplph.2017.07.004

Keller JD, Loescher WH (1989) Nonstructural carbohydrate partitioning in perennial parts of sweet cherry. J Am Soc Hort Sci 114 (6):969-975

Klein T, Vitasse Y, Hoch G (2016) Coordination between growth, phenology and carbon storage in three coexisting deciduous tree species in a temperate forest. Tree Physiol 36 (7):847-855. doi:10.1093/treephys/tpw030

Kozarewa I, Ibáñez C, Johansson M, Ögren E, Mozley D, Nylander E, Chono M, Moritz T, Eriksson ME (2010) Alteration of PHYA expression change circadian rhythms and timing of bud set in *Populus*. Plant Mol Biol 73 (1-2):143-156. doi:10.1007/s11103-010-9619-2

Lang GA (1987) Dormancy - A new universal terminology. HortScience 22 (5):817-820

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo, para-, and ecodormancy: Physiological terminology and classification for dormancy research. HortScience 22:371-377

Lavee S, May P (1997) Dormancy of grapevine buds - Facts and speculation. Aust J Grape Wine R 3 (1):31-46. doi:10.1111/j.1755-0238.1997.tb00114.x

Lenth R (2018) emmeans: Estimated Marginal Means, aka Least-Squares Means. R. Package. 1.2.3. edn

Luedeling E (2012) Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci Hortic 144:218-229. doi:10.1016/j.scienta.2012.07.011

Luedeling E, Brown PH (2011) A global analysis of the comparability of winter chill models for fruit and nut trees. Int J Biometeorol 55 (3):411-421. doi:10.1007/s00484-010-0352-y

Luedeling E, Gassner A (2012) Partial Least Squares Regression for analyzing walnut phenology in California. Agric For Meteorol 158:43-52. doi:10.1016/j.agrformet.2011.10.020

Luedeling E, Girvetz EH, Semenov MA, Brown PH (2011) Climate change affects winter chill for temperate fruit and nut trees. PLoS One 6 (5):13. doi:10.1371/journal.pone.0020155

Luedeling E, Zhang MH, Girvetz EH (2009a) Climatic changes lead to declining winter chill for fruit and nut trees in California during 1950-2099. PLoS One 4 (7):9. doi:10.1371/journal.pone.0006166

Luedeling E, Zhang MH, Luedeling V, Girvetz EH (2009b) Sensitivity of winter chill models for fruit and nut trees to climatic changes expected in California's Central Valley. Agric Ecosyst Environ 133 (1-2):23-31. doi:10.1016/j.agee.2009.04.016

Marchi S, Tognetti R, Minnocci A, Borghi M, Sebastiani L (2008) Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (*Prunus persica*) and an evergreen sclerophyllous Mediterranean shrub (*Olea europaea*). Trees-Struct Funct 22 (4):559-571. doi:10.1007/s00468-008-0216-9

Morin X, Ameglio T, Ahas R, Kurz-Besson C, Lanta V, Lebourgeois F, Miglietta F, Chuine I (2007) Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. Tree Physiol 27 (6):817-825. doi:10.1093/treephys/27.6.817

Naor A, Flaishman M, Stern R, Moshe A, Erez A (2003) Temperature effects on dormancy completion of vegetative buds in apple. J Am Soc Hort Sci 128 (5):636-641

Naschitz S, Naor A, Genish S, Wolf S, Goldschmidt EE (2010) Internal management of non-structural carbohydrate resources in apple leaves and branch wood under a broad range of sink and source manipulations. Tree Physiol 30 (6):715-727. doi:10.1093/treephys/tpq028

Parankusam S, Adimulam SS, Bhatnagar-Mathur P, Sharma KK (2017) Nitric Oxide (NO) in plant heat stress tolerance: Current knowledge and perspectives. Front Plant Sci 8. doi:10.3389/fpls.2017.01582

Perez FJ, Burgos B (2004) Alterations in the pattern of peroxidase isoenzymes and transient increases in its activity and in H_2O_2 levels take place during the dormancy cycle of grapevine buds: The effect of hydrogen cyanamide. Plant Growth Regul 43 (3):213-220. doi:10.1023/B:GROW.0000046003.33194.ac

Perez FJ, Lira W (2005) Possible role of catalase in post-dormancy bud break in grapevines. J Plant Physiol 162 (3):301-308. doi:10.1016/j.jplph.2004.07.011

Perez FJ, Vergara R, Or E (2009) On the mechanism of dormancy release in grapevine buds: A comparative study between hydrogen cyanamide and sodium azide. Plant Growth Regul 59 (2):145-152. doi:10.1007/s10725-009-9397-5

Portrat K, Mathieu C, Motta C, Petel G (1995) Changes in plasma membrane properties of peach tree buds and stands during dormancy. J Plant Physiol 147 (3-4):346-350. doi:10.1016/s0176-1617(11)82165-6

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 1:331-332

Rinne PLH, van der Schoot C (2003) Plasmodesmata at the crossroads between development, dormancy, and defense. Can J Bot-Rev Can Bot 81 (12):1182-1197. doi:10.1139/b03-123

Rios G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in perennial plants. Front Plant Sci 5:6. doi:10.3389/fpls.2014.00247

Rodrigo J, Hormaza JI, Herrero M (2000) Ovary starch reserves and flower development in apricot (*Prunus armeniaca*). Physiol Plant 108 (1):35-41. doi:10.1034/j.1399-3054.2000.108001035.x

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12 (5):217-223. doi:10.1016/j.tplants.2007.03.012

Ruiz D, Campoy JA, Egea J (2007) Chilling and heat requirements of apricot cultivars for flowering. Environ Exp Bot 61 (3):254-263. doi:10.1016/j.envexpbot.2007.06.008

Saure MC (2011) Dormancy release in deciduous fruit trees. In: Janick J (ed) Horticultural Reviews. doi: 10.1002/9781118060735.ch6

Sivaci A (2006) Seasonal changes of total carbohydrate contents in three varieties of apple (*Malus sylvestris* Miller) stem cuttings. Sci Hortic 109 (3):234-237. doi:10.1016/j.scienta.2006.04.012

Sperling O, Silva LCR, Tixier A, Theroux-Rancourt G, Zwieniecki MA (2017) Temperature gradients assist carbohydrate allocation within trees. Sci Rep 7:10. doi:10.1038/s41598-017-03608-w

Tixier A, Roxas AA, Godfrey J, Saa S, Lightle D, Maillard P, Lampinen B, Zwieniecki MA (2017a) Role of bark color on stem temperature and carbohydrate management during dormancy break in Persian walnut. J Am Soc Hort Sci 142 (6):454-463. doi:10.21273/jashs04216-17

Tixier A, Sperling O, Orozco J, Lampinen B, Roxas AA, Saa S, Earles JM, Zwieniecki MA (2017b) Spring bud growth depends on sugar delivery by xylem and water recirculation by phloem Munch flow in *Juglans regia*. Planta 246 (3):495-508. doi:10.1007/s00425-017-2707-7

Vitra A, Lenz A, Vitasse Y (2017) Frost hardening and dehardening potential in temperate trees from winter to budburst. New Phytol 216 (1):113-123. doi:10.1111/nph.14698

Wang SY, Faust M (1988a) Changes of fatty-acids and sterols in apple buds during bud break induced by a plant bioregulator, thidiazuron. Physiol Plant 72 (1):115-120. doi:10.1111/j.1399-3054.1988.tb06631.x

Wang SY, Faust M (1988b) Metabolic-activities during dormancy and blooming of deciduous fruit-trees. Israel Journal of Botany 37 (2-4):227-243

Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56:122-128

Wickham H (2016) ggplot2: Elegant graphics for data analysis. R. Package. Springer-Verlag, New York

Yu DJ, Hwang JY, Chung SW, Oh HD, Yun SK, Lee HJ (2017) Changes in cold hardiness and carbohydrate content in peach (*Prunus persica*) trunk bark and wood tissues during cold acclimation and deacclimation. Sci Hortic 219:45-52. doi:10.1016/j.scienta.2017.02.038

Zheng CL, Acheampong AK, Shi ZW, Halaly T, Kamiya Y, Ophir R, Galbraith DW, Or E (2018) Distinct gibberellin functions during and after grapevine bud dormancy release. J Exp Bot 69 (7):1635-1648. doi:10.1093/jxb/ery022

Chapter 3

Prospects of decreasing winter chill for deciduous fruit production in Chile throughout the 21st century

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Introduction

Some perennial tree species enter a dormancy period during winter, which allows them to survive cold temperatures and continue growth in the following season. During dormancy, the meristem in a bud or in other parts of the plant is unable to undergo cell division and expansion (Cooke et al. 2012). To release dormancy and continue vegetative and reproductive growth in spring, woody deciduous species require cold temperatures during winter (Lang et al. 1987). This need is expressed by the concept of chill requirements (CR), i.e. the amount of chill required in a certain species and/or variety to reach bud break (Luedeling 2012). Chill requirements are typically quantified with one of a small set of common chill models and expressed in the models' units, i.e. Chilling Hours (CH) for the Chilling Hours model (Bennett 1949; Weinberger 1950), Chill Units (CU) for the Utah model (Richardson et al. 1974), and Chill Portions (CP) for the Dynamic model (Erez et al. 1990; Fishman et al. 1987a, b). All of these models use only autumn and winter temperatures as input values and do not consider any physiological parameters (Campoy et al. 2011; Luedeling 2012). The lack of such parameters may partly be responsible for well-documented low accuracy and low transferability of CR estimates, as evidenced by observations of bud break after accumulating much less chill than what was determined as the CR for the same tree variety elsewhere (Luedeling and Brown 2011). For instance, Benmoussa et al. (2017) reported a CR of 14.4 CP for almonds cv. Ferragnes in Tunisia, while Ramírez et al. (2010) estimated a need of 32 CP in the Central Valley of Chile. In Spain and for the same variety, a CR of 558 CU has been estimated (Egea et al. 2003, about 46 CP according to conversion factors between chill models reported in Luedeling and Brown 2011). Improving our understanding of dormancy control might allow updates to current CR estimates.

Commonly, the CR is estimated through techniques such as the analysis of long-term phenological datasets (Guo et al. 2015b; Luedeling and Gassner 2012; Pope et al. 2014) or with the use of twigs or young potted trees exposed to different amounts of chill and then introduced into forcing conditions until they reach bud break (Campoy et al. 2013; Egea et al. 2003). Despite the uncertainty of CR estimates and the chilling models' lack of general validity, along with the site dependency of the methods to estimate it, CR is a widely accepted parameter used in forestry (Junttila and Hänninen 2012), agricultural research (Benmoussa et al. 2017; Campoy et al. 2013), and agricultural planning (Luedeling 2012). In orchard planning, a common practice for selecting species and varieties is to compare the chill requirement of a given species and/or variety with the historical availability of winter chill in the orchard. This comparison provides important support to decisions related to the planning of long-term orchard investments (Luedeling 2012). Understanding chill requirements and matching them to future winter chill may be critical for the management of deciduous fruit tree orchards in a changing climate.

Overwhelming scientific evidence has underscored the notion that the Earth is warming (IPCC 2014). For projecting future conditions, the Intergovernmental Panel on Climate Change (IPCC) describes four Representative Concentration Pathways (RCPs) of atmospheric greenhouse gas (GHG) concentrations (IPCC 2014). These four RCPs include a stringent mitigation scenario (RCP2.6), two intermediate scenarios (RCP4.5 and RCP6.0), and one scenario with very high GHG concentrations (RCP8.5, IPCC 2014). Global surface temperature increases expected by the end of the 21st century range from 0.3 to 1.7 °C in the RCP2.6 scenario to 2.6 to 4.8 °C in the RCP8.5 scenario (IPCC 2014). Thus, even in the lowest GHG concentration scenario (RCP2.6), a marked increase in global temperature is projected. This is widely expected to impact winter chill availability in many regions, including some of the world's most important production regions of temperate fruit trees (Chmielewski et al. 2012; Darbyshire et al. 2016).

Areas with Mediterranean climate, such as the Mediterranean Basin, the Central Valley of California, and the Central Valley of Chile, among others, make important contributions to regional and global food security. Chile is an important actor regarding global food supply due mainly to its position in the southern hemisphere and its ability to produce certain foods in seasons when production in the northern hemisphere is negligible. According to FAOSTAT (2017), Chile ranked 1st globally in grape exports, 3rd for walnuts, 5th for apples, and 1st for sweet cherries in 2016. Fruit production contributes 38% to Chile's gross agricultural product, with 318,500 ha cultivated in 2016 (ODEPA 2017). The most common species are deciduous trees such as table grapes, walnuts, apples, and sweet cherries (ODEPA 2017). Roughly two thirds of Chile's agricultural area presents a Mediterranean climate and is located between the Coquimbo (29° S, 71° W) and Los Rios (39° S, 73° W) regions (ODEPA 2017).

A number of studies have projected the impact of climate change on winter chill availability, e.g. for California (Baldocchi and Wong 2008; Kerr et al. 2018; Luedeling et al. 2009d), Germany (Luedeling et al. 2009a), the Arabian Peninsula (Luedeling et al. 2009b), Australia (Darbyshire et al. 2016), and at the global scale (Luedeling et al. 2011a). According to Luedeling et al. (2009c) and based on a greenhouse gas emission scenario comparable with RCP8.5 (IPCC 2014), winter chill in the Central Valley of California is likely to decrease by 30 – 60% in relation to 1950 by mid-century and up to 80% by the end of the century. Mediterranean areas such as the Central Valley of Chile have shown a reduction from 60 CP in 1975 to 45 CP by 2000 (Luedeling et al. 2011a). The region is expected to lose at least 50% of available winter chill by the end of the 21st century (Luedeling et al. 2011a). Detailed information on the impacts of climate change, generated for an updated version of future GHG scenarios and using a wider set of global climate models along the considerable climate gradient that characterizes the Chilean growing regions, is still missing.

The main aim of this work was to project the impact of climate change on winter chill, a major factor determining what deciduous fruit species and cultivars can be grown in which locations. Additionally, we projected the risk of spring frost threatening to affect major species cultivated at each location.We analyzed historical change in winter chill for nine sites in Chile and anticipated future changes using an ensemble of climate scenarios. We employed the Dynamic model for chill quantification (Erez et al. 1990; Fishman et al. 1987b, a), since it has regularly emerged from model comparison studies as the most plausible model (Luedeling et al. 2009d; Ruiz et al. 2007; Zhang and Taylor 2011). We evaluated past changes in winter chill and projected future chill availability for the middle and the end of the 21st century for two RCP scenarios (RCP4.5 and RCP8.5) using 15 global climate models.

Materials and methods

Site selection and weather data

To analyze past and prospective future levels of winter chill, we used daily minimum and maximum temperature records for nine sites of present and future interest for deciduous fruit tree production from the Center for Climate and Resilience Research ([CR]^2) sponsored by the University of Chile (www.cr2.cl). These sites were Vallenar and Ovalle in the north of Chile; Quillota, Rengo, Curicó, Talca, and Chillán in central Chile; and Temuco and Osorno in the south of the country. At each site, we selected one weather station as primary source of data according to its percentage of complete data between 1967 and 2017 (Table 3-1, Fig. 3-1).

Table 3-1. Weather stations used as primary sources of weather data for nine fruit tree production sites in Chile

Site	Station Name	Responsible organization	Latitude	Longitude	Elevation (above sea level)	Percentage of dataset complete[a]
Vallenar	Santa Juana	DGA	28.66 °S	70.66 °W	560 m	93.3 %
Ovalle	Embalse La Paloma	DGA	30.69 °S	71.03 °W	320 m	97.5 %
Quillota	Quillota	DGA	32.89 °S	71.20 °W	130 m	75.1 %
Rengo	Rengo	DGA	34.42 °S	70.86 °W	310 m	75.7 %
Curicó	General Freire	DMC	34.96 °S	71.21 °W	225 m	99.9 %
Talca	Talca U.C.	DGA	35.43 °S	71.61 °W	130 m	67.2 %
Chillán	Bernardo O'Higgins	DMC	36.58 °S	72.04 °W	151 m	99.6 %
Temuco	Manquehue	DMC	38.77 °S	72.63 °W	92 m	99.2 %
Osorno	Canal Bajo	DMC	40.60 °S	73.06 °W	61 m	99.1 %

DGA: Dirección General de Aguas (General Direction of Water Resources); DMC: Dirección Meteorológica de Chile (Meteorology Direction of Chile)

[a] Percentage of the dataset complete considering the period between 01/01/1967 and 31/12/2017 (37,256 observations of minimum and maximum temperatures)

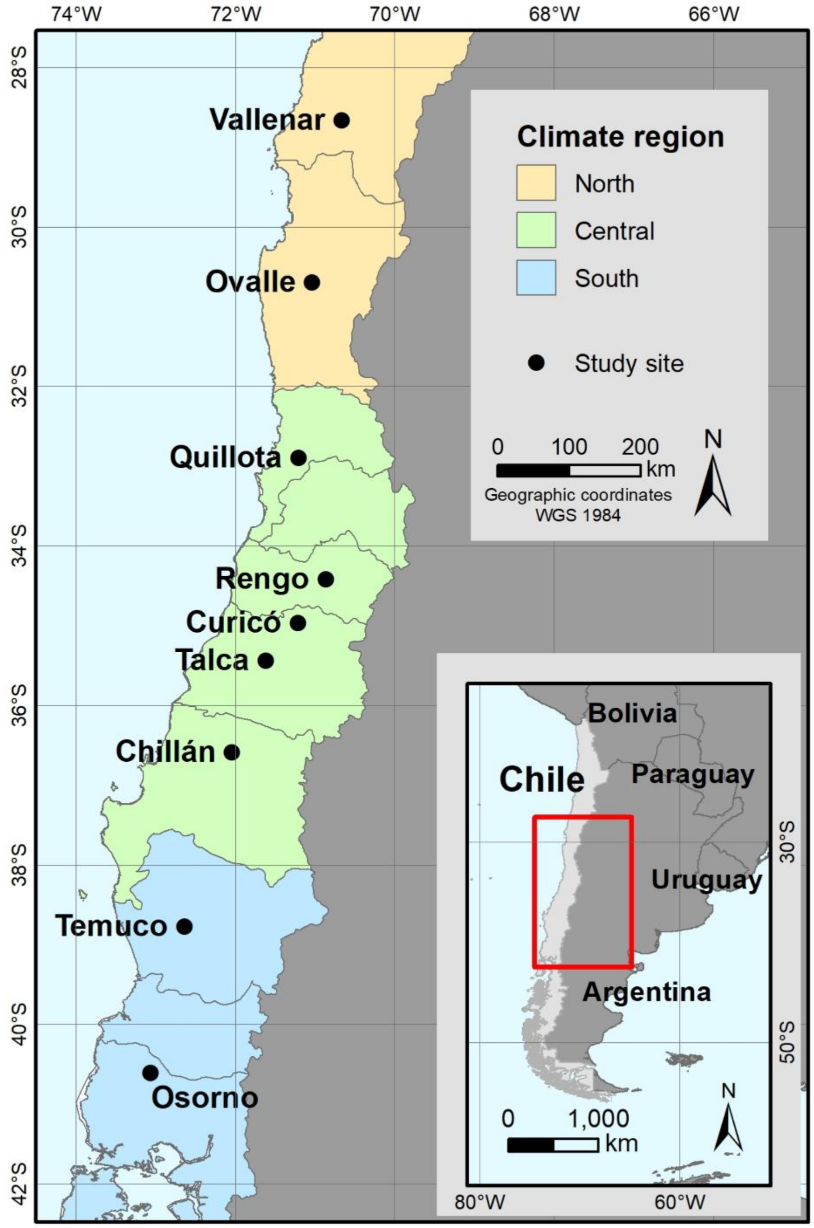

Figure 3-1. Location of the nine weather stations used as sources of primary information for assessment of future chill for fruit tree production sites in Chile

Additionally, due to gaps in the daily temperature records for the primary stations, we selected the 24 closest weather stations to each primary location and used them as auxiliary sources of data. The average auxiliary weather station was located 73.9 km from the primary weather station (the furthest was in Vallenar at 147.9 km away from the primary station). Using the function *patch_daily_temperatures* contained in the chillR package (Luedeling 2018) for R (R Core Team 2019), we filled the gaps in the record for the primary weather stations with data from the auxiliary stations. For each auxiliary station, this function determines temperature differences between sites and uses these to correct for between-station bias. Gaps in the primary station's records are then filled with data from the auxiliary site. This process is applied sequentially for all auxiliary stations, until all gaps have been filled or no more stations are available. To avoid including non-representative data, the maximum acceptable bias of the auxiliary records for daily minimum and maximum temperatures compared to the primary station was set to 5 °C. The procedure described above resulted in complete records for six locations, but seven, six, and one gaps remained in the data sets for three of the nine sites (Vallenar, Ovalle, and Curicó, respectively). We filled these gaps through linear interpolation according to procedures proposed in Luedeling et al. (2013) using the function *fix_weather* contained in the chillR package (Luedeling 2018). This resulted in complete datasets containing historic daily minimum and maximum temperature records for 51 years for all nine locations.

Temperature generation, future scenarios, and climate models

To analyze past trends in winter chill, we computed chill for each station and for each winter season of the historic record. In such analyses, however, long-term trends are often obscured by inter-annual variation, and, for any given year, it is difficult to determine the risk of particular temperature-related phenomena, such as chill shortfalls. Yet knowledge of this risk can be crucial for orchard managers taking strategic decisions, such as what species or cultivars to plant. To fill this information gap, we used the RMAWGEN weather generator (Cordano and Eccel 2016), which is accessed by the chillR package, to produce representative temperature scenarios for several points in time. These scenarios consisted of 100 replicates of plausible weather that might have occurred in the year of interest, given the local climate at the time. To produce these data, we trained the weather generator using all historic data between 1967 and 2017. Typical historic scenarios for each of the 51 years of the weather record were then produced by generating separate linear regression equations for the mean daily minimum and maximum temperatures of each month. We then applied these equations to compute typical values for mean daily temperature extremes of each month for all the years of interest and used these scenarios as inputs to the weather generator to obtain 100 replicates of plausible weather for each scenario year.

To enable analysis of future agroclimatic conditions, we obtained future projections for all locations from the ClimateWizard database maintained by the International Center of Tropical Agriculture (CIAT) via an application programming interface (https://github.com/CIATDAPA/climate_wizard_api), using functions integrated into chillR (Luedeling 2018). This database contains projections by 15 global climate models (Table 3-2) for the two Representative Concentration Pathways (RCPs) RCP4.5 and RCP8.5, which represent total radiative forcings of 4.5 W m^{-2} and 8.5 W m^{-2}, respectively, by 2100 (IPCC 2014). The RCP4.5 scenario represents a situation, in which authorities institute some policies that are effective in reducing emissions (IPCC 2014), whereas the RCP8.5 scenario is a strong warming scenario (IPCC 2014). For both RCPs, we obtained future temperature scenarios for the middle of the 21st century, represented by mean conditions between 2035 and 2065, and for the end of the 21st century (between 2070 and 2100). These scenarios were represented by their central years 2050 and 2085 and fed into the weather generator, which was used to produce 100 replicate years for each combination of site, RCP, year, and climate model.

Table 3-2. Climate models, whose projections were used to generate future temperature data

Name	Abbreviation	Reference and/or link
Beijing Climate Center – Climate System Model 1.1	bcc-csm1-1	Wu (2012) http://forecast.bcccsm.ncc-cma.net/web/channel-43.htm
Geophysical Fluid Dynamics Laboratory – Earth System Models	GFDL-ESM2G GFDL-ESM2M	Delworth et al. (2006) https://www.gfdl.noaa.gov/earth-system-model/
	GFDL-CM3	Donner et al. (2011)
Institute of Numerical Mathematics Climate Model version 4	inmcm4	Volodin et al. (2010)
Institute Pierre – Simon Laplace – Climate Model 5[a]	IPSL-CM5A-LR IPSL-CM5A-MR	https://cmc.ipsl.fr/ipsl-climate-models/ipsl-cm5/
Community Climate System Model 4	CCSM4	http://www.cesm.ucar.edu/models/ccsm4.0/
Community Earth System Model version 1 – BioGeoChemical model enabled	CESM1-BGC	Lindsay et al. (2014)
Beijing Normal University – Earth System Model	BNU-ESM	Ji et al. (2014)
Canadian Earth System Model 2	CanESM2	Chylek et al. (2011)
Model for Interdisciplinary Research On Climate – Earth System Model	MIROC-ESM	Watanabe et al. (2011)
Centre National de Recherches Météorologiques – Climate Model 5	CNRM-CM5	http://www.umr-cnrm.fr/spip.php?article126&lang=en
Australian Community Climate and Earth-System Simulator 1.0	ACCESS1-0	Bi et al. (2013)
Commonwealth Scientific and Industrial Research Organisation – Mark3.6.0	CSIRO-Mk3-6-0	Rotstayn et al. (2010)

Past and projected future winter chill and percentage of years with spring frost

To summarize chill, we considered the winter season between 1st of May and 30th of September in each year and at each site. We computed winter chill according to the Dynamic Model (expressed as Chill Portions – CP, Erez et al. 1990; Fishman et al. 1987b, a), using the *Dynamic_Model* function in chillR (Luedeling 2018). This calculation was performed (i) for each year of the historic dataset, (ii) for each year of each past scenario, and (iii) for each future scenario year (2050 and 2085), for both RCPs, and for each of 15 climate models. The resulting chill accumulation in each year was expressed by an array of 100 values. For each site, we also estimated safe winter chill, which is the 10% quantile of these winter chill distributions and represents the amount of chill that can reasonably be expected to be exceeded 90% of the time (Luedeling et al. 2009c).

As the incidence of spring frosts depends on the sensitivity of tree species and the phenological state, as well as on the time spent below a given temperature threshold, we only estimated the percentage of years with spring frost events according to Leolini et al. (2018). This metric was estimated for an early period in the past (from 1967 to 1980), a recent past period (from 2000 to 2014), and for each representative future year, RCP scenario, and climate model at each site. Specifically, we defined a frost event as a day with minimum temperature < 0 °C during the blooming period of the most representative deciduous fruit tree species cultivated at each site. In Vallenar and Ovalle, we estimated this metric between the 1st and 31st of August based on the most plausible budburst dates reported for table grapes (extracted from Selles and Muena 2017). For Quillota, Rengo, Curicó, Talca, and Chillán, we used the period between 1st and 30th of September, while for Temuco and Osorno, we used 1st to 31st of October based on bloom dates reported for similar sites of Chile for sweet cherry trees (Córdova et al. 2005; Serri et al. 2005). We then summarized this metric using the percentage of years where at least one frost event occurred during the frost-sensitive period.

To determine frost risk for the past periods, we used the weather generator to produce 100 years of plausible weather for each of the 15 years within the periods and quantified the probability of frost events during the sensitive period for each year.

Results

Historic winter chill

We detected substantial variation in historic chill levels across the study sites (left panels in Fig. 3-2). The lowest chill amounts were found in Vallenar (Fig. 3-2), the northernmost site (Fig. 3-1), while Osorno, the southernmost location, logged the highest number of Chill Portions (Fig. 3-2). The observed decrease in the mean Chill Portion count between the past period (between 1967 and 1977) and the recent period (between 2007 and 2017) was 18% in Vallenar, 18% in Ovalle, 29% in Quillota, 9% in Rengo, 5% in Curicó, 0% in Talca, 1% in Chillán, 0% in Temuco, and 1% in Osorno. Based on the simulation of historic winter scenarios, the most likely chill availability (90% confidence interval - CI) in 1970 was lowest, at between 23 and 39 CP, for Vallenar and highest, between 105 and 113 CP, for Osorno. The greatest historic change was observed in Quillota, where the 90% CI of chill diminished from 82 – 97 CP to 38 – 58 CP between 1967 and 2017. In other central regions such as Rengo, Curicó, and Talca, past variation was lower compared with northern sites and higher than at the southern sites used in this study. Chillán, the southernmost site in the central region, showed a reduction of winter chill comparable with southern sites.

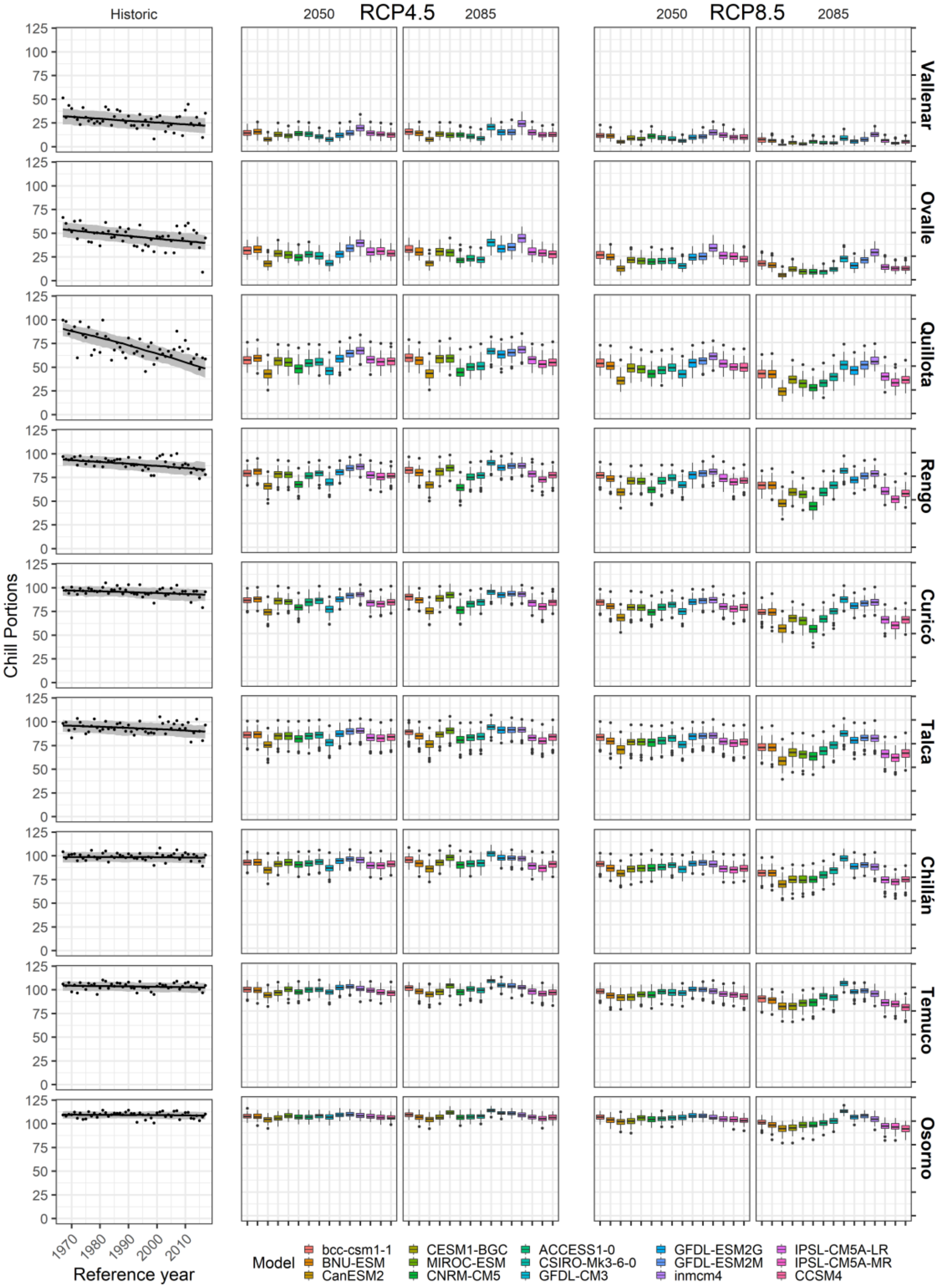

Figure 3-2. Historic and projected future winter chill for the major agricultural sites of Chile. In the historic panels (left), the grey areas represent typical chill ranges for each year (90% probability that chill falls within this range, determined with a weather generator), the black line shows the central values of these ranges, while dots indicate actual chill computed from historic weather data. The other panels show future scenarios defined by Representative Concentration Pathway (RCP4.5 or RCP8.5) and future year (2050 and 2085). In each panel, boxplots illustrate the plausible chill distributions for each of 15 climate models, determined with a weather generator (black dots in these plots represent outliers)

Future winter chill

Model results show substantial chill decreases in the northern sites Vallenar and Ovalle, as well as in the central sites Quillota, Rengo, Curicó, and Talca by 2050 and 2085 in both RCP scenarios (Fig. 3-2). For the RCP4.5 scenario, projected winter chill in 2050 was between 0 and 25 CP for Vallenar and between 13 and 38 CP for Ovalle. For the same scenario, Quillota was projected to experience between 38 and 63 CP, and Rengo between 63 and 88 CP (Fig. 3-2). Most winter chill was projected for Chillán (from 80 to 97 CP), Temuco (from 88 to 103 CP), and Osorno (from 99 to 110 CP). Relatively minor additional changes were projected for RCP4.5 by 2085 (Fig. 3-2).

For RCP8.5, considerable changes in winter chill were projected for both 2050 and 2085. For Vallenar, we projected between 0 and 12 CP by 2050 and from 0 to less than 10 CP by 2085. Similarly, models forecast a chill reduction by 36% from 2050 to 2085 for Ovalle in the RCP8.5 scenario, with chill levels between 5 and 27 CP projected for 2085.

We found high variability across the different climate models used to project future temperature scenarios in northern sites such as Vallenar and Ovalle, as well as in Quillota (Fig. 3-2). In these areas, the models projecting the lowest CP count were CanESM2 and GFDL-CM3. For central regions such as Rengo, the models CanESM2 and CNRM-CM5 forecasted the lowest chill (Fig. 3-2). In southern sites, all the models tended to predict similar quantities of winter chill (Fig. 3-2).

Safe winter chill

Projected safe winter chill is expected to decrease for all sites by 2050 and 2085 for both RCP scenarios (Fig. 3-3). In general, northern regions and the northernmost sites in the central region are expected to reach the lowest levels compared with the other sites. The southern sites of Temuco and Osorno appear to be the safest locations reaching safe chill levels greater than 75 CP in most of the future scenarios. For all sites, we expect a considerable reduction in safe winter chill in future scenarios compared with levels observed in the past. Minor changes are expected between 2050 and 2085 in the RCP4.5 scenario, while strong chill losses are projected between 2050 and 2085 in the RCP8.5 scenario for most of the climate models. Regarding differences between models, CanESM2 consistently projected lower safe winter chill in all sites for both RCP scenarios and for both years. In contrast, GFDL-CM3 projected higher values in both RCP scenarios by 2085 compared with other models.

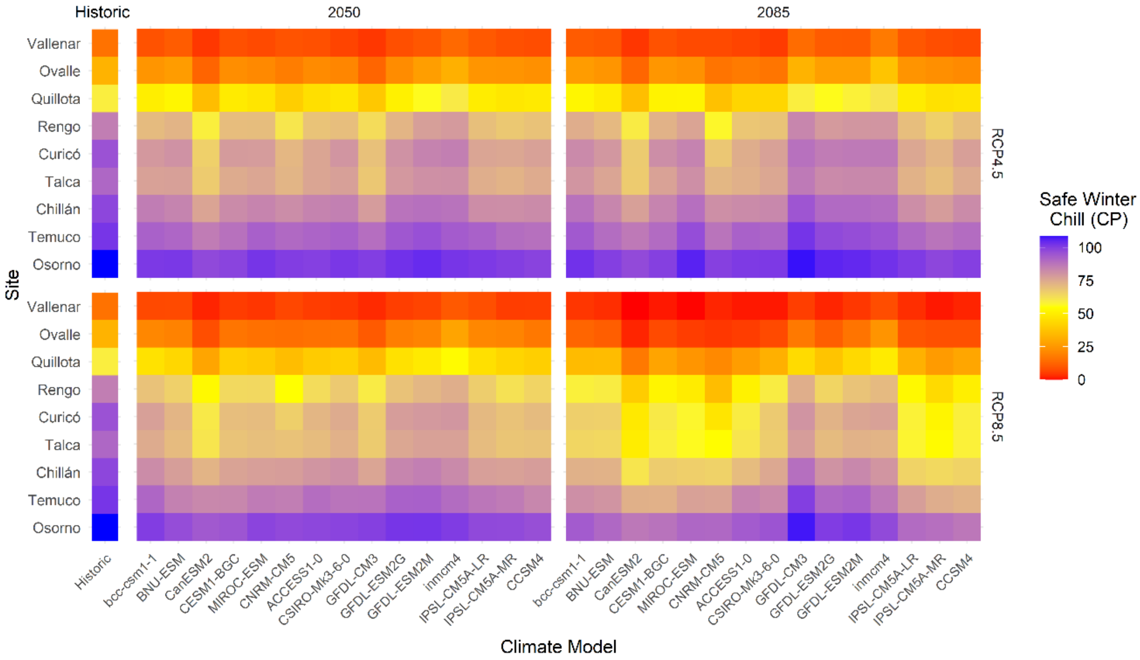

Figure 3-3. Safe winter chill expressed as Chill Portions in each of nine fruit production sites in Chile, for two RCP scenarios (RCP4.5 and RCP8.5) for three time horizons (historic = 1967 – 2017, 2050, and 2085) and 15 climate models. Safe winter chill is the amount of chill expected to be exceeded in 90% of years

Historic and projected future risk of spring frost

The number of years with at least one spring frost event (days with minimum temperatures below 0 °C) during the most plausible period of budburst for deciduous fruit trees is expected to decrease considerably in the future for all sites (Fig. 3-4). Between 1967 and 1981, the percentage of years with frost events was less than 15% in Vallenar and Ovalle; between 60 and 80% in Quillota, Rengo, Curicó, Talca, and Chillán; and between 80 and 90% in Temuco and Osorno. In the recent past period, Rengo, Curicó, Talca, and Chillán showed a strong reduction in the risk of frost events compared with the early past period. These sites reached between 40 and 60% of years with frost events during the period 2000 to 2014. Model results showed that Vallenar and Ovalle may continue to be the safest regions, with low probabilities of frost events in the future. In Quillota, for the RCP4.5 scenario by 2050, the probability of years with frost events ranges from 10 to 60% depending on the climate model, but only minor changes are expected by 2085. Finally, the highest probabilities of frost events are expected in Temuco and Osorno in all possible scenarios. Nonetheless, considerable differences are projected for these sites for the RCP8.5 scenario by 2085.

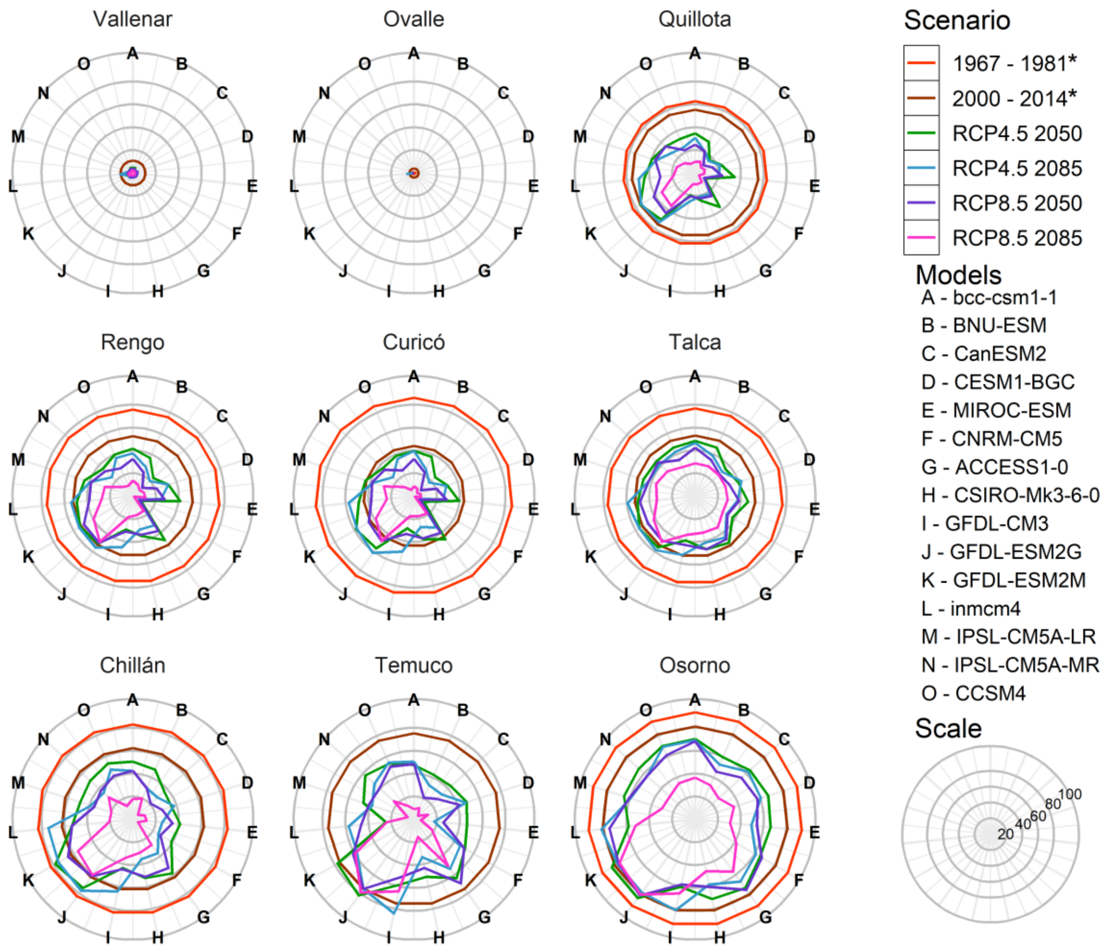

Figure 3-4. Probability of spring frost (daily minimum temperature below 0 °C during the frost-sensitive period) projected by 15 climate models for different sites of agricultural interest in Chile, for two past and four future climate scenarios. In each polar plot, letters represent the 15 climate models used in this study, while each gray ring represents 20% frost probability (maximum of 100% from the center to the periphery); colors represent different scenarios. Asterisk: In past scenarios, the median number of spring frost events for each period is presented

Discussion

Our results indicate that chill availability (estimated as Chill Portions) has declined substantially in some sites of Chile. These findings are in agreement with previous studies (Luedeling et al. 2011a; Luedeling et al. 2009c). Quillota, which is an area of Mediterranean climate (Kottek et al. 2006), showed the greatest decline (28.57%) between 1967 and 2017. Surprisingly, our results identified lower reductions of past chill in semi-arid regions of northern Chile, such as Vallenar and Ovalle, than in Quillota's Mediterranean climate. The global analysis performed by Luedeling et al. (2011a) reported a decrease by 15 CP in the Central Valley of Chile between 1975 and 2000. Our projections show a more dramatic decrease by 42 CP for Quillota (the northernmost site in the central region) from 1967 to 2017. This might be explained by our analysis including data after 2000 and using a wider range of specific locations for the same country than was considered in the earlier study. Earlier reports indicating that areas with temperate climates have not experienced major changes in winter chill (Luedeling et al. 2011a) were confirmed by our findings for Chile.

Our models forecast different levels of winter chill among sites and future scenarios. We found the lowest values in the RCP8.5 scenario by 2085 for all sites. Nonetheless, also for the intermediate scenario (e.g. RCP4.5), considerable chill losses are expected by 2050 in northern and central sites of Chile, while the southern sites are unlikely to experience significant changes. This difference between sites concurs with earlier reports of relatively stable future chill levels in temperate-zone locations (Luedeling et al. 2011a). Thus, major agricultural sites of Chile may experience adverse consequences of winter warming in the relatively near future.

According to our analysis, future cultivation of some deciduous fruit trees may no longer be viable in northern regions close to Vallenar and Ovalle. At projected chill levels, farmers can expect bud abortion, low percentage of budburst, staggered bloom, and reduction in yields of nut and fruit trees (Erez 2000; Lavee and May 1997). Horikoshi et al. (2017) demonstrated that thermal fluctuations during endo-dormancy of pear trees reduce the survival of buds and budburst. It is possible that there will not be enough winter chill to cultivate woody fruit species with low chill requirement such as grapes, for which CRs of 15 to 35 CP (depending on the variety) have been determined in the cold-hardy grapevine germplasm collection in Geneva, New York (Londo and Johnson 2014). Some almond cultivars can tolerate low-chill conditions, requiring only 22 to 23 CP in the Central Valley of Chile (Ramírez et al. 2010). Tree cultivars with such low CRs are widely cultivated today in low-chill regions. Given future chill expectations for such regions, however, even these cultivars may only thrive in specific microclimates in the future (Luedeling 2012; Pathak et al. 2018). Conversely, in the southernmost sites in the central region such as Chillán, and in the south of Chile (Temuco and Osorno), projected chill should be sufficient for many crops with high CR, such as apple (e.g. approximately 50 CP for cv. Golden Delicious, according to Erez 2000), pear, prune, kiwifruit, walnut, and sweet cherry (e.g. approximately 70 CP for cv. Sam, according to Alburquerque et al. 2008). Despite prospects of future warming, spring frost events are expected to remain common, especially in Temuco and Osorno, indicating considerable risk of frost damage during pollination and fruit set. It is important to note that the susceptibility of tree crops to frost damage is greatly influenced by the timing of sensitive developmental phases. The most critical phases occur after budburst, which has been shown to shift in response to temperature changes (Badeck et al. 2004; Martinez-Lüscher et al. 2017). In evaluating frost risk, we did not take such shifts into account, because the extent (Martinez-Lüscher et al. 2017) and even the direction (Guo et al. 2015a) of such shifts are difficult to forecast, and it seems highly likely that growers would respond to early indications of such shifts by adopting tree cultivars that are adapted to future conditions.

In some sites of the Central Valley of Chile (Rengo, Curicó, and Talca), projected safe winter chill is expected to decrease to between 69 and 75 CP by 2050 in the RCP4.5 scenario and to 64–70 CP by the same year in the RCP8.5 scenario. Facing such changes, growers may need to develop adaptation strategies, such as the use of dormancy-breaking products (Perez et al. 2009), in order to maintain currently cultivated crops with high CR, such as kiwifruits, sweet cherries, pears, walnuts, and apples. Introduction of cultivars with lower CR, possibly produced in local breeding programs, is likely to facilitate the adaptation process. For the northernmost site in the central region (Quillota), replacement of the current stock with low-chill varieties may be the only viable option for growers of deciduous fruits and nuts. Nevertheless, generating new low-chill cultivars requires a better understanding of dormancy control under current and future conditions.

It should be noted that the Dynamic Model has not been validated or even parameterized under Chilean (or many other) conditions, or for the species cultivated there. Model validation, however, requires extensive physiological and/or phenological datasets, or experiments under controlled conditions. This kind of information is still missing, especially for the range of climates expected in the future and for the large geographic scope we addressed. Chill models developed so far have demonstrated low accuracy and transferability among climates (Benmoussa et al. 2017; Campoy et al. 2011; Luedeling and Brown 2011), and it seems that slightly different models should be used for different species or even varieties. Site-specific parametrization and extensive validation would be desirable. While the Dynamic Model (Erez et al. 1990; Fishman et al. 1987b, a) has consistently outperformed all other available models in comparative studies (Luedeling 2012), we therefore point out that accurate quantification of chill accumulation and chill requirements is still a prominent knowledge gap, which should urgently be addressed in order to facilitate quantitatively appropriate adaptation to imminent changes in winter conditions.

Climate change does not only impact on winter chill, but it may also positively or negatively affect other production-relevant factors such as water availability, the risk of spring frost events, fruit development, and pest pressure (through earlier appearance at the start of the growing season, or greater number of pest generations per season). Among the beneficial impacts that may arise are a reduced frequency and severity of frost damage in spring and advanced fruit maturity in response to warmer temperatures (Tombesi et al. 2010). On the other hand, high temperatures during spring might decrease the final size of peach fruits (Lopez et al. 2007), which may constitute a reduction in fruit quality. A review by Pathak et al. (2018) and a study by Luedeling et al. (2011b) indicate a possible increase in the incidence and severity of pests and diseases with rising temperatures during the growing season.

Farmers, industry, and governments should be prepared to take action to preserve deciduous fruit tree production. Urgent measures in terms of identifying appropriate varieties, species, and management methods may be required given the many possible impacts of climate change on fruit trees. The modeling approaches applied here can help in devising meaningful adaptation strategies for growers of deciduous fruit trees in Chile. They can also be applied to other crops and in other regions.

Conclusions

To overcome risks and exploit new opportunities arising from climate change, farmers and planners are in need of decision support for orchard planning and other impact mitigation strategies. The methodologies outlined in this study may be useful to assess effects of global warming on various tree crops in Chile and elsewhere. They can also aid geneticists and breeders in generating cultivars able to face warm winters and physiologists in improving their understanding of dormancy under possible future conditions. Our projections suggest that winter chill will decrease rapidly across the major agricultural sites of Chile, most of which are characterized by a Mediterranean climate. The greatest decline is expected for the northern and north-central regions. Our expectation of considerable chill reductions in these regions indicates that some currently cultivated low-chill species, such as table grapes and almonds, may experience severe impacts on yield and growth. The safest sites appear to be the southern regions, followed by central regions, which are expected to be moderately affected by climate change. The modeling approach demonstrated here holds promise for helping farmers, industry, and governments take action to ensure that deciduous fruit tree production will be viable in the future.

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Conflict of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

Author contributions

EL developed the code for future projections of winter chill. CW and EF performed the projections and made the figures. EF wrote the manuscript. IC, CW, and EL improved the manuscript.

References

Alburquerque N, Garcia-Montiel F, Carrillo A, Burgos L (2008) Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. Environ Exp Bot 64 (2):162-170. doi:10.1016/j.envexpbot.2008.01.003

Badeck FW, Bondeau A, Bottcher K, Doktor D, Lucht W, Schaber J, Sitch S (2004) Responses of spring phenology to climate change. New Phytol 162 (2):295-309. doi:10.1111/j.1469-8137.2004.01059.x

Baldocchi D, Wong S (2008) Accumulated winter chill is decreasing in the fruit growing regions of California. Clim Change 87:S153-S166. doi:10.1007/s10584-007-9367-8

Benmoussa H, Ghrab M, Ben Mimoun M, Luedeling E (2017) Chilling and heat requirements for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a warm Mediterranean location based on 30 years of phenology records. Agric For Meteorol 239:34-46. doi:10.1016/j.agrformet.2017.02.030

Bennett JP (1949) Temperature and bud rest period. Calif Agric 3 (11):9-12

Bi DH, Dix M, Marsland SJ, O'Farrell S, Rashid HA, Uotila P, Hirst AC, Kowalczyk E, Golebiewski M, Sullivan A, Yan HL, Hannah N, Franklin C, Sun ZA, Vohralik P, Watterson I, Zhou XB, Fiedler R, Collier M, Ma YM, Noonan J, Stevens L, Uhe P, Zhu HY, Griffies SM, Hill R, Harris C, Puri K (2013) The ACCESS coupled model: Description, control climate and evaluation. Aust Meteorol Oceanogr J 63 (1):41-64. doi:10.22499/2.6301.004

Campoy JA, Ruiz D, Egea J (2011) Dormancy in temperate fruit trees in a global warming context: A review. Sci Hortic 130 (2):357-372. doi:10.1016/j.scienta.2011.07.011

Campoy JA, Ruiz D, Nortes MD, Egea J (2013) Temperature efficiency for dormancy release in apricot varies when applied at different amounts of chill accumulation. Plant Biol 15:28-35. doi:10.1111/j.1438-8677.2012.00636.x

Chmielewski FM, Blumel K, Palesova I (2012) Climate change and shifts in dormancy release for deciduous fruit crops in Germany. Clim Res 54 (3):209-219. doi:10.3354/cr01115

Chylek P, Li J, Dubey MK, Wang M, Lesins G (2011) Observed and model simulated 20th century Artic temperature variability: Canadian Earth System Model CanESM2. Atmos Chem Phys Discuss 11:22893 - 22907. doi:10.5194/acpd-11-22893-2011

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. Plant Cell Environ 35 (10):1707-1728. doi:10.1111/j.1365-3040.2012.02552.x

Cordano E, Eccel E (2016) Tools for stochastic weather series generation in R environment. Ital J Agrometeorol-Riv Ital Agrometeorol 21 (3):31-42. doi:10.19199/2016.3.2038-5625.031

Córdova C, Reginato G, Fernandez P, Alessandrini L (2005) Introducción del cerezo en la décima región. In: Lemus G (ed) El cultivo del cerezo, vol 133. Boletín INIA Santiago de Chile, pp 211-220

Darbyshire R, Measham P, Goodwin I (2016) A crop and cultivar-specific approach to assess future winter chill risk for fruit and nut trees. Clim Change 137 (3-4):541-556. doi:10.1007/s10584-016-1692-3

Delworth TL, Broccoli AJ, Rosati A, Stouffer RJ, Balaji V, Beesley JA, Cooke WF, Dixon KW, Dunne J, Dunne KA, Durachta JW, Findell KL, Ginoux P, Gnanadesikan A, Gordon CT, Griffies SM, Gudgel R, Harrison MJ, Held IM, Hemler RS, Horowitz LW, Klein SA, Knutson TR, Kushner PJ, Langenhorst AR, Lee HC, Lin SJ, Lu J, Malyshev SL, Milly PCD, Ramaswamy V, Russell J, Schwarzkopf MD, Shevliakova E, Sirutis JJ, Spelman MJ, Stern WF, Winton M, Wittenberg AT, Wyman B, Zeng F, Zhang R (2006) GFDL's CM2 global coupled climate models. Part I: Formulation and simulation characteristics. J Clim 19 (5):643-674. doi:10.1175/jcli3629.1

Donner LJ, Wyman BL, Hemler RS, Horowitz LW, Ming Y, Zhao M, Golaz JC, Ginoux P, Lin SJ, Schwarzkopf MD, Austin J, Alaka G, Cooke WF, Delworth TL, Freidenreich SM, Gordon CT, Griffies SM, Held IM, Hurlin WJ, Klein SA, Knutson TR, Langenhorst AR, Lee HC, Lin YL, Magi BI, Malyshev SL, Milly PCD, Naik V, Nath MJ, Pincus R, Ploshay JJ, Ramaswamy V, Seman CJ, Shevliakova E, Sirutis JJ, Stern WF, Stouffer RJ, Wilson RJ, Winton M, Wittenberg AT, Zeng FR (2011) The dynamical core, physical parameterizations, and basic simulation characteristics of the atmospheric component AM3 of the GFDL global coupled model CM3. J Clim 24 (13):3484-3519. doi:10.1175/2011jcli3955.1

Egea J, Ortega E, Martinez-Gomez P, Dicenta F (2003) Chilling and heat requirements of almond cultivars for flowering. Environ Exp Bot 50 (1):79-85. doi:10.1016/s0098-8472(03)00002-9

Erez A (2000) Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics. In: Erez A (ed) Temperate Fruit Crops in Warm Climates. Springer Netherlands, Dordrecht, pp 17-48. doi:10.1007/978-94-017-3215-4_2

Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. Acta Hort 276:165-174

FAOSTAT (2017) Rankings: Countries by commodity. http://www.fao.org/faostat/en/#rankings/countries_by_commodity_exports. Accessed 18-07-2018

Fishman S, Erez A, Couvillon GA (1987a) The temperature-dependence of dormancy breaking in plants - Computer-simulation of processes studied under controlled temperatures. J Theor Biol 126 (3):309-321. doi:10.1016/s0022-5193(87)80237-0

Fishman S, Erez A, Couvillon GA (1987b) The temperature-dependence of dormancy breaking in plants - Mathematical-analysis of a 2-step model involving a cooperative transition. J Theor Biol 124 (4):473-483. doi:10.1016/s0022-5193(87)80221-7

Guo L, Dai JH, Wang MC, Xu JC, Luedeling E (2015a) Responses of spring phenology in temperate zone trees to climate warming: A case study of apricot flowering in China. Agric For Meteorol 201:1-7. doi:10.1016/j.agrformet.2014.10.016

Guo L, Xu JC, Dai JH, Cheng JM, Luedeling E (2015b) Statistical identification of chilling and heat requirements for apricot flower buds in Beijing, China. Sci Hortic 195:138-144. doi:10.1016/j.scienta.2015.09.006

Horikoshi HM, Sekozawa Y, Sugaya S (2017) Inhibition of carbohydrate metabolism by thermal fluctuations during endodormancy lead to negative impacts on bud burst and incidence of floral necrosis in 'Housur' Japanese pear flower buds. Sci Hortic 224:324-331. doi:10.1016/j.scienta.2017.06.018

IPCC (2014) Climate Change 2014: Synthesis report. Contributions of working groups I, II and III to the fifth assesment report of the Intergovernmental Panel on Climate Change IPCC, Geneva, Switzerland

Ji D, Wang L, Feng J, Wu Q, Cheng H, Zhang Q, Yang J, Dong W, Dai Y, Gong D, Zhang RH, Wang X, Liu J, Moore JC, Chen D, Zhou M (2014) Description and basic evaluation of Beijing Normal University Earth System Model (BNU-ESM) version 1. Geosci Model Dev 7 (5):2039-2064. doi:10.5194/gmd-7-2039-2014

Junttila O, Hänninen H (2012) The minimum temperature for budburst in *Betula* depends on the state of dormancy. Tree Physiol 32 (3):337-345. doi:10.1093/treephys/tps010

Kerr A, Dialesandro J, Steenwerth K, Lopez-Brody N, Elias E (2018) Vulnerability of California specialty crops to projected mid-century temperature changes. Clim Change 148 (3):419-436. doi:10.1007/s10584-017-2011-3

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F (2006) World map of the Koppen-Geiger climate classification updated. Meteorol Z 15 (3):259-263. doi:10.1127/0941-2948/2006/0130

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo, para-, and ecodormancy: Physiological terminology and classification for dormancy research. HortScience 22:371-377

Lavee S, May P (1997) Dormancy of grapevine buds - Facts and speculation. Aust J Grape Wine R 3 (1):31-46. doi:10.1111/j.1755-0238.1997.tb00114.x

Leolini L, Moriondo M, Fila G, Costafreda-Aumedes S, Ferrise R, Bindi M (2018) Late spring frost impacts on future grapevine distribution in Europe. Field Crops Res 222:197-208. doi:10.1016/j.fcr.2017.11.018

Lindsay K, Bonan GB, Doney SC, Hoffman FM, Lawrence DM, Long MC, Mahowald NM, Moore JK, Randerson JT, Thornton PE (2014) Preindustrial-control and twentieth-century carbon cycle experiments with the Earth System Model CESM1(BGC). J Clim 27 (24):8981-9005. doi:10.1175/jcli-d-12-00565.1

Londo JP, Johnson LM (2014) Variation in the chilling requirement and budburst rate of wild *Vitis* species. Environ Exp Bot 106:138-147. doi:10.1016/j.envexpbot.2013.12.012

Lopez G, Girona J, Del Campo J, Marsal J (2007) Effects of relative source-sink position within peach trees on fruit growth under water stress conditions. J Horticult Sci Biotechnol 82 (1):140-148

Luedeling E (2012) Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci Hortic 144:218-229. doi:10.1016/j.scienta.2012.07.011

Luedeling E (2018) chillR: Statistical methods for phenology analysis in temperate fruit trees. R package version 0.70.17

Luedeling E, Blanke M, Gebauer J (2009a) Climate change effects on winter chill for fruit crops in Germany. Erwerbs-Obstbau 51 (3):81-94. doi:10.1007/s10341-009-0085-4

Luedeling E, Brown PH (2011) A global analysis of the comparability of winter chill models for fruit and nut trees. Int J Biometeorol 55 (3):411-421. doi:10.1007/s00484-010-0352-y

Luedeling E, Gassner A (2012) Partial Least Squares Regression for analyzing walnut phenology in California. Agric For Meteorol 158:43-52. doi:10.1016/j.agrformet.2011.10.020

Luedeling E, Gebauer J, Buerkert A (2009b) Climate change effects on winter chill for tree crops with chilling requirements on the Arabian Peninsula. Clim Change 96 (1-2):219-237. doi:10.1007/s10584-009-9581-7

Luedeling E, Girvetz EH, Semenov MA, Brown PH (2011a) Climate change affects winter chill for temperate fruit and nut trees. PLoS One 6 (5):13. doi:10.1371/journal.pone.0020155

Luedeling E, Kunz A, Blanke MM (2013) Identification of chilling and heat requirements of cherry trees - A statistical approach. Int J Biometeorol 57 (5):679-689. doi:10.1007/s00484-012-0594-y

Luedeling E, Steinmann KP, Zhang MH, Brown PH, Grant J, Girvetz EH (2011b) Climate change effects on walnut pests in California. Global Change Biol 17 (1):228-238. doi:10.1111/j.1365-2486.2010.02227.x

Luedeling E, Zhang MH, Girvetz EH (2009c) Climatic changes lead to declining winter chill for fruit and nut trees in California during 1950-2099. PLoS One 4 (7):9. doi:10.1371/journal.pone.0006166

Luedeling E, Zhang MH, Luedeling V, Girvetz EH (2009d) Sensitivity of winter chill models for fruit and nut trees to climatic changes expected in California's Central Valley. Agric Ecosyst Environ 133 (1-2):23-31. doi:10.1016/j.agee.2009.04.016

Martinez-Lüscher J, Hadley P, Ordidge M, Xu XM, Luedeling E (2017) Delayed chilling appears to counteract flowering advances of apricot in southern UK. Agric For Meteorol 237:209-218. doi:10.1016/j.agrformet.2017.02.017

ODEPA (2017) Chilean Agriculture Overview. Office of Agricultural Studies and Policies, Santiago, Chile

Pathak TB, Maskey ML, Dahlberg JA, Kearns F, Bali KM, Zaccaria D (2018) Climate change trends and impacts on California agriculture: A detailed review. Agronomy-Basel 8 (3):27. doi:10.3390/agronomy8030025

Perez FJ, Vergara R, Or E (2009) On the mechanism of dormancy release in grapevine buds: A comparative study between hydrogen cyanamide and sodium azide. Plant Growth Regul 59 (2):145-152. doi:10.1007/s10725-009-9397-5

Pope KS, Da Silva D, Brown PH, DeJong TM (2014) A biologically based approach to modeling spring phenology in temperate deciduous trees. Agric For Meteorol 198:15-23. doi:10.1016/j.agrformet.2014.07.009

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Version 3.5.3. Vienna, Austria

Ramírez L, Sagredo KX, Reginato GH (2010) Prediction models for chilling and heat requirements to estimate full bloom of almond cultivars in the Central Valley of Chile. Acta Horticulturae (ISHS) 872:107-112. doi:10.17660/ActaHortic.2010.872.12

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 1:331-332

Rotstayn LD, Collier MA, Dix MR, Feng Y, Gordon HB, O'Farrell SP, Smith IN, Syktus J (2010) Improved simulation of Australian climate and ENSO-related rainfall variability in a global climate model with an interactive aerosol treatment. Int J Climatol 30 (7):1067-1088. doi:10.1002/joc.1952

Ruiz D, Campoy JA, Egea J (2007) Chilling and heat requirements of apricot cultivars for flowering. Environ Exp Bot 61 (3):254-263. doi:10.1016/j.envexpbot.2007.06.008

Selles G, Muena V (2017) Requerimientos hídricos en uva de mesa. In: Torres A (ed) Manual del cultivo de uva de mesa, vol Boletín INIA N° 18. INIA-INDAP, Santiago, Chile, pp 55-73

Serri H, Venegas A, Ocampo J (2005) El cerezo en la VIII región de Chile. In: Lemus G (ed) El cultivo del cerezo, vol 133. Boletín INIA, Santiago de Chile, Chile, pp 185-209

Tombesi S, Scalia R, Connell J, Lampinen B, DeJong TM (2010) Fruit development in almond is influenced by early spring temperatures in California. J Horticult Sci Biotechnol 85 (4):317-322. doi:10.1080/14620316.2010.11512674

Volodin EM, Dianskii NA, Gusev AV (2010) Simulating present-day climate with the INMCM4.0 coupled model of the atmospheric and oceanic general circulations. Izv Atmos Ocean Phys 46 (4):414-431. doi:10.1134/s000143381004002x

Watanabe S, Hajima T, Sudo K, Nagashima T, Takemura T, Okajima H, Nozawa T, Kawase H, Abe M, Yokohata T, Ise T, Sato H, Kato E, Takata K, Emori S, Kawamiya M (2011) MIROC-ESM 2010: Model description and basic results of CMIP5-20c3m experiments. Geosci Model Dev 4 (4):845-872. doi:10.5194/gmd-4-845-2011

Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56:122-128

Wu TW (2012) A mass-flux cumulus parameterization scheme for large-scale models: Description and test with observations. Clim Dyn 38 (3-4):725-744. doi:10.1007/s00382-011-0995-3

Zhang JL, Taylor C (2011) The Dynamic Model provides the best description of the chill process on 'Sirora' pistachio trees in Australia. HortScience 46 (3):420-425

Chapter 4

The importance of chill model selection — A multi-site analysis

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Introduction

Winter chill is a major regulator of dormancy in deciduous trees. This makes chill availability a critical characteristic of fruit and nut production in both Mediterranean and temperate climate regions. Dormancy is a stage defined as the absence of growth in any meristem within a bud or other part of the plant (Cooke et al. 2012; Rohde and Bhalerao 2007). This stage is commonly divided into endo- and eco-dormancy (Lang 1987). According to Faust et al. (1997), the main driver of endo-dormancy is low temperature, whereas eco-dormancy is modulated by heat (Harrington et al. 2010; Luedeling 2012). To overcome endo-dormancy and maintain their productive potential in the following season, deciduous fruit and nut trees require cold temperatures during winter (Lang 1987; Lang et al. 1987). In this phase buds accumulate a tree-specific amount of chill (called chill requirement; CR; Faust et al. 1997; Luedeling 2012), after which they respond to heat until reaching budburst (Alburquerque et al. 2008; Lang et al. 1987). Evidence suggests that chill and heat may compensate for each other to some extent (Darbyshire et al. 2016b; Harrington et al. 2010; Kaufmann and Blanke 2019).

Chill requirements have been estimated for several species and varieties in various climate conditions by applying statistical approaches to large phenology datasets (Benmoussa et al. 2017a; Guo et al. 2015; Luedeling et al. 2013; Pope et al. 2014) or with chilling-forcing experiments using shoots or young potted trees (Alburquerque et al. 2008; Campoy et al. 2013; Egea et al. 2003; Ruiz et al. 2007). However, these reported estimates often differ among climates for the same species and varieties. For example, estimations of chill requirements by Viti et al. (2010) for apricot cv. Currot and cv. Orange Red in Italy differ by up to 20% from earlier studies by Ruiz et al. (2007) on the same cultivars in Spain, when quantified in units of the Utah model (Richardson et al. 1974). Similar differences can be found among CR estimations done for almonds cv. Ferragnes in Tunisia (Benmoussa et al. 2017a), the Central Valley of Chile (Ramírez et al. 2010) and in Spain (Egea et al. 2003). This low level of accuracy and transferability between regions might be explained either by site-specificity of CR (Campoy et al. 2011) or by problems with the way that winter chill accumulation is quantified (Luedeling 2012). Many of the observed differences may arise from inaccuracies within the chill models, which are merely empirical approximations of a biological phenomenon that so far defies direct observation and is therefore difficult to model (Luedeling 2012).

Approximating the response of winter-dormant plants to cold temperatures is difficult, because buds do not exhibit any visible change during the endo-dormancy phase. Although some efforts have been made to correlate chill accumulation with morphological changes (Fadón et al. 2018) or to review the metabolomics and genetics involved in dormancy progression (Beauvieux et al. 2018; Rios et al. 2014), available chill models do not yet include this knowledge. So far, all chill models rely entirely on temperature data.

The most widely used chill models in Horticulture are the Chilling Hours model (CHM, Bennett 1949; Weinberger 1950), the Utah model (UM, Richardson et al. 1974) and the Dynamic model (DM, Erez et al. 1990; Fishman et al. 1987a, b). While evidence suggests that the DM is the most plausible chill model (Benmoussa et al. 2017a; Luedeling et al. 2009c; Ruiz et al. 2007; Zhang and Taylor 2011), a number of publications use only the UM or the CHM model to quantify chill (Horikoshi et al. 2017; Park et al. 2018; Sawamura et al. 2017). These models are also widely used by farmers for orchard management. The Utah and Chilling Hours models have been shown to be very sensitive to temperature changes, predicting chill losses between 29 and 39% (CHM) and between 21 and 35% (UM) during the period 1950–2050 for the Central Valley of California, whereas the DM predicted more moderate losses of only between 14 and 21% for the same period (Luedeling et al. 2009c). Moreover, Luedeling et al. (2009b) showed that the ratio between seasonal chill accumulations according to the CHM and DM varied strongly over time, indicating that models are not equivalent. Along the same lines, Luedeling and Brown (2011) reported that ratios between winter chill metrics vary substantially around the world, clearly indicating that chill models differ greatly in their accuracy, with severe implications for the validity of CR estimates from one location in other growing regions. This is highly problematic when farmers import varieties for which CRs have been estimated elsewhere, even when using the same chill model for the importing region.

Alternatives to common chill models have been presented in various studies exploring the phenology of deciduous forest and fruit trees. The North Carolina model (NCM, Shaltout and Unrath 1983), the Positive Utah model (PUM, Linsley-Noakes et al. 1994) and the Low Chill model (LCM, Gilreath and Buchanan 1981) use the same structure as the UM but introduce some modifications. The NCM uses a wider range of temperatures, and it includes a stronger chill negation (up to a factor of −2) for temperatures above 23.3 °C. The PUM has no chill negation, considering all temperatures between 1.4 and 12.4 °C as effective for chill accumulation, whereas the LCM changes the temperature thresholds for each chill unit. The Modified Utah model (MUM, Linvill 1990), which uses a continuous function to estimate chill, considers all temperatures between 0 and 14 °C as effective and temperatures above 14 °C as contributing negatively (maximum chill negation for temperatures > 21 °C). Other agricultural and forest models that use daily extreme or hourly temperatures as inputs have been proposed in phenological studies for predicting budburst dates for various tree species (Cesaraccio et al. 2004; Chmielewski et al. 2011; Hänninen 1990; Harrington et al. 2010; Legave et al. 2013). These models use different functions to estimate chill and heat during dormancy. As with other common chill models, none of the models we have described here includes any physiological parameters.

Representative Concentration Pathways (RCPs) are scenarios for the future development of atmospheric greenhouse gas concentrations. They are named after the total radiative forcing (W m^{-2}) expected by the end of the 21st century (e.g. RCP4.5 stands for a forcing of 4.5W m^{-2} by 2100; IPCC, 2014). Such radiative forcing is projected to decrease winter chill in the future in many regions, with Mediterranean climate areas in particular facing severe climate change impacts (Baldocchi and Wong 2008; Chmielewski et al. 2012; Darbyshire et al. 2016a; Kerr et al. 2018; Luedeling et al. 2009a; Luedeling et al. 2011a; Rodríguez et al. 2019).

Despite the importance of future chill availability for decision-making, only a few sets of chill models have been compared in earlier studies with regard to their suitability for projecting future chill levels (Darbyshire et al. 2013; Luedeling et al. 2009b). Alternatives to these evaluated chill models have not yet been analyzed across different sites and future scenarios. In fact, most model development efforts have attempted to validate such approaches with local phenological data. The resulting CR estimations rarely correspond across regions (Luedeling 2012). An effective and globally applicable metric to estimate CR and to project winter chill might help dormancy researchers and growers devise strategies to face the impacts of global warming on temperate trees. Therefore, we set out to demonstrate the importance of model choice as a step toward a generalizable chill model. The work aims to provide an analysis of the major sources of variation in assessments of future chill by comparing common and alternative chill models for past and future climate scenarios in three countries (Chile, Tunisia and Germany). In each of the three countries, we selected three sites where deciduous fruit trees are commonly cultivated. At each site we analyzed winter chill for the past, and we projected future chill availability by the end of the 21st century for two RCP scenarios (RCP4.5 and RCP8.5) using 15 global climate models and 13 chill models.

Materials and methods

All analyses, which include the use of specific software packages and functions, as well as the figures, were developed in the R programing language (R Core Team 2019).

Site selection and weather data collection

In each country we selected three relevant sites for deciduous fruit tree production. These sites were Quillota, Curicó and Chillán in Chile, Ben Arous, Sfax and Mellita in Tunisia, and the Altes Land, Rhineland and Lake Constance growing regions in Germany (Fig. 4-1). One weather station was selected as primary data source for each site (Table 4-1). For sites in Chile, we downloaded weather data from the website of the Center for Climate and Resilience Research ($[CR]^2$) sponsored by the University of Chile (www.cr2.cl). For sites in Tunisia we obtained weather data from the Global Summary of the Day (GSOD) database of the National Oceanic and Atmospheric Administration (NOAA) through functions contained in the chillR package (Luedeling 2019). For Lake Constance and Altes Land in Germany we downloaded the data from the Climate Data Center for Germany (https://opendata.dwd.de/climate_environment/CDC/). For the Rhineland region, we obtained records from the Campus Klein-Altendorf North weather station of the University of Bonn. We selected daily minimum and maximum records between 1973 and 2017 for all sites.

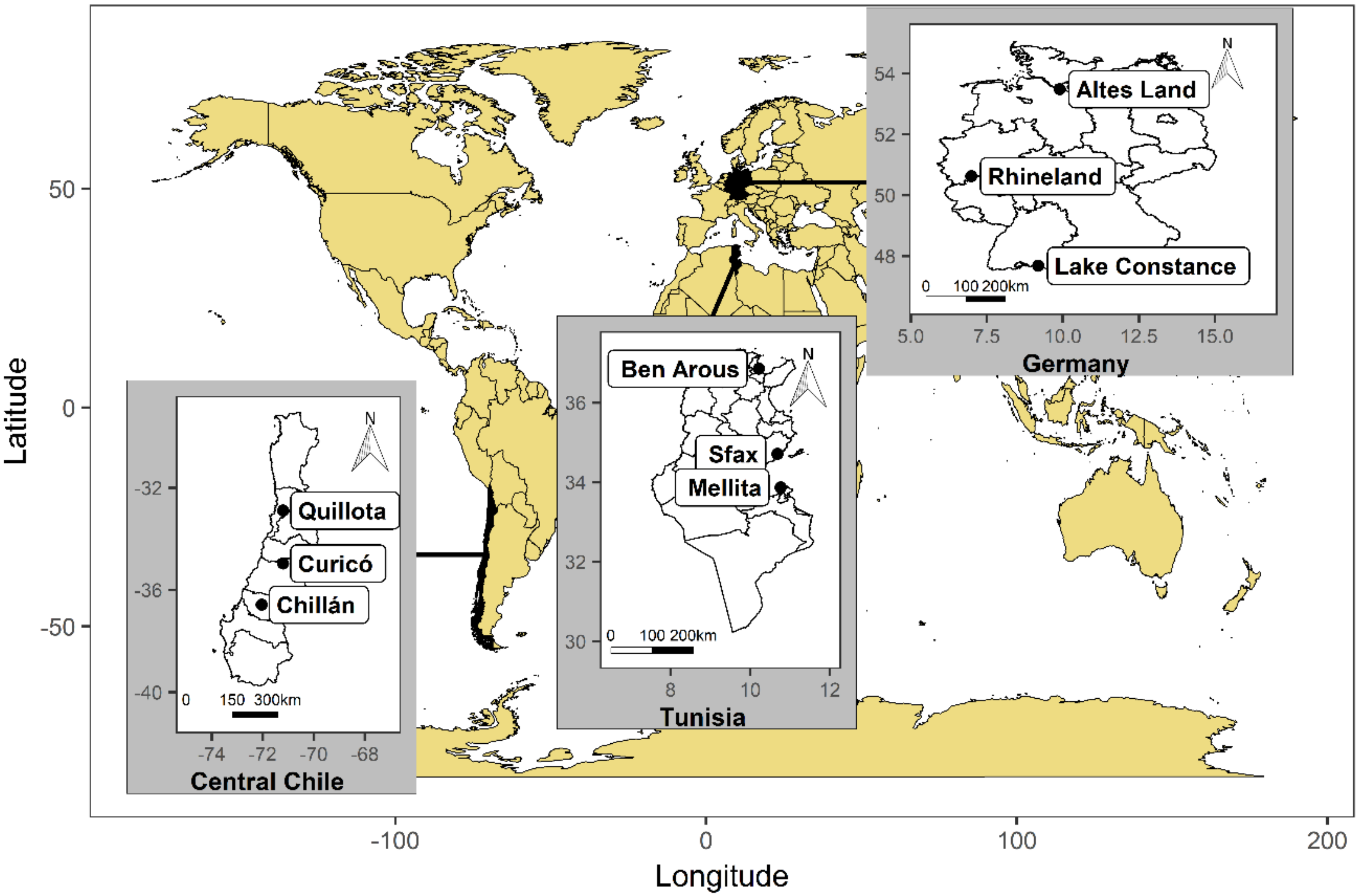


Figure 4-1. Locations of nine study sites used in Chile, Tunisia and Germany. Boxes show locations of the sites within the respective countries. Sites were Quillota, Curicó and Chillán in Chile (left panel), Ben Arous, Sfax and Mellita in Tunisia (center panel), and Altes Land, Rhineland, and Lake Constance in Germany (right panel). Black dots in the panels represent the weather stations used as sources of primary information

Table 4-1. Information regarding the weather stations used as primary data sources for three tree fruit production sites in Chile, Tunisia and Germany. Data are from the Dirección General de Aguas (General Direction of Water Resources, DGA), the Dirección Meteorológica de Chile (Meteorology Direction of Chile, DMC), the National Oceanic and Atmospheric Administration of the United States (NOAA), the Climate Data Center Germany (CDC), and Campus Klein-Altendorf, an experimental station of the University of Bonn (CKA, northern weather station)

Country	Site	Station Name	Source organization	Latitude	Longitude	Elevation (above sea level)	Percentage of dataset complete[a]
Chile	Quillota	Quillota	DGA	32.89 °S	71.20 °W	130 m	75.1%
	Curicó	General Freire	DMC	34.96 °S	71.21 °W	225 m	99.9%
	Chillán	Bernardo O'Higgins	DMC	36.58 °S	72.04 °W	151 m	99.6%
Tunisia	Ben Arous	Carthage	NOAA	36.85 °N	10.22 °E	7 m	99.7%
	Sfax	Thyna	NOAA	34.71 °N	10.69 °E	19 m	99.7%
	Mellita	Zarzis	NOAA	33.87 °N	10.77 °E	1 m	99.3%
Germany	Altes Land	Neuwiedenthal	CDC	53.48 °N	9.90 °E	3 m	100%
	Rhineland	Klein-Altendorf	CKA	50.62 °N	6.99 °E	178 m	100%
	Lake Constance	Konstanz	CDC	47.68 °N	9.19 °E	443 m	100%

[a] Percentage of the dataset that was complete for the period between 01/01/1973 and 31/12/2017 (32,872 observations of minimum and maximum temperatures)

We filled gaps in the daily records from each primary weather station (Table 4-1) with data from a set of auxiliary stations. These were the 24 closest weather stations that had data for the period of interest. On average, these stations were located 69 km from the primary stations in Chile and 133 km away in Tunisia. Elevation differences were, on average, −143 m (standard deviation of 186 m) for sites in Chile and −136 m (standard deviation of 17 m) in Tunisia. For Germany, use of auxiliary stations was not necessary since data for all relevant days were available for the primary weather stations. Auxiliary stations were used to fill gaps in order of their proximity to the primary station, until all gaps had been filled or no more stations were available. To avoid including non-representative data, we set the maximum acceptable bias of the auxiliary records for daily minimum and maximum temperatures, compared to the primary station, to 4 °C, and we corrected for this between-location bias using functions from the chillR package (Luedeling 2019). Specific information on the quality check (i.e. mean bias and standard deviation bias) for the filling procedure at each site, as well as mean distance and elevation differences, are provided as supplementary material accompanying this chapter. The few remaining gaps in all sites were then filled by linear interpolation as proposed in Luedeling (2018). These procedures resulted in complete records of daily minimum and maximum temperatures for the 45 years between 1973 and 2017.

Historic weather scenarios

We estimated chill metrics for each season of the historic record between 1973 and 2017. However, analyzing these single-season estimations may obscure long-term trends due to year-to-year variation. To better identify long-term trends in historic records, we generated historic weather scenarios to characterize typical agroclimatic conditions for several points in time. We applied a modified version of the procedure reported by Fernandez et al. (2020). In brief, we trained a weather generator with historically recorded data to produce representative scenarios for 10 years spanning the period of observed temperatures. For each of these years, we determined typical mean daily minimum and maximum temperatures for each month by applying a running mean function across all recorded monthly extreme temperatures for the respective month for all years on record. We then applied these scenarios to the weather generator to obtain 100 replicates of plausible weather data for each scenario year.

Future weather scenarios

Similar to the procedure described in section Historic weather scenarios, future weather scenarios were obtained according to the methods described by Fernandez et al. (2020). We downloaded future projections for each site from the Climate Wizard database maintained by the International Center of Tropical Agriculture (CIAT). This was done via an application programming interface (https://github.com/CIAT-DAPA/climate_wizard_api), using functions contained in the chillR package (Luedeling 2019). Specifically, this database contains projections for two RCP scenarios (RCP4.5 and RCP8.5) obtained from fifteen Global Climate Models (GCMs, Table 4-2). In both RCP scenarios, temperature projections were obtained for the period 2035–2065, and for the period 2070−2100. These periods were represented by their central years 2050 and 2085, respectively. Data for 100 replicate years for each combination of site, RCP, year and climate model were then obtained by using these scenarios as inputs to the weather generator (Benmoussa et al. 2018).

Table 4-2. Information on the Global Climate models from the Climate Wizard database that were used to generate future temperature data for three tree fruit production sites in Chile, Tunisia and Germany (adapted from Fernandez et al. 2020)

Name	Abbreviation	Reference and/or link
Beijing Climate Center – Climate System Model 1.1	bcc-csm1-1	Wu (2012) http://forecast.bcccsm.ncc-cma.net/web/channel-43.htm
Geophysical Fluid Dynamics Laboratory – Earth System Models	GFDL-ESM2G	Delworth et al. (2006)
	GFDL-ESM2M	https://www.gfdl.noaa.gov/earth-system-model/
	GFDL-CM3	Donner et al. (2011)
Institute of Numerical Mathematics Climate Model version 4	inmcm4	Volodin et al. (2010)
Institute Pierre – Simon Laplace – Climate Model 5[a]	IPSL-CM5A-LR IPSL-CM5A-MR	https://cmc.ipsl.fr/ipsl-climate-models/ipsl-cm5/
Community Climate System Model 4	CCSM4	http://www.cesm.ucar.edu/models/ccsm4.0/
Community Earth System Model version 1 – BioGeoChemical model enabled	CESM1-BGC	Lindsay et al. (2014)
Beijing Normal University – Earth System Model	BNU-ESM	Ji et al. (2014)
Canadian Earth System Model 2	CanESM2	Chylek et al. (2011)
Model for Interdisciplinary Research On Climate – Earth System Model	MIROC-ESM	Watanabe et al. (2011)
Centre National de Recherches Météorologiques – Climate Model 5	CNRM-CM5	http://www.umr-cnrm.fr/spip.php?article126&lang=en
Australian Community Climate and Earth-System Simulator 1.0	ACCESS1-0	Bi et al. (2013)
Commonwealth Scientific and Industrial Research Organisation – Mark3.6.0	CSIRO-Mk3-6-0	Rotstayn et al. (2010)

Chill estimation

We estimated seasonal winter chill for i) each year of the historic record, ii) each year of each past scenario, and iii) each future scenario year (2050 and 2085), for the two RCPs, and for each of the 15 GCMs. For locations in the southern hemisphere, we defined a winter season as the period between 1st of May and 31st of August in each year, while for sites in the northern hemisphere, the period between 1st of November and 28th of February was used.

To describe the wide range of available options for chill assessment, we estimated winter chill according to 13 chill models, described in Table 4-3 and in more detail in the supplementary material. We calculated the Chilling Hours model, the Utah model and the Dynamic model using the respective functions contained in the chillR package (Luedeling 2019). For the remaining models we programmed new R functions (supplementary material). For those models using hourly records as input, we derived hourly temperatures with functions contained in the chillR package (Luedeling 2019). In brief, these functions, which use latitude and daily temperature extremes as inputs, estimate hourly temperature based on an idealized daily temperature curve consisting of a sine function for daytime warming and a logarithmic decay function for nighttime cooling (Almorox et al. 2005; Linvill 1990).

Chill distributions were then summarized into six scenarios per site and chill model. Past observed contains the actual chill accumulation computed from the historic temperature records for 45 years (1973–2018). Past simulated summarizes chill estimates for the ten representative scenario years spanning the period of observed temperatures (a total of 1,000 synthetic years). We also treated each combination of RCP and year as a possible scenario (i.e. RCP4.5 2050), with each one containing 1,500 chill estimations computed from data for 100 synthetic years for each of 15 GCMs. Finally, future chill projections were compared with chill levels of the past. We computed the median chill level for the past scenario (1974–2016) by using data from the ten representative past scenarios generated in section Historic weather scenarios. We then estimated the change (absolute and in percent) relative to this baseline.

To determine the major sources of variation in future projections, we performed an analysis of variance (ANOVA) on the dataset generated by this study (see summary in supplementary material). Rather than significance levels, we focused on the effect sizes for the factors site, scenario, chill model and climate model. Since climate model showed a small effect size (0.5% of the total sum of squares among factors) we grouped the models according to behavior by applying a hierarchical cluster analysis to the median change in chill accumulation relative to the period 1974–2016 (in percentage) as a grouping variable. We estimated the Euclidean distance between climate models and used Ward’s minimum variance method to define three categories (Murtagh and Legendre 2014). These three climate model categories were cool (with incm4, GFDL-ESM2M and GFDL-ESM2G models), moderate (with CESM1-BGC, ACCESS1-0, IPSL-CM5A-LR, IPSL-CM5AMR, bcc-csm1-1, and CCSM4 models) and warm (with models CanESM2, CSIRO-Mk3-6-0, MIROC-ESM, GFDL-CM3, BNU-ESM, and CNRM-CM5).

Table 4-3. Information about the models used to quantify chill accumulation at each site

Model name	Unit name	Disciplinary origin	Time step	Chill negation by heat	Author/Reference
Dynamic model (DM)	Chill Portions (CP)	Horticulture	Hourly	Yes	Erez et al. (1990), Fishman et al. (1987a, b)
Chilling Hours model (CHM)	Chilling Hours (CH)	Horticulture	Hourly	No	Bennett (1949) and Weinberger (1950)
Utah model (UM)	Chill Units (CU)	Horticulture	Hourly	Yes	Richardson et al. (1974)
Positive Utah model (PUM)	Chill Units (PCU)	Horticulture	Hourly	No	Linsley-Noakes et al. (1994)
North Carolina model (NCM)	Chill Units (CU)	Horticulture	Hourly	Yes	Shaltout and Unrath (1983)
Modified Utah model (MUM)	Chill Units (CU)	Horticulture	Hourly	Yes	Linvill (1990)
Low Chill model (LCM)	Chill Units (CU)	Horticulture	Hourly	Yes	Gilreath and Buchanan (1981)
Chill Days model (CDM)	Chill Days (CD)	Horticulture and Forestry	Daily	No	Cesaraccio et al. (2004)
Chilling Rate function (CRF)	Rate of Chilling (RC)	Horticulture	Daily	No	Chmielewski et al. (2011)
Triangular Chill function (TCFL)	Chill Function (CF)	Horticulture	Daily	No	Legave et al. (2013)
Exponential Chill function (ECF)	Chill Function (CF)	Horticulture	Daily	No	Legave et al. (2013)
Chilling function (CFH)	Chilling Units (Cu)	Forestry	Hourly	No	Harrington et al. (2010)
Triangular Chill function (TCFH)	Rate of Chill (Rc)	Forestry	Daily	No	Hänninen (1990)

Results

Differences between chill models

To facilitate visualization of results, in this chapter we only show chill model differences for three of the nine sites. Figures for the other locations are provided in the supplementary materials accompanying chapter 4.

The response of the chill models evaluated in this study differed according to the site and past or future scenario (Figs. 4-2 and 4-3). Some models (i.e. the Dynamic, Utah, Positive Utah, Modified Utah, Low Chill and Chill Days) estimated a slightly higher absolute chill level in Curicó (a Mediterranean climate area) compared with Lake Constance (a temperate climate site). For instance, the Dynamic model computed a median of 81 Chill Portions (CP) in Curicó and 74 CP at Lake Constance for the historic records.

Most of the models forecasted the greatest future chill availability among the regions analyzed for the temperate growing regions in Germany. At Lake Constance, five of the 13 chill models tended to forecast higher levels of chill in the RCP8.5 scenario by 2085 compared to the other RCP scenario and time horizon. Conversely, the models Chilling Hours, Chilling Rate function, Triangular Chill function (both TCFL and TCFH), Chill Days and Exponential Chill function (ECF) forecasted the lowest amounts of chill for the RCP8.5 scenario by 2085. Nonetheless, CHM, CRF and CDM reached values similar to those estimated for past scenarios. At Lake Constance, the models North Carolina and Chilling function (CFH) displayed a tendency to predict relatively stable amounts of chill in all possible future scenarios. Analyzing the first and third distribution quartiles, projections with the North Carolina model ranged from 1,813 to 2,042 CU, whereas according to the Chilling Function model chill accumulation ranged between 1,951 and 2,178 Chilling units. In this site, the Dynamic model forecasted between 79 (percentile 25% – P25%) and 85 (P75%) CP, the Chilling Hours model between 1,602 and 1,856 CH (P25% and P75%), and the Utah model between 1,592 (P25%) and 1,940 (P75%) CU by 2085 for the RCP4.5 scenario.

For Curicó and Mellita all models showed substantial and consistent reductions in all future scenarios (Figs. 4-2 and 4-3). While in Curicó no model projected extremely low chill values, Mellita showed the lowest levels of chill for all chill models. The North Carolina, Utah, Modified Utah and Low Chill models projected negative seasonal chill totals for past and future scenarios. Along the same lines, the Chilling Hours model, the Chilling Rate function and the Triangular Chill function (TCFH) forecasted amounts of chill near zero by 2050 in the RCP4.5 scenario at this site. The Dynamic model and the Positive Utah model projected 12 (P25%) to 20 (P75%) CP and 310 (P25%) to 430 (P75%) PCU, respectively, for the same future scenario.

Only the Triangular Chill function (TCFL) and the Exponential Chill function consistently showed chill reductions for the future at all sites. Using the percentiles 25% and 75%, the TCFL computed between 96 and 101 chill function units for Lake Constance, between 73 and 77 chill function units for Curicó and between 41 and 46 chill function units in Mellita for the RCP4.5 scenario by 2050 (Fig. 4-2).

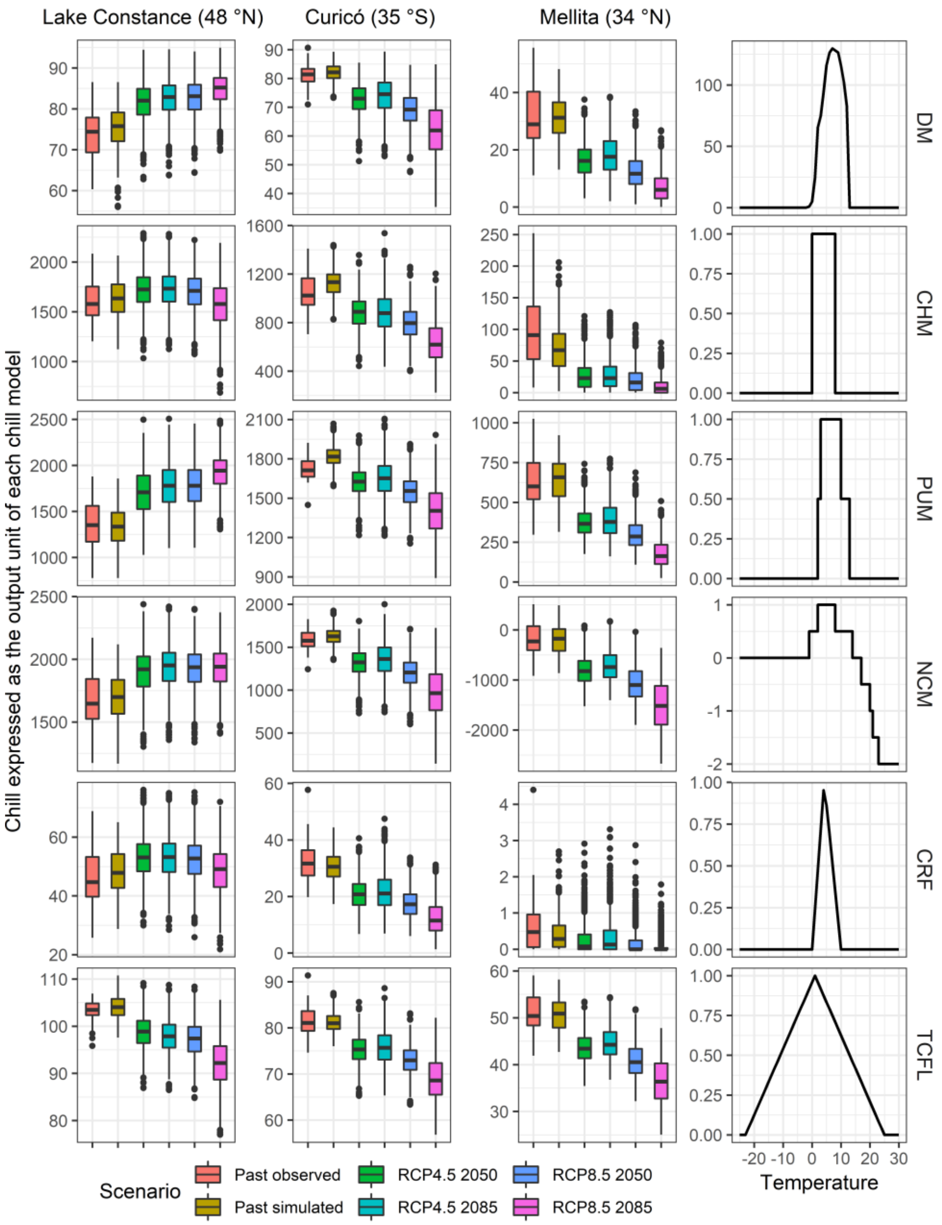


Figure 4-2. Winter chill estimations from six chill models (rows) at Lake Constance (Germany), Curicó (Chile) and Mellita (Tunisia, columns) for six past and future scenarios (boxplots). Chill models were: DM = Dynamic model, CHM = Chilling Hours model, PUM = Positive Utah model, NCM = North Carolina model, CRF = Chilling Rate function and TCFL = Triangular Chill function. Among the scenarios, past observed corresponds to the actual estimations of chill computed from the weather data (n = 45). Past simulated corresponds to chill calculations from the data generated as described in section Historic weather scenarios (n = 1,000). RCP4.5 and RCP8.5 correspond to the possible future scenarios of total radiative forcing according to IPCC (2014), while 2050 and 2085 represent the year used as central year for each RCP scenario (n = 1,500 each). The column to the right shows chill effectiveness of chill models (y) for a range of temperatures (x). CHM, PUM and NCM use a step function while the remaining models use a continuous function. Effectiveness is expressed as the output unit of each model. In TCFL and CRF, temperature corresponds to the daily mean, whereas for DM, CHM, PUM and NCM, temperature corresponds to the hourly temperature. The DM does not contain a simple weight function. For this model, the effectiveness curve shows chill accumulated after 100 continuous hours at the specified temperature

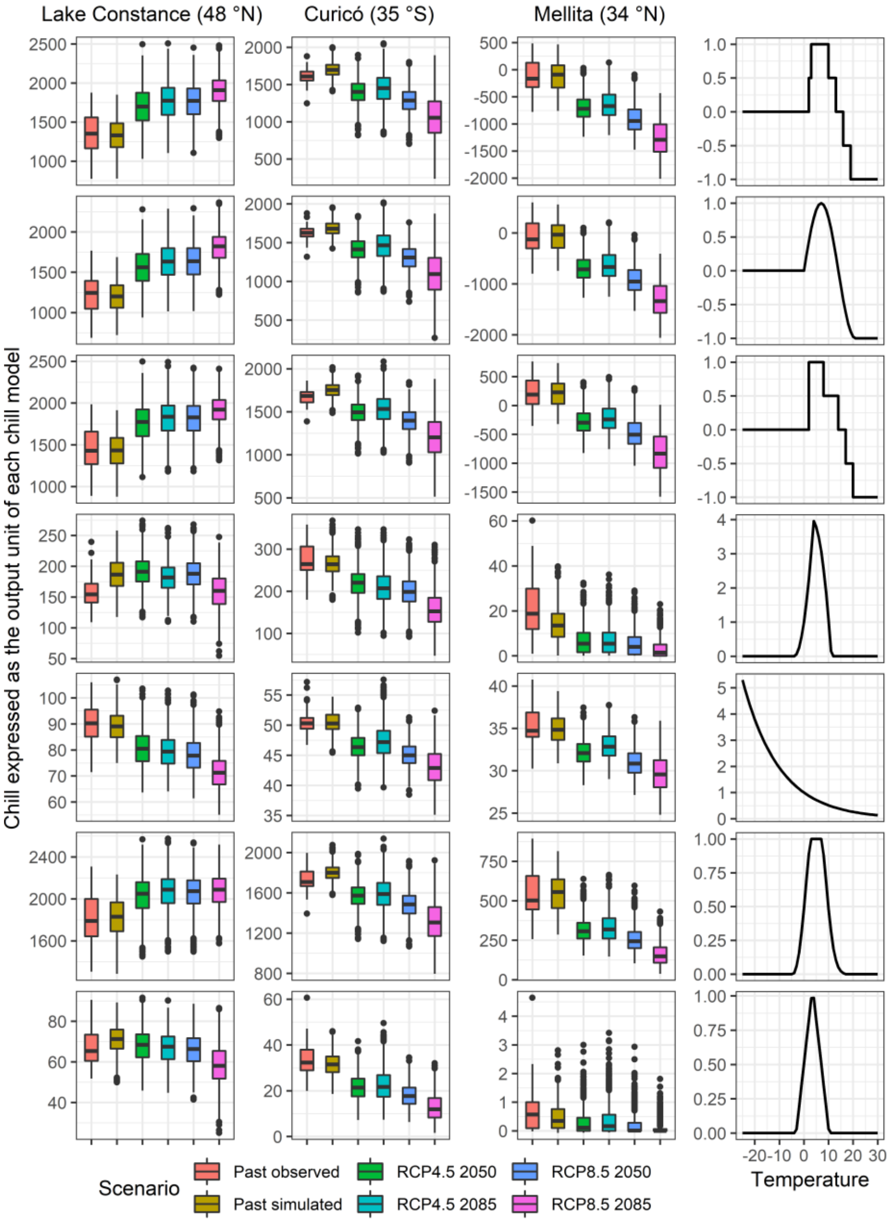


Figure 4-3. Winter chill estimations from seven chill models (rows) at Lake Constance (Germany), Curicó (Chile) and Mellita (Tunisia, columns) for six past and future scenarios (boxplots). Chill models were: UM = Utah model, MUM = Modified Utah model, LCM = Low Chill model, CDM = Chill Days model, ECF = Exponential Chill function, CFH = Chilling function and TCFH = Triangular Chill function. Among the scenarios, past observed corresponds to the actual estimations of chill computed from the weather data (n = 45). Past simulated corresponds to chill calculations from the data generated as described in section Historic weather scenarios (n = 1,000). RCP4.5 and RCP8.5 correspond to the possible future scenarios of total radiative forcing according to IPCC (2014), while 2050 and 2085 represent the year used as central year for each RCP scenario (n = 1,500 each). The column to the right shows chill effectiveness of chill models (y) for a range of temperatures (x). UM and LCM use a step function while the remaining models use a continuous function. Effectiveness is expressed as the output unit of each model. In CDM, ECF and TCFH, temperature corresponds to daily records (mean, maximum and mean, respectively), whereas for UM, MUM, LCM and CFH, temperature corresponds to the hourly temperature

Projected chill changes

Chill changes projected for future scenarios compared to the past depended strongly on the group of climate models we used for quantifying chill (Fig. 4-4). For sites in Chile and Tunisia, warm climate models consistently projected more severe negative changes for all chill models and future scenarios compared to other climate model groups. For Germany, this climate model group showed a tendency to project the greatest increases in chill accumulation relative to the period 1974–2016 for all future scenarios and sites. At Lake Constance, the variation observed was between −12 and +36% for the RCP4.5 scenario by 2050 and between −23 and +57% for the RCP8.5 scenario by 2085, depending on the chill model.

The greatest increases for German locations were predicted by the Utah, Positive Utah, Modified Utah and Low Chill models for the RCP8.5 scenario by 2085 (from +6 to +57% according to the site), while the CHM, CDM and TCFH models predicted the greatest decreases (about −30%) for the same RCP scenario and year. The Dynamic model, North Carolina model and Chilling function (CFH) estimated the smallest increases in chill availability among future scenarios and years in Germany. For Tunisia and one site in Chile (Quillota), some models projected negative absolute values for future scenarios (i.e. UM, NCM, MUM and LCM). In Tunisia, the Triangular Chill function (TCFL), the Exponential Chill function and the Dynamic model showed the smallest forecasted changes. In Quillota (Chile), the Chilling Rate function and Triangular Chill function (TCFH) indicated important chill losses for future scenarios. The remaining locations in Chile (Curicó and Chillán) showed a similar trend for future conditions. At these sites, none of the chill models forecast any major changes for any climate model. TCFL, ECF, CFH, and PUM projected the smallest changes relative to the past for all sites in Chile.

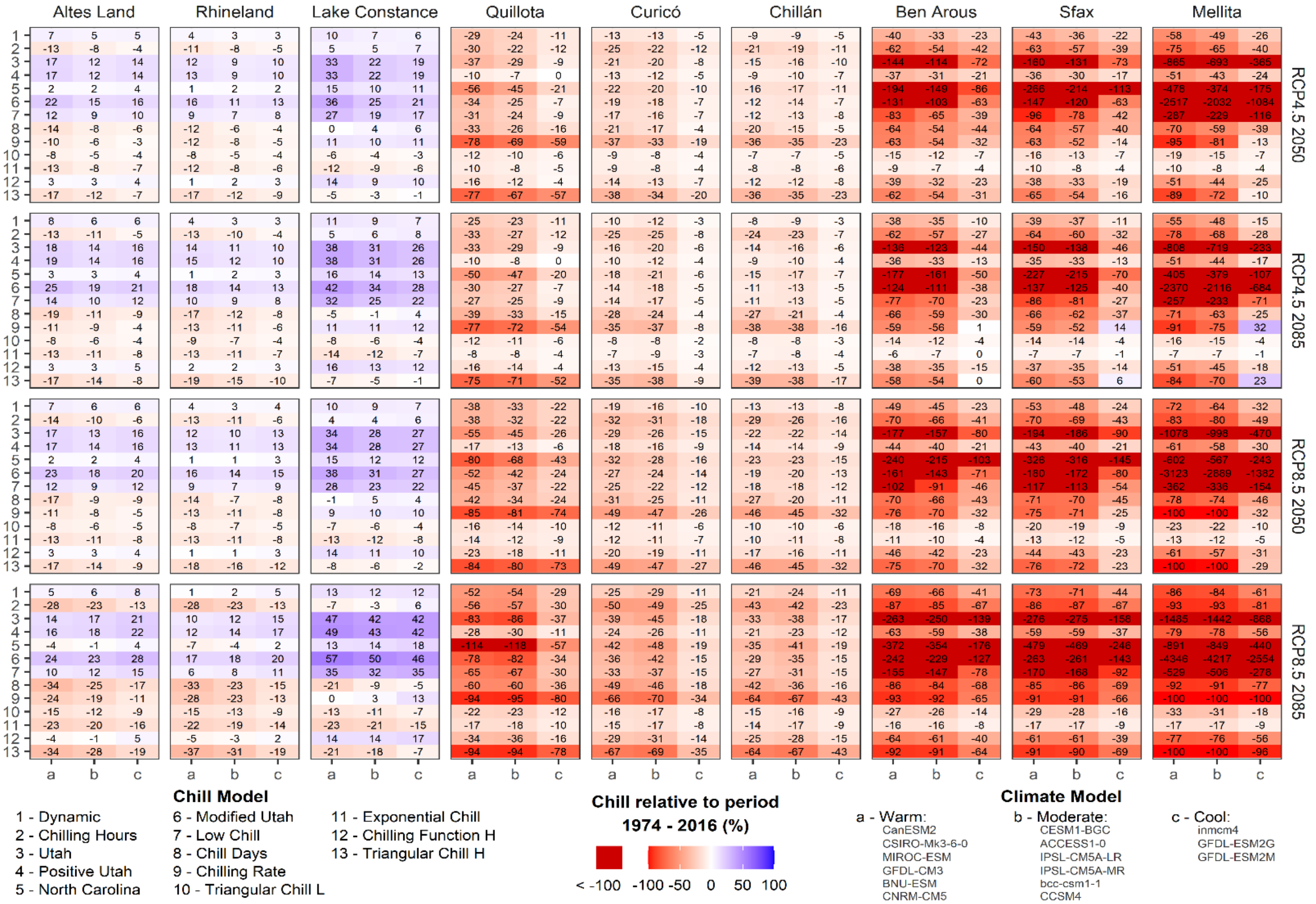
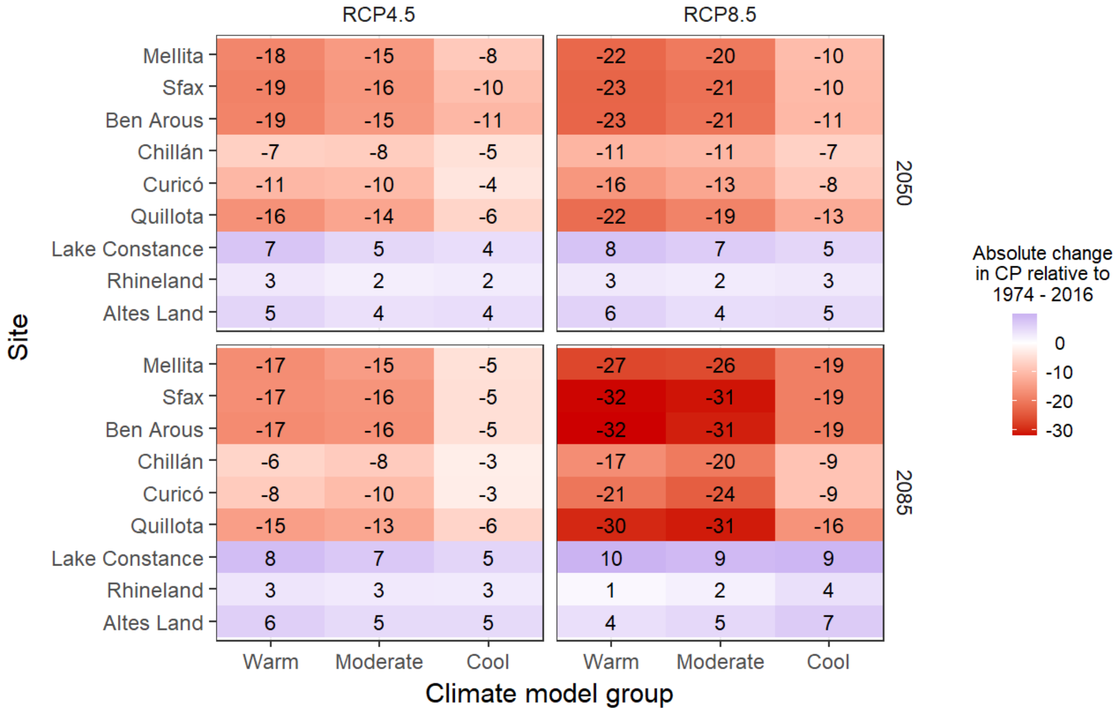


Figure 4-4. Changes in chill accumulation relative to the period 1974 – 2016 according to thirteen chill models and three groups of climate models for four possible future conditions at three locations in Germany (Altes Land, Rhineland and Lake Constance), Chile (Quillota, Curicó and Chillán) and Tunisia (Ben Arous, Sfax and Mellita). Change was computed as percentage by using median values of chill distributions projected for each scenario and climate model class (see methods section for grouping details). Note that for some models that allow negative chill accumulation (Utah, North Carolina, Modified Utah and Low Chill), losses can be greater than 100%

Substantial variation in unit values across the chill models makes concise presentation of absolute change projections difficult. We therefore only present results for the Dynamic model here (Fig. 4-5), while results for all other models can be found in the supplementary materials for this chapter. The Dynamic model indicated a wide range of change prospects across the study sites. Locations in Germany were projected to gain up to 10 CP, while other regions could experience chill losses. In the warmest scenario we evaluated, this could amount to up to 32 CP for RCP8.5 by 2085 (extreme cases in Sfax and Ben Arous, Fig. 4-5).



Horizon	Site	RCP4.5 Warm	RCP4.5 Moderate	RCP4.5 Cool	RCP8.5 Warm	RCP8.5 Moderate	RCP8.5 Cool
2050	Mellita	-18	-15	-8	-22	-20	-10
2050	Sfax	-19	-16	-10	-23	-21	-10
2050	Ben Arous	-19	-15	-11	-23	-21	-11
2050	Chillán	-7	-8	-5	-11	-11	-7
2050	Curicó	-11	-10	-4	-16	-13	-8
2050	Quillota	-16	-14	-6	-22	-19	-13
2050	Lake Constance	7	5	4	8	7	5
2050	Rhineland	3	2	2	3	2	3
2050	Altes Land	5	4	4	6	4	5
2085	Mellita	-17	-15	-5	-27	-26	-19
2085	Sfax	-17	-16	-5	-32	-31	-19
2085	Ben Arous	-17	-16	-5	-32	-31	-19
2085	Chillán	-6	-8	-3	-17	-20	-9
2085	Curicó	-8	-10	-3	-21	-24	-9
2085	Quillota	-15	-13	-6	-30	-31	-16
2085	Lake Constance	8	7	5	10	9	9
2085	Rhineland	3	3	3	1	2	4
2085	Altes Land	6	5	5	4	5	7

Figure 4-5. Absolute chill changes (in Chill Portions - CP) relative to the past period (1974–2016) estimated with the Dynamic model for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and three groups of climate models. Values shown in each cell represent the median value according to the climate models included in the respective climate model group (grouped by cluster analysis)

Discussion

This study is novel in the use of 13 approaches for analyzing past and future chill accumulation in a global comparison. The wide range of chill models we explored allowed us to determine that most of the variation in future chilling assessments (about 52% of the total sum of squares among factors) is generated by model choice and the use of different sites (about 46%), with only minor effects of climate model and future scenario (0.5 and 0.7%, respectively). This analysis extends the findings reported in Luedeling and Brown (2011) and presents updated methods for future chill assessments.

Our findings for north and central Chile and Tunisia concur with earlier projections of major chill losses in Mediterranean climate areas (Baldocchi and Wong 2008; Darbyshire et al. 2016a; Luedeling et al. 2011a). In these regions, the lack of winter chill due to warm winters is likely to affect bloom times (Chmielewski et al. 2012), budburst percentages (Campoy et al. 2019) and yield potentials of many of the fruit tree species that are currently cultivated there. In contrast, temperate climate sites (e.g. Lake Constance) are expected to be the safest places regarding future winter chill (Luedeling et al. 2011a). Major efforts, such as long-term breeding programs, and improved understanding of dormancy management under warm conditions, are needed in Mediterranean-climate areas to overcome the impacts of climate change on deciduous fruit orchards.

For the Utah, North Carolina, Modified Utah and Low Chill models, we computed negative chill totals for some scenarios in warm-winter climates. These models include chill negation effects for temperatures above certain thresholds (15.9 °C for the UM, 16.5 °C for the NCM, 14 °C for the MUM, and 17 °C for the LCM). Warm winters can lead to accumulation of more negative units than positive units, leading to a negative total for the season. This negation effect can be strongest in the North Carolina model, which assigns negative chill effectiveness weights up to −2 to warm temperatures (Shaltout and Unrath 1983). A review by Luedeling (2012) highlighted that model comparisons in some regions (i.e. South Africa, Spain, Chile, California, Australia) have usually found these models to perform poorly in explaining phenological dates of several species. Such models are thus unlikely to be useful in warm-winter regions considering that deciduous fruit and nut trees are still grown in these places (Benmoussa et al. 2017b). Nonetheless, their performance may be acceptable in sites with similar climates to those in which they were developed.

The Chilling Hours model forecasted smaller chill decreases in warm-winter regions but greater decreases in Curicó, Altes Land and Rhineland compared to the UM or similar models. These results differ from those reported in previous studies where the CHM has emerged as the most temperature-sensitive model (Luedeling et al. 2009c) and the one performing most poorly in explaining walnut phenology records in California (Luedeling et al. 2009d) in comparisons that involved the UM. This might be explained by the CHM's lack of a chill negation effect for high winter temperatures, which frequently occur in sites such as Quillota, Ben Arous, Sfax and Mellita. However, for most of the sites, the CHM still showed greater sensitivity to future warming when compared with the DM. The Chilling Rate function (CRF) reported by Chmielewski et al. (2011) for Germany and the Triangular Chill function (TCFH) reported by Hänninen (1990) showed relatively high variation and very low chill values in warm-winter locations compared with other models. This might be explained by the low optimum temperature for chill accumulation in these models of about 4 °C (daily mean), which is rarely observed in Mediterranean climate areas. For some climate models, the CRF predicted chill increases of up to 80% relative to the past period for Mellita, the warmest region evaluated. Such findings result from comparisons between very low values (i.e. Rate of Chilling of 0.28 for the past period and 0.51 for the future scenario).

For temperate climate locations (i.e. the German sites), some of the models predicted an increase in chill availability in response to warming. This finding is in agreement with earlier observations of past records in Germany (Luedeling et al. 2011b), as well as future projections reported by Luedeling et al. (2011a) for sites such as Southern Sweden. In such regions, the frequency of frost events, which are not considered effective for chill accumulation by most models (Luedeling et al. 2011a), is expected to decrease as winter temperatures increase. Depending on the model, fewer frost events may substantially increase the number of hours with temperatures that are effective for chill accumulation in these regions. For the area around Lake Constance, several chill models forecasted substantial increases in winter chill. However, the DM consistently indicated a weaker increase compared with other chill models such as the UM and the PUM. This may be due to the wider range of temperatures for maximum chill accumulation for the PUM and the NCM compared with the DM (Erez et al. 1990; Fishman et al. 1987a, b; Linsley-Noakes et al. 1994; Shaltout and Unrath 1983).

The only two chill models that showed the same trend in future chill projections across all sites were the Exponential Chill function and the Triangular Chill function (Legave et al. 2013). Relative to the period 1974–2016, the TCFL forecasted chill losses between 8 and 33% for all sites under the RCP8.5 scenario by 2085. The wide range of effective temperatures for chill accumulation in this model (−20 to about 25 °C, Legave et al. 2013) may explain this result. With such a wide range, chill accumulation only depends on the observed temperature at each site without the kind of thresholds for accumulation or chill negation that are contained in other models. Thus, chill reduction coincides with the expected future temperature increase in all the sites. Even though a positive effect of temperatures between −5 and 0 °C on chill accumulation has been suggested (Kaufmann and Blanke 2019), we are not aware of evidence that extreme cold temperatures (i.e. −20 °C) during dormancy contribute to chill accumulation. On the warm end of the temperature spectrum considered effective by the TCFL, it seems unlikely that temperatures above 20 °C can contribute to chill accumulation, since negative effects of warm temperatures on dormancy-related processes and budburst have also been reported (Horikoshi et al. 2017).

In recent years, the DM has increasingly been accepted as the most accurate model available for estimating chill requirements in temperate fruit trees (Alburquerque et al. 2008; Ruiz et al. 2007; Zhang and Taylor 2011), for explaining budburst dates of walnuts (Luedeling et al. 2009d), in a global comparison of three chill models (Luedeling and Brown 2011) and in future chill assessments (Darbyshire et al. 2013). The theory underlying the DM seems more plausible than the concepts behind the other chill models (Luedeling 2012). However, the DM is still far from being a fully reliable model. None of the currently available chill models, the DM included, considers advances in understanding of tree dormancy physiology obtained over at least three decades of dormancy-related research. Examples of such omissions include the role of oxidative cues (Beauvieux et al. 2018) and epigenetic regulation (Rios et al. 2014). Further parameterization of the DM, using a wider array of species and varieties, as well as a broader range of climates, might help improve model accuracy. Until a fully credible model becomes available, orchard managers, dormancy researchers, geneticists and breeders should be wary of the substantial differences between models when selecting chill models.

In this study, we were unable to validate chill models with observed phenological data – an analysis that would have greatly helped in improving our understanding on chill model performance. However, earlier studies have demonstrated that even though some models perform better than others in explaining phenological observations, they cannot necessarily predict phenological stages accurately (Luedeling et al. 2009d). Moreover, while historic observations are sometimes available, observations under conditions that are representative of future climates are much harder to obtain, making model validation for the purpose of projecting future chill very difficult. Reliable validation for this purpose would require observational records from multiple sites along temperature gradients, with long-term data for similar or even identical species and varieties. Such data are usually scarce, since cultivated species are rarely the same across sites, and few sites have reliable long-term data.

In general, chill models have performed poorly in explaining historic phenological records for forest and fruit trees. Some research suggests that chill and heat, which are both involved in determining bloom dates, interact with each other during part or all of the dormancy period (Darbyshire et al. 2016b; Harrington et al. 2010; Pope et al. 2014). Specifically, Harrington et al. (2010) reported that additional heat during eco-dormancy might partially compensate for chill deficiencies during endo-dormancy. In this regard, Darbyshire et al. (2016b) demonstrated that an overlap model performs better compared to a sequential approach in explaining phenological observations of apple trees in Australia. This compensation effect may explain part of the inaccuracy in CR estimations across climates reported earlier (Benmoussa et al. 2017a; Ramírez et al. 2010; Viti et al. 2010). For instance, in warmer places such as Tunisia the compensation could be stronger than in a colder location such as Chile, leading to underestimation of the chill requirement of a given species at that location. Differences in chill model performance among climates may also explain part of the reported inaccuracy. A process-based model that includes the chill accumulation process and considers its interaction with the heat phase may achieve accuracy gains in bloom date predictions. Such a model would facilitate the anticipation of chill-related risks, the transfer of species and cultivars between regions and the development of dormancy management strategies to assist growers around the world in adapting to the challenges of climate change.

Conclusions

Chill model and site were the most relevant factors explaining the variation we observed in future chill estimations. These forecasts can vary greatly depending on the chill model selected for the analysis, even for the same site, RCP scenario and scenario year. While some models project complete chill losses in warm-winter regions such as Mellita, others indicate reductions of only about 25% for the most severe warming scenario (RCP8.5 by 2085). The Dynamic model, which is widely considered the most credible model currently available, was among the least sensitive in Mediterranean regions (i.e. Tunisia and Chile) and projected only minor chill increases in temperate regions (i.e. Germany). Even though the developers of the DM already had tree physiology in mind, there is a need to synthesize recent advances in dormancy knowledge into a more accurate model. Such a model would facilitate dormancy management and selection of appropriate germplasm for orchards. As long as no fully reliable models are available, however, orchard managers and advisors, researchers working on dormancy-related topics, geneticists and breeders should be wary of the high variability among chill models when selecting trees for their orchards or making forecasts of future chill levels.

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Conflict of 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.

Author contributions

EF developed this research with the guidance of EL. EF performed the analysis with the help of CW while EL developed the code for obtaining future climate projections. EF wrote the manuscript with CW and EL commenting on and editing the draft.

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References

Alburquerque N, Garcia-Montiel F, Carrillo A, Burgos L (2008) Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. Environ Exp Bot 64 (2):162-170. doi:10.1016/j.envexpbot.2008.01.003

Almorox J, Hontoria C, Benito M (2005) Statistical validation of daylength definitions for estimation of global solar radiation in Toledo, Spain. Energy Convers Manag 46 (9):1465-1471. doi:10.1016/j.enconman.2004.07.007

Baldocchi D, Wong S (2008) Accumulated winter chill is decreasing in the fruit growing regions of California. Clim Change 87:S153-S166. doi:10.1007/s10584-007-9367-8

Beauvieux R, Wenden B, Dirlewanger E (2018) Bud dormancy in perennial fruit tree species: A pivotal role for oxidative cues. Front Plant Sci 9:13. doi:10.3389/fpls.2018.00657

Benmoussa H, Ben Mimoun M, Ghrab M, Luedeling E (2018) Climate change threatens central Tunisian nut orchards. Int J Biometeorol 62 (12):2245-2255. doi:10.1007/s00484-018-1628-x

Benmoussa H, Ghrab M, Ben Mimoun M, Luedeling E (2017a) Chilling and heat requirements for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a warm Mediterranean location based on 30 years of phenology records. Agric For Meteorol 239:34-46. doi:10.1016/j.agrformet.2017.02.030

Benmoussa H, Luedeling E, Ghrab M, Ben Yahmed J, Ben Mimoun M (2017b) Performance of pistachio (*Pistacia vera* L.) in warming Mediterranean orchards. Environ Exp Bot 140:76-85. doi:10.1016/j.envexpbot.2017.05.007

Bennett JP (1949) Temperature and bud rest period. Calif Agric 3 (11):9-12

Bi DH, Dix M, Marsland SJ, O'Farrell S, Rashid HA, Uotila P, Hirst AC, Kowalczyk E, Golebiewski M, Sullivan A, Yan HL, Hannah N, Franklin C, Sun ZA, Vohralik P, Watterson I, Zhou XB, Fiedler R, Collier M, Ma YM, Noonan J, Stevens L, Uhe P, Zhu HY, Griffies SM, Hill R, Harris C, Puri K (2013) The ACCESS coupled model: Description, control climate and evaluation. Aust Meteorol Oceanogr J 63 (1):41-64. doi:10.22499/2.6301.004

Campoy JA, Darbyshire R, Dirlewanger E, Quero-Garcia J, Wenden B (2019) Yield potential definition of the chilling requirement reveals likely underestimation of the risk of climate change on winter chill accumulation. Int J Biometeorol 63 (2):183-192. doi:10.1007/s00484-018-1649-5

Campoy JA, Ruiz D, Egea J (2011) Dormancy in temperate fruit trees in a global warming context: A review. Sci Hortic 130 (2):357-372. doi:10.1016/j.scienta.2011.07.011

Campoy JA, Ruiz D, Nortes MD, Egea J (2013) Temperature efficiency for dormancy release in apricot varies when applied at different amounts of chill accumulation. Plant Biol 15:28-35. doi:10.1111/j.1438-8677.2012.00636.x

Cesaraccio C, Spano D, Snyder RL, Duce P (2004) Chilling and forcing model to predict bud-burst of crop and forest species. Agric For Meteorol 126 (1-2):1-13. doi:10.1016/j.agrformet.2004.03.002

Chmielewski FM, Blumel K, Henniges Y, Blanke M, Weber RWS, Zoth M (2011) Phenological models for the beginning of apple blossom in Germany. Meteorol Z 20 (5):487-496. doi:10.1127/0941-2948/2011/0258

Chmielewski FM, Blumel K, Palesova I (2012) Climate change and shifts in dormancy release for deciduous fruit crops in Germany. Clim Res 54 (3):209-219. doi:10.3354/cr01115

Chylek P, Li J, Dubey MK, Wang M, Lesins G (2011) Observed and model simulated 20th century Artic temperature variability: Canadian Earth System Model CanESM2. Atmos Chem Phys Discuss 11:22893 - 22907. doi:10.5194/acpd-11-22893-2011

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. Plant Cell Environ 35 (10):1707-1728. doi:10.1111/j.1365-3040.2012.02552.x

Darbyshire R, Measham P, Goodwin I (2016a) A crop and cultivar-specific approach to assess future winter chill risk for fruit and nut trees. Clim Change 137 (3-4):541-556. doi:10.1007/s10584-016-1692-3

Darbyshire R, Pope K, Goodwin I (2016b) An evaluation of the chill overlap model to predict flowering time in apple tree. Sci Hortic 198:142-149. doi:10.1016/j.scienta.2015.11.032

Darbyshire R, Webb L, Goodwin I, Barlow EWR (2013) Impact of future warming on winter chilling in Australia. Int J Biometeorol 57 (3):355-366. doi:10.1007/s00484-012-0558-2

Delworth TL, Broccoli AJ, Rosati A, Stouffer RJ, Balaji V, Beesley JA, Cooke WF, Dixon KW, Dunne J, Dunne KA, Durachta JW, Findell KL, Ginoux P, Gnanadesikan A, Gordon CT, Griffies SM, Gudgel R, Harrison MJ, Held IM, Hemler RS, Horowitz LW, Klein SA, Knutson TR, Kushner PJ, Langenhorst

AR, Lee HC, Lin SJ, Lu J, Malyshev SL, Milly PCD, Ramaswamy V, Russell J, Schwarzkopf MD, Shevliakova E, Sirutis JJ, Spelman MJ, Stern WF, Winton M, Wittenberg AT, Wyman B, Zeng F, Zhang R (2006) GFDL's CM2 global coupled climate models. Part I: Formulation and simulation characteristics. J Clim 19 (5):643-674. doi:10.1175/jcli3629.1

Donner LJ, Wyman BL, Hemler RS, Horowitz LW, Ming Y, Zhao M, Golaz JC, Ginoux P, Lin SJ, Schwarzkopf MD, Austin J, Alaka G, Cooke WF, Delworth TL, Freidenreich SM, Gordon CT, Griffies SM, Held IM, Hurlin WJ, Klein SA, Knutson TR, Langenhorst AR, Lee HC, Lin YL, Magi BI, Malyshev SL, Milly PCD, Naik V, Nath MJ, Pincus R, Ploshay JJ, Ramaswamy V, Seman CJ, Shevliakova E, Sirutis JJ, Stern WF, Stouffer RJ, Wilson RJ, Winton M, Wittenberg AT, Zeng FR (2011) The dynamical core, physical parameterizations, and basic simulation characteristics of the atmospheric component AM3 of the GFDL global coupled model CM3. J Clim 24 (13):3484-3519. doi:10.1175/2011jcli3955.1

Egea J, Ortega E, Martinez-Gomez P, Dicenta F (2003) Chilling and heat requirements of almond cultivars for flowering. Environ Exp Bot 50 (1):79-85. doi:10.1016/s0098-8472(03)00002-9

Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. Acta Hort 276:165-174

Fadón E, Rodrigo J, Herrero M (2018) Is there a specific stage to rest? Morphological changes in flower primordia in relation to endodormancy in sweet cherry (*Prunus avium* L.). Trees-Struct Funct 32 (6):1583-1594. doi:10.1007/s00468-018-1735-7

Faust M, Erez A, Rowland LJ, Wang SY, Norman HA (1997) Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance, and release. HortScience 32 (4):623-629

Fernandez E, Whitney C, Cuneo IF, Luedeling E (2020) Prospects of decreasing winter chill for deciduous fruit production in Chile throughout the 21st century. Clim Change 159 (3):423-439. doi:10.1007/s10584-019-02608-1

Fishman S, Erez A, Couvillon GA (1987a) The temperature-dependence of dormancy breaking in plants - Computer-simulation of processes studied under controlled temperatures. J Theor Biol 126 (3):309-321. doi:10.1016/s0022-5193(87)80237-0

Fishman S, Erez A, Couvillon GA (1987b) The temperature-dependence of dormancy breaking in plants - Mathematical-analysis of a 2-step model involving a cooperative transition. J Theor Biol 124 (4):473-483. doi:10.1016/s0022-5193(87)80221-7

Gilreath PR, Buchanan DW (1981) Rest prediction model for low-chilling 'Sungold' nectarine. J Am Soc Hort Sci 106 (4):426-429

Guo L, Xu JC, Dai JH, Cheng JM, Luedeling E (2015) Statistical identification of chilling and heat requirements for apricot flower buds in Beijing, China. Sci Hortic 195:138-144. doi:10.1016/j.scienta.2015.09.006

Hänninen H (1990) Modelling bud dormancy release in trees from cool and temperate regions. Acta For Fenn 213:1-47. doi:10.14214/aff.7660

Harrington CA, Gould PJ, St Clair JB (2010) Modeling the effects of winter environment on dormancy release of Douglas-fir. For Ecol Manage 259 (4):798-808. doi:10.1016/j.foreco.2009.06.018

Horikoshi HM, Sekozawa Y, Sugaya S (2017) Inhibition of carbohydrate metabolism by thermal fluctuations during endodormancy lead to negative impacts on bud burst and incidence of floral necrosis in 'Housur' Japanese pear flower buds. Sci Hortic 224:324-331. doi:10.1016/j.scienta.2017.06.018

IPCC (2014) Climate Change 2014: Synthesis report. Contributions of working groups I, II and III to the fifth assesment report of the Intergovernmental Panel on Climate Change IPCC, Geneva, Switzerland

Ji D, Wang L, Feng J, Wu Q, Cheng H, Zhang Q, Yang J, Dong W, Dai Y, Gong D, Zhang RH, Wang X, Liu J, Moore JC, Chen D, Zhou M (2014) Description and basic evaluation of Beijing Normal University Earth System Model (BNU-ESM) version 1. Geosci Model Dev 7 (5):2039-2064. doi:10.5194/gmd-7-2039-2014

Kaufmann H, Blanke M (2019) Substitution of winter chilling by spring forcing for flowering using sweet cherry as model crop. Sci Hortic 244:75-81. doi:10.1016/j.scienta.2018.09.021

Kerr A, Dialesandro J, Steenwerth K, Lopez-Brody N, Elias E (2018) Vulnerability of California specialty crops to projected mid-century temperature changes. Clim Change 148 (3):419-436. doi:10.1007/s10584-017-2011-3

Lang GA (1987) Dormancy - A new universal terminology. HortScience 22 (5):817-820

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo, para-, and ecodormancy: Physiological terminology and classification for dormancy research. HortScience 22:371-377

Legave JM, Blanke M, Christen D, Giovannini D, Mathieu V, Oger R (2013) A comprehensive overview of the spatial and temporal variability of apple bud dormancy release and blooming phenology in Western Europe. Int J Biometeorol 57 (2):317-331. doi:10.1007/s00484-012-0551-9

Lindsay K, Bonan GB, Doney SC, Hoffman FM, Lawrence DM, Long MC, Mahowald NM, Moore JK, Randerson JT, Thornton PE (2014) Preindustrial-control and twentieth-century carbon cycle experiments with the Earth System Model CESM1(BGC). J Clim 27 (24):8981-9005. doi:10.1175/jcli-d-12-00565.1

Linsley-Noakes G, Allan P, Matthee G (1994) Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J S Afr Soc Hort Sci 4:13-15

Linvill DE (1990) Calculating chilling hours and chill units from daily maximum and minimum temperature observations. HortScience 25 (1):14-16

Luedeling E (2012) Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci Hortic 144:218-229. doi:10.1016/j.scienta.2012.07.011

Luedeling E (2018) Interpolating hourly temperatures for computing agroclimatic metrics. Int J Biometeorol 62 (10):1799-1807. doi:10.1007/s00484-018-1582-7

Luedeling E (2019) chillR: Statistical methods for phenology analysis in temperate fruit trees. R package version 0.70.21

Luedeling E, Brown PH (2011) A global analysis of the comparability of winter chill models for fruit and nut trees. Int J Biometeorol 55 (3):411-421. doi:10.1007/s00484-010-0352-y

Luedeling E, Gebauer J, Buerkert A (2009a) Climate change effects on winter chill for tree crops with chilling requirements on the Arabian Peninsula. Clim Change 96 (1-2):219-237. doi:10.1007/s10584-009-9581-7

Luedeling E, Girvetz EH, Semenov MA, Brown PH (2011a) Climate change affects winter chill for temperate fruit and nut trees. PLoS One 6 (5):13. doi:10.1371/journal.pone.0020155

Luedeling E, Kunz A, Blanke M (2011b) Mehr Chilling für Obstbäume in wärmeren Wintern? Erwerbs-Obstbau 53 (4):145-155. doi:10.1007/s10341-011-0148-1

Luedeling E, Kunz A, Blanke MM (2013) Identification of chilling and heat requirements of cherry trees - A statistical approach. Int J Biometeorol 57 (5):679-689. doi:10.1007/s00484-012-0594-y

Luedeling E, Zhang MH, Girvetz EH (2009b) Climatic changes lead to declining winter chill for fruit and nut trees in California during 1950-2099. PLoS One 4 (7):9. doi:10.1371/journal.pone.0006166

Luedeling E, Zhang MH, Luedeling V, Girvetz EH (2009c) Sensitivity of winter chill models for fruit and nut trees to climatic changes expected in California's Central Valley. Agric Ecosyst Environ 133 (1-2):23-31. doi:10.1016/j.agee.2009.04.016

Luedeling E, Zhang MH, McGranahan G, Leslie C (2009d) Validation of winter chill models using historic records of walnut phenology. Agric For Meteorol 149 (11):1854-1864. doi:10.1016/j.agrformet.2009.06.013

Murtagh F, Legendre P (2014) Ward’s hierarchical agglomerative clustering method: Which algorithms implement Ward’s criterion? J Classif 31 (3):274-295. doi:10.1007/s00357-014-9161-z

Park Y, Lee B, Park HS (2018) Predicted effects of Climate Change on winter chill accumulation by temperate trees in South Korea. Horticult J 87 (2):166-173. doi:10.2503/hortj.OKD-089

Pope KS, Da Silva D, Brown PH, DeJong TM (2014) A biologically based approach to modeling spring phenology in temperate deciduous trees. Agric For Meteorol 198:15-23. doi:10.1016/j.agrformet.2014.07.009

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Version 3.5.3. Vienna, Austria

Ramírez L, Sagredo KX, Reginato GH (2010) Prediction models for chilling and heat requirements to estimate full bloom of almond cultivars in the Central Valley of Chile. Acta Horticulturae (ISHS) 872:107-112. doi:10.17660/ActaHortic.2010.872.12

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 1:331-332

Rios G, Leida C, Conejero A, Badenes ML (2014) Epigenetic regulation of bud dormancy events in perennial plants. Front Plant Sci 5:6. doi:10.3389/fpls.2014.00247

Rodríguez A, Pérez-López D, Sánchez E, Centeno A, Gómara I, Dosio A, Ruiz-Ramos M (2019) Chilling accumulation in fruit trees in Spain under climate change. Nat Hazards Earth Syst Sci 19 (5):1087-1103. doi:10.5194/nhess-19-1087-2019

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12 (5):217-223. doi:10.1016/j.tplants.2007.03.012

Rotstayn LD, Collier MA, Dix MR, Feng Y, Gordon HB, O'Farrell SP, Smith IN, Syktus J (2010) Improved simulation of Australian climate and ENSO-related rainfall variability in a global climate model with an interactive aerosol treatment. Int J Climatol 30 (7):1067-1088. doi:10.1002/joc.1952

Ruiz D, Campoy JA, Egea J (2007) Chilling and heat requirements of apricot cultivars for flowering. Environ Exp Bot 61 (3):254-263. doi:10.1016/j.envexpbot.2007.06.008

Sawamura Y, Suesada Y, Sugiura T, Yaegaki H (2017) Chilling requirements and blooming dates of leading peach cultivars and a promising early maturing peach selection, Momo Tsukuba 127. Horticult J 86 (4):426-436. doi:10.2503/hortj.OKD-052

Shaltout AD, Unrath CR (1983) Rest completion prediction model for 'Starkrimson Delicious' apples. J Am Soc Hort Sci 108 (6):957-961

Viti R, Andreini L, Ruiz D, Egea J, Bartolini S, Iacona C, Campoy JA (2010) Effect of climatic conditions on the overcoming of dormancy in apricot flower buds in two Mediterranean areas: Murcia (Spain) and Tuscany (Italy). Sci Hortic 124 (2):217-224. doi:10.1016/j.scienta.2010.01.001

Volodin EM, Dianskii NA, Gusev AV (2010) Simulating present-day climate with the INMCM4.0 coupled model of the atmospheric and oceanic general circulations. Izv Atmos Ocean Phys 46 (4):414-431. doi:10.1134/s000143381004002x

Watanabe S, Hajima T, Sudo K, Nagashima T, Takemura T, Okajima H, Nozawa T, Kawase H, Abe M, Yokohata T, Ise T, Sato H, Kato E, Takata K, Emori S, Kawamiya M (2011) MIROC-ESM 2010: Model description and basic results of CMIP5-20c3m experiments. Geosci Model Dev 4 (4):845-872. doi:10.5194/gmd-4-845-2011

Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56:122-128

Wu TW (2012) A mass-flux cumulus parameterization scheme for large-scale models: Description and test with observations. Clim Dyn 38 (3-4):725-744. doi:10.1007/s00382-011-0995-3

Zhang JL, Taylor C (2011) The Dynamic Model provides the best description of the chill process on 'Sirora' pistachio trees in Australia. HortScience 46 (3):420-425

Chapter 5

Mild water stress makes apple buds more likely to flower and more responsive to artificial forcing — Impacts of an unusually warm and dry summer in Germany

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Introduction

Apple production, and the cultivation of other temperate fruit trees, is affected by short and long-term weather variation, which is increasingly being modulated by anthropogenic climate change. In Germany, annual mean temperature has risen by approximately 1.9 °C since 1881 (based on a linear regression until 2018 according to Deutsche Wetterdienst 2019). Relative to the period 1971–2000, mean winter temperature is expected to keep increasing throughout the 21st century, with a total increment between 1.2 and 3.2 °C projected for the representative concentration pathway (RCP) scenario RCP4.5 (characterized by the expectation of 4.5 W m^{-2} of additional radiative forcing by 2100 compared to pre-industrial conditions, Jacob et al. 2017). Compared to 1986–2005 and using the scenario RCP4.5, total precipitation during April–September is predicted to change between about −20% (25th percentile of multi-model distribution) and +10% (75th percentile) in central Europe by 2046–2065 (Van Oldenborgh et al. 2013). These changes may result in more frequent extreme events, similar to the extraordinary heat and dry weather observed during the apple growing season in June–September of 2018. Such seasons do not only have immediate impacts, but they may also affect orchard performance in the following growth period. In fact, tree development in a given season depends on processes that occurred during the previous year, including the accumulation of reserves and dormancy induction, as well as flower initiation and differentiation (Wilkie et al. 2008; Chai et al. 2015).

Apple trees are cultivated in a wide range of climates, including temperate and tropical regions (Wilkie et al. 2008). Accordingly, growing techniques have been adapted to a range of temperature and water regimes. Under drought conditions, where water scarcity limits apple production, regulated deficit irrigation (RDI) allows maintenance of productive orchards (Chai et al. 2015; Behboudian and Mills 1997), although this practice may have undesirable implications for fruit maturity, quality, and shelf life (Ebel et al. 1993). On the other hand, RDI has been proposed as a useful approach to control excessive vegetative growth (Ebel et al. 1995). In general, water scarcity during the growing season may affect fruit production by reducing fruit yield and quality (Berman and DeJong 1996), as well as by modifying morphological and physiological traits of trees (Bolat et al. 2014). According to Naor et al. (2008), the yield of apple trees decreased by 65% in response to reducing irrigation from 7 to 1 mm day^{-1} under a medium crop load of ~300 apples per tree. This observation may be explained by water stress directly affecting fruit growth by influencing the cell division process (Failla et al. 1992), reducing cell turgor (Kramer 1983) and/or by limiting energetic resources due to a decrease in photosynthetic rate (Naschitz et al. 2010). Along the same lines, Bolat et al. (2014), working on one-year-old M9 apple rootstocks, demonstrated that cutting irrigation by 50% between mid-July and the onset of dormancy decreased relative shoot length by 67%, probably caused by a reduction in auxin concentrations. A decrease in soil water content has been associated with physiological modifications, such as hormone signaling and the activity of reactive oxygen species (ROS) in plants (Cruz de Carvalho 2008). Chen et al. (2019) concluded that water stress promoted growth cessation during summer and affected the ontogeny of apple shoots by increasing the production of abscisic acid (ABA) in roots (Sobeih et al. 2004).

The end of the growing season in deciduous forest and fruit trees is characterized by leaf fall and dormancy induction. During dormancy, all meristems in buds or other plant structures are unable to perform cell division and cell expansion (Rohde and Bhalerao 2007; Cooke et al. 2012). To resume growth, buds require exposure to low and subsequent warm temperatures (Lang 1987). These thermal needs, denoted by the concepts of chill and heat requirements (CR and HR, respectively, Faust et al. 1997, Luedeling 2012), have been widely studied in the past (Campoy et al. 2013; Benmoussa et al. 2017; Guo et al. 2015) and used in orchard planning and management (Luedeling 2012). Most studies on CR and HR estimation assume a sequential relationship between chilling and heating phases, defining a specific need for each of them (Alburquerque et al. 2008). However, some evidence suggests that chill and heat may compensate for each other under particular circumstances (Harrington et al. 2010; Pope et al. 2014). The effect of temperature on dormancy completion is commonly evaluated by experiments with shoots exposed to forcing conditions (Fadón and Rodrigo 2018) or through statistical approaches applied to long-term datasets (Benmoussa et al. 2017; Luedeling et al. 2013). An accurate temperature response model would allow estimation of thermal requirements, as well as the relationship between chill and heat accumulation during dormancy. This may then facilitate the adaptation of species and/or varieties to different regions and future climate conditions (Luedeling 2012).

In deciduous fruit trees, flower initiation and flowering occur in different growing seasons (Kurokura et al. 2013), since dormancy interrupts flower development during the winter months. Flowers in apple trees normally differentiate during summer in apical buds on spurs (short shoots) and current-season shoots (long shoots). However, apical buds can also remain vegetative under particular circumstances (e.g. in shoots with less than 16 vegetative buds in cv. Golden Delicious, Wilkie et al. 2008). The transition from vegetative to flower buds is modulated by both environmental and physiological factors. Wilkie et al. (2008) highlighted that floral initiation in apples is affected by light conditions, crop load of the previous season, and leaf area. These factors may lead to physiological changes, such as increasing concentrations of gibberellins (GAs) due to high crop load, preventing flower induction. Similarly, smaller leaf area due to high crop load has been proposed to decrease the concentration of starch in almond spurs, with negative effects on flower initiation and flowering rate in the following season (Fernandez et al. 2018). After dormancy has been established, buds only resume growth after fulfilling their temperature requirements (CR and HR, Rohde and Bhalerao 2007) during a period when trees do not have mature leaves. This implies that bud burst and flowering rely on stored reserves (Dietze et al. 2014). Starch has been proposed to play a key role in flower development (Rodrigo et al. 2000) and dormancy progression (Fernandez et al. 2019). Starch content in the pistil and anther has been suggested to sustain growth and cellular differentiation in both *Arabidopsis thaliana* (Hedhly et al. 2016), the annual model plant, and temperate fruit trees (Pimienta and Polito 1982; Rodrigo and Herrero 1998). The starch detected in flowers before bloom has been associated with dormancy and flower quality (Rodrigo et al. 2000; Alcaraz et al. 2013), as well as with key reproductive processes, such as pollination (Rodrigo et al. 2009; Herrero and Dickinson 1979) and fruit set (Alcaraz et al. 2013; Ruiz et al. 2001; Iglesias et al. 2003). Flower differentiation and the storage of reserves, which occur during the previous growing season, may be affected by unfavorable environmental conditions during summer and early fall (Hedhly et al. 2009).

Apple is the most important fruit crop in Germany, representing about 50% of the country's total fruit production (Statistisches Bundesamt 2019). To evaluate the impacts of the unusual conditions in summer of 2018 in Germany on apple trees, we compared two orchard blocks under different irrigation regimes (irrigated vs. non-irrigated). While fruit development during the season allowed maintaining adequate yield in both blocks, we focused the attention on how natural drought affected bud performance in the following season. This general aim has been achieved by the following approaches: i) morphological observations of vegetative growth and reproductive buds; ii) evaluation of dormancy response and temperature requirements under greenhouse and field conditions; iii) estimation of the level of reserves (starch) accumulated in the flower meristems prior to flowering; and iv) estimation of the probability of buds to flower in the subsequent spring.

Materials and methods

Site and plant material

This study was conducted in an experimental orchard of the University of Bonn at Campus Klein-Altendorf (CKA) in Germany (6° 59' 32" E, 50° 37' 51" N, and 160 m.a.s.l.). CKA is located in the temperate oceanic climate zone, according to the Köppen-Geiger classification (Peel et al. 2007). Specific climatic conditions of this zone are mild winters and mild summers without dry periods. The records of the years 1956–2014 show an annual mean temperature of 9.4 °C, total annual precipitation of 603 mm, and a growing season length between 165 and 170 days. The orchard soil is a loess loam (silty loam), with relatively high water retention capacity.

To evaluate summer drought effects on flowering during the following season, we investigated six-year-old apple trees cv. Elstar grafted onto M9 rootstock, cultivated with a spacing of 1.5 m within rows, and 3.5 m between rows. The experiment was carried out from June 2018 to May 2019, with the beginning of this period (June–September 2018) constituting an extraordinarily warm (Fig. 5-1 A) and dry (Fig. 5-1 B) growth period. Two independent blocks with similar soil conditions and crop load were exposed to different irrigation regimes (treatments). One of the blocks (of 0.27 ha and approximately 505 trees) was not irrigated, only receiving water through rainfall: 49 mm in June, 29 mm in July, 19 mm in August, and 37 mm in September. The other block (of 0.39 ha and approximately 743 trees) was irrigated according to standard orchard procedures, receiving an additional 63 mm in July and 70 mm in August (Fig. 5-1 B), split over a total of nine dates. According to a weather station located in the orchard, reference evapotranspiration (ET_0) was 102 mm in June, 172 mm in July, 136 mm in August, and 95 mm in September (Fig. 5-1 B). Since recommended crop coefficients (kc) for computing the water needs of apple during the growing season range between 0.9 and 1.2 (Allen et al. 1998), these figures closely approximate the trees' water needs, indicating that trees in this orchard experienced water shortage during all summer months. Neither of the blocks was fertilized during the growing season to preclude nutrition-related effects on tree development. Similarly, selected trees for this experiment were not pruned during winter to preserve the total number of buds formed during the previous season. In this study, we made use of what may be considered a natural experiment, exploiting the conditions that presented themselves in the aftermath of the 2018 drought.

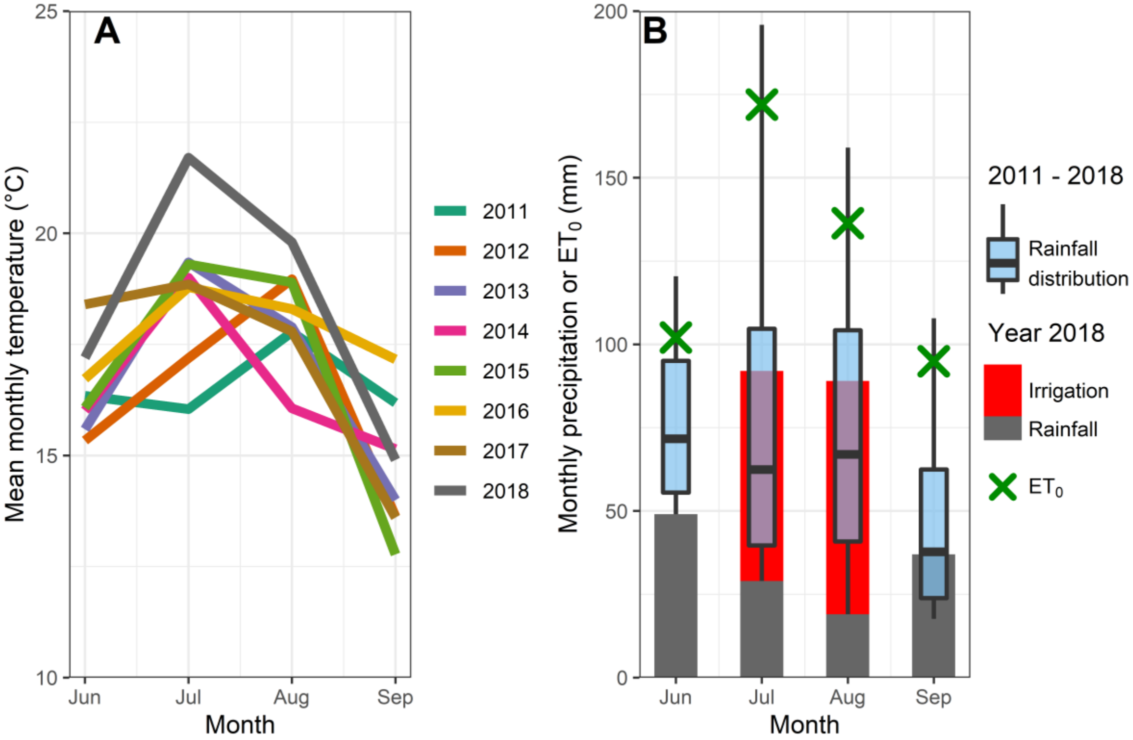

Figure 5-1. Weather conditions at Campus Klein-Altendorf from 2011 to 2018 for June, July, August, and September. **A**: Mean monthly temperature (°C) for each year. **B**: Precipitation, irrigation, and reference evapotranspiration (ET_0, in mm, indicated by green crosses). In **B**, boxplots correspond to the distribution of monthly precipitation for the period 2011–2018. Grey bars represent the precipitation for the year 2018, whereas red sections correspond to the additional water supplied by irrigation in July and August

Morphological and developmental observations on shoots and apical buds in the field

Once dormancy had been established in December, a total of 240 one-year-old shoots were randomly selected and labelled in a set of 12 trees per treatment (120 one-year-old shoots per block). These shoots were characterized by measuring their length, their diameter, at 2 cm from the branch insertion, and the number of buds. Shoot length and number of buds were used to compute the average internode length.

Another set of seven trees in each treatment was used for weekly bud sampling from January to April, when natural bud burst occurred. In these seven trees, a total of four apical buds were randomly collected on each date from one-year-old shoots and spurs (short shoots < 5 cm in length and older than one year) and subsequently dissected and observed under a stereoscopic microscope (Zeiss Discovery, V12 Stereo Microscope, Germany) to determine the developmental stage of the flower primordia. Micrographs were taken with a digital camera (The Imaging Source Camera DFK41BU02.H, Germany), linked to image processing software (IC Capture 2.4, Germany). Similarly, a total of 60 apical buds were used to determine, through microscopic observations, the number of flower primordia that composed the cyme.

Phenology observations under greenhouse and field conditions

The phenology of the vegetative and reproductive buds was recorded according to the BBCH scale (Meier 2001), which has recently been adapted for apple trees (Martinez et al. 2019). The phenology of the vegetative buds was covered by the scale for principal growth stage 0: bud development (sub-stages from 00 to 09) and principal growth stage 1: leaf development (sub-stages from 10 to 19). The phenology of the reproductive buds was described by the scale for principal growth stage 5: inflorescence emergence (sub-stages from 50 to 59), and bloom time was classified by the scale for principal growth stage 6: flowering (sub-stages from 60 to 69, Meier 2001).

To estimate the chill requirement of apple buds and to study its relationship with the heat accumulation phase of dormancy, we observed bud phenology under greenhouse conditions after buds had been exposed to different chill levels under field conditions. To this end, we applied the procedure described in Fernandez et al. (2019) with some modifications. In brief, 24 (12 from each block) out of the previously selected shoots (Section Morphological and developmental observations on shoots and apical buds in the field of Materials and Methods) were sampled weekly from 7th December 2018 to 8th February 2019. After sampling, shoots were transferred into a heated greenhouse (environmental conditions: 13.8 ± 5.1 °C; 72.9 ± 12.5% relative humidity) and placed vertically in 1 L containers with 250 mL of a 5% (w/v) sucrose solution after a cut under water to avoid cavitation and disinfection treatment (3% CaCl2 w/v). Shoots were maintained inside the greenhouse for 10 weeks or until an advanced developmental stage (BBCH 60) was recorded in most of the shoots. The cut was refreshed and disinfected, and the sucrose solution changed every week.

In addition, flower bud phenology was tracked directly on the trees under field conditions. Twenty individual apical buds were selected and labelled in two trees (10 buds each) per irrigation treatment. Half of the buds were located on one-year-old shoots and the other half on spurs, where fruits are usually produced. Observations were carried out weekly from early March to late April.

Proportion of the buds that generated a cyme

The proportion of apical flower buds that developed a cyme was evaluated in the field on three trees per treatment, for which five main branches per tree at different heights were selected and labeled. On 19th February 2019, while trees were still dormant, we determined the total number of apical flower buds on shoots and spurs. In total, 602 buds were recorded. Out of these, about 65% were located on one-year-old shoots and about 35% on spurs. At full bloom, on 22nd April 2019, we recorded the number of buds with fully developed (BBCH 65) cymes.

Chill and heat quantification and chill requirement estimation

Hourly temperatures were obtained from a weather station placed in the orchard and recorded with a data logger (Tinytag TGP-4500) inside the greenhouse to estimate chill and heat accumulation. We used the R package chillR (Luedeling 2019) to compute chill in Chill Portions (CP) according to the Dynamic Model (Erez et al. 1990; Fishman et al. 1987a, b), since this model appears to be the most accurate available model of chill accumulation during tree dormancy (Zhang and Taylor 2011; Luedeling et al. 2011b). Winter chill was quantified from the 1st of November, the approximate onset of the dormant season, until the day before shoot sampling (Table 5-1). Once shoots had been transferred into the greenhouse, heat accumulation was quantified according to the Growing Degree Hour model (Anderson et al. 1986). Following common practice (Fernandez et al. 2019), we used the default parameters proposed in the original publications for both the Dynamic Model and the Growing Degree Hour model.

Table 5-1. Chill accumulation (in Chill Portions–CP) in the orchard according to the Dynamic Model

Date of Sampling	Chill Received in the Field (in CP)
7th December 2018	24.9
14th December 2018	29.7
21st December 2018	34.4
28th December 2018	39.4
4th January 2019	44.9
11th January 2019	49.6
18th January 2019	55.5
25th January 2019	56.3
2nd February 2019	63.7
8th February 2019	68.6

The date of dormancy breaking, or the date the chill requirement was fulfilled, was defined as the moment (sampling date) for which most of the buds showed a steady response to heat in the greenhouse and presented at least stage BBCH 51 at the end of the experiment. This was determined by visual assessment of the heat response plots (shown in Section Bud development on one-year-old shoots under greenhouse conditions of the Results).

We concluded that the chilling requirement was fulfilled when additional chill accumulation did not lead to further changes in the buds' responsiveness to heat (as indicated by the shape of the heat response curves)

Starch evaluation on king flower primordia

A total of 48 apical buds were sampled on four dates (24th January, 1st and 13th February, and 7th March) from shoots and spurs of both the irrigated and non-irrigated treatments (six buds per treatment per date, three from spurs plus three from one-year-old shoots). These buds were fixed in Carnoy fixative (ethanol:acetic acid, 3 to 1 v/v) for 24 h and then moved to 75% ethanol and stored at 4 °C.

King flower primordia were separated from the fixed buds and directly stained using a whole-mount clearing and starch staining solution. Herr's $4\frac{1}{2}$ clearing solution makes plant samples transparent (Herr 1971), allowing detection of starch stained with lugol (Herr 1972). This solution is composed of lactic acid, chloral hydrate, phenol, clove oil, xylene, iodine, and potassium iodide (mixed at a weight-by-weight ratio of 2:2:2:2:1:0.1:0.5). The solution was applied for 30–45 min, after which the excess liquid was absorbed with a blotting paper. The stained primordia were photographed under identical light conditions and camera settings under a stereoscopic microscope (Zeiss Discovery, V12 Stereo Microscope, Germany) with a digital camera (The Imaging Source Camera DFK41BU02.H, Germany) linked to image processing software (IC Capture 2.4, Germany). Starch was qualitatively evaluated by comparing the photographs and classifying them according to a three-level color scale: low (Fig. 5-2 A), medium (Fig. 5-2 B), and high (Fig. 5-2 C) starch content.

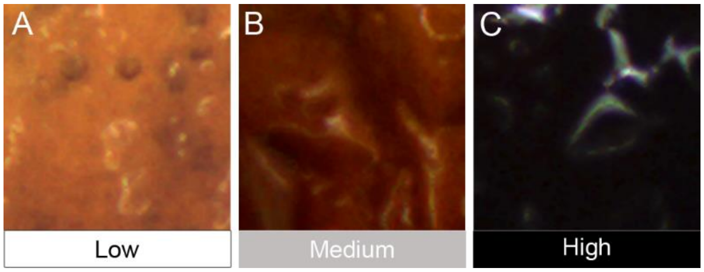

Figure 5-2. Qualitative starch evaluation according to a three-level color scale. **A**: Low starch content (light brown). **B**: Medium starch content (brown). **C**: High starch content (dark grey-black). Samples were taken from flower primordia of apical buds of apple trees

Statistical analysis

All analyses and figures were developed in the R programming environment (version 3.5.3, R Core Team 2019). Morphological variables, such as the length of internodes and the diameter, were modeled via one-way analysis of variance (ANOVA). The number of flower primordia observed within the apical buds was analyzed through generalized linear models (GLMs), assuming a Poisson distribution and using treatment, bud type, and the interaction of both factors as predictors.

Variation in starch concentrations was analyzed using the Wilcoxon–Mann–Whitney test (Hollander et al. 2015). We performed separate analyses for each sampling date to test for the effect of irrigation treatment on the starch concentration. Additionally, we treated the date as a factor to test for differences in the concentration of starch during winter, independent of the irrigation treatment. We compared starch concentrations in the flower primordia between all subsequent pairs of sampling dates. Since this implied testing for multiple null hypotheses (i.e. no differences between observations on subsequent dates), we applied Bonferroni's method (Bonferroni 1936) to adjust the criteria for identifying statistical significance (*p* value correction).

Phenological observations under both greenhouse and field conditions were analyzed using ordinal linear regression models (also known as proportional odds models). This approach assumes that the phenological stages (which are ordered from 0 to 69) and the transitions between them depend on either continuous or discrete variables, in this case on chill, heat, and irrigation treatment. A small variation of this approach is the use of nominal effects, which allows the thresholds between stages to vary in response to some predictors. In brief, we modeled the BBCH stages as a function of chill and heat accumulation (and their interaction) and used the irrigation treatment as a nominal effect factor. This enabled us to evaluate whether the irrigation treatment shifted the thresholds between BBCH stages of apple buds. With the original phenology classification (the BBCH scale), the fitting procedure of the cumulative link model (maximum likelihood method) did not converge on a well-defined set of parameters for data collected under greenhouse conditions. Following common procedures to address this computational problem (Berhe et al. 2017), we reclassified the phenology data. We defined six new classes according to BBCH stages: 1 for buds at BBCH stage 0, 2 for buds at BBCH stages 51 and 52, 3 for buds at BBCH stages 53 and 54, 4 for buds at BBCH stages 55 and 56, 5 for buds at BBCH stages 57 and 59, and 6 for buds at BBCH stage 60. Data collected under field conditions did not need reclassification, since the analysis worked with the original classes. We then compared the thresholds between BBCH states in irrigated and non-irrigated treatments under greenhouse and field conditions.

Finally, the probability of success in buds to generate a cyme in the following spring was modelled using GLMs, assuming a binomial distribution of the data and including shoot type and treatment as factors. In the case of the total number of buds (independent of shoot type), we only considered treatment as a factor. To identify differences between groups (i.e. irrigated versus non-irrigated), we contrasted means using Tukey's test (Tukey 1970).

Results

Morphological observations on shoots and microscopic monitoring of apical buds

Shoot growth during the summer period was clearly affected by the irrigation treatment. Although shoots presented similar numbers of buds in both treatments (median of 11 buds per shoot), shoots from the irrigated treatment had longer internodes (median of 2.27 cm) than those from the non-irrigated treatment (median of 1.37 cm, $p < 0.001$, Fig. 5-3 A). In contrast, the shoot diameter was shorter (but no significant effect was detected using this data set, $p = 0.11$) in the irrigated treatment (median of 4.37 mm) than in the non-irrigated treatment (median of 4.76 mm, Fig. 5-3 B).

Apical buds placed on shoots and spurs presented cymes composed of 3–6 flower primordia, surrounded by leaf primordia and numerous bud scales. No clear effect of irrigation or bud type on the number of flower primordia that composed the cymes was determined in this experiment. The majority of cymes (78–100%) presented 4–5 flower primordia (Fig. 5-3 C). It is worth noting that in irrigated trees, buds with six-flower cymes only differentiated at a low rate (6%, Fig. 5-3 C).

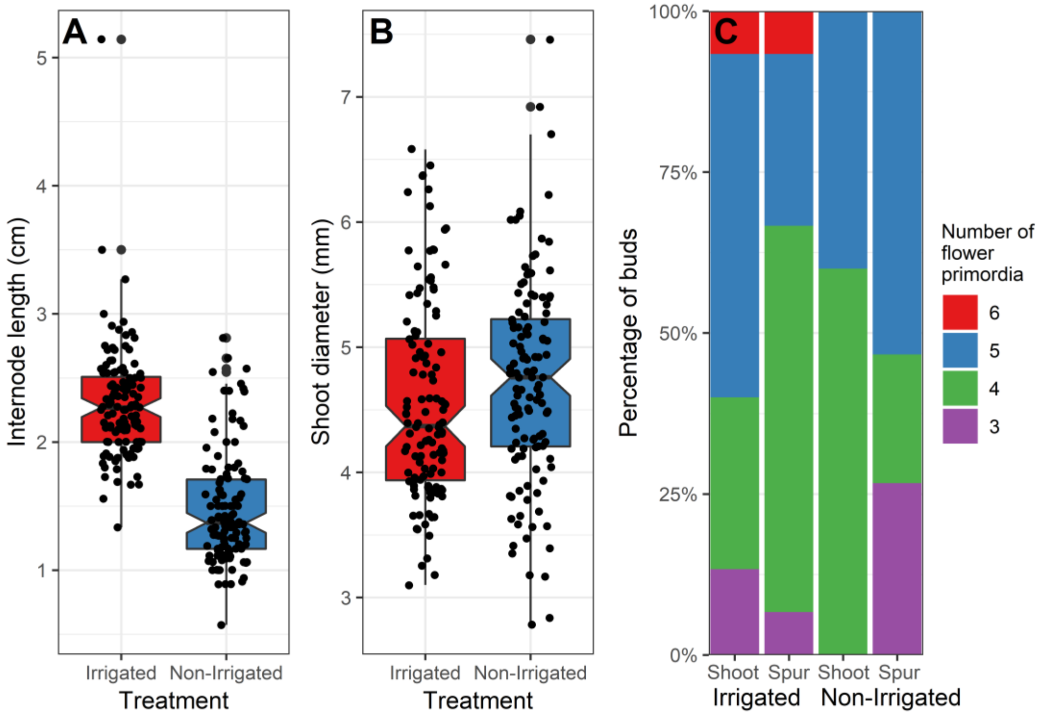

Figure 5-3. Morphological traits of one-year-old apple shoots cv. Elstar sampled during the winter season of 2018/2019 (Table 5-1). Shoots were sampled from trees grown in Campus Klein-Altendorf, exposed to two water regimes during the growing season of 2018. **A**: Internode length. **B**: Shoot diameter. In **A** and **B**, boxplots represent the distribution of the data, while black dots represent actual observations. In boxplots, the colored box represents 50% of the data (percentile 25%, 50%, and 75%), whereas vertical black lines mean the distance between the percentile (25% or 75%) and the smallest or largest value, respectively. Notches represent an approximation of the 95% confidence interval for comparing medians. In total, 240 shoots were sampled for internode length and shoot diameter measurements. (**C**): Percentage of buds with a given number of flower primordia per cyme (colors) in one-year-old shoots and spurs considering 60 apical buds in total

Morphological observations of the king flower primordia before bud burst revealed that flower primordia developed within the dormant buds (phenological stage BBCH 00) showed no major differences between irrigated and non-irrigated trees. The general development of the flower primordia of both treatments can thus be illustrated with only one set of photographs (Fig. 5-4). During winter, the king flower (kf) primordia were centrally placed and significantly larger than primordia of lateral flowers (lf, Fig. 5-4 A). The sepals (se) that enclosed the flower primordia presented abundant trichomes (tr). Pistils and stamens were not differentiated and appeared as rounded domes (rd) in the king flower primordium (Fig. 5-4 B). By the end of February and early March, flower primordia had grown significantly and differentiated despite the absence of external phenological changes. Stamens presented short filaments and the anther acquired its characteristic shape with two thecae and four locules. The five styles (st) were also distinguished at this moment (Fig. 5-4 C). Once buds showed the first signs of burst, by the end of March or early April (BBCH 51), the anthers (an) acquired a yellowish appearance and the stigmas (ss), composed of numerous papillae, were distinguishable (Fig. 5-4 D).

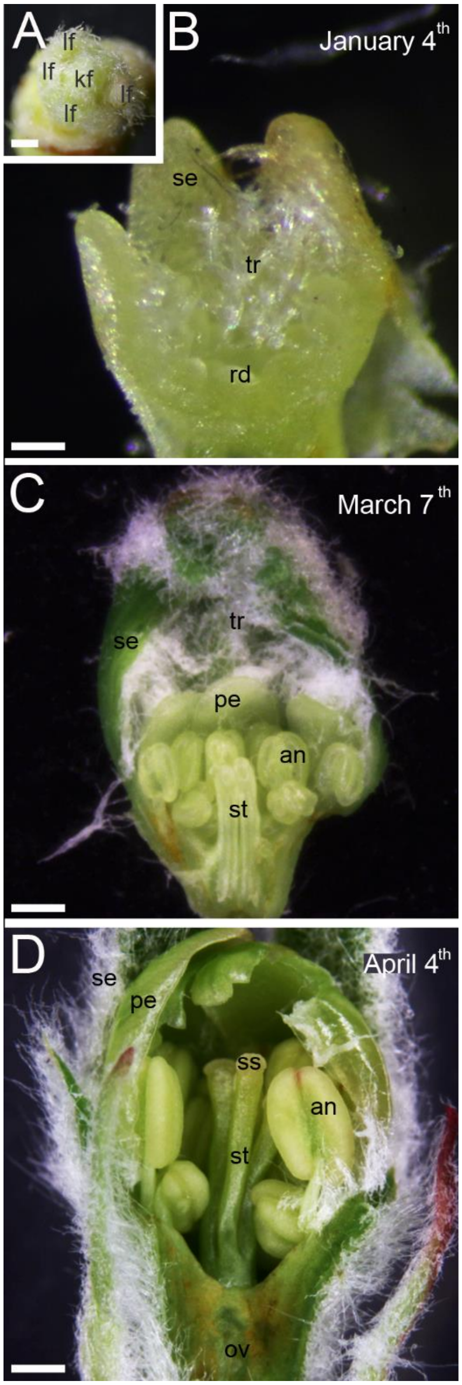

Figure 5-4. Flower development and growth during dormancy until bud burst. **A**: Apple cyme during winter (4th of January 2019). **B**: King flower primordia presenting sepals with numerous trichomes and undifferentiated flower verticiles during winter (4th of January 2019). **C**: Distinguishable stamens and pistils at the end of winter (7th of March 2019). **D**: Anthers with yellowish appearance and stigmas at the end of the styles (4th of April 2019). Abbreviations: lateral flower (lf), king flower (kf), sepals (se), trichomes (tr), rounded dome (rd), petals (pe), anthers (an), style (st), stigma (ss), and ovary (ov). Scale bars: A, C, and D = 500 µm, B = 100 µm

Starch concentrations in the king flower primordia changed over the course of the observation period (Fig. 5-5). These dynamics were comparable across irrigation treatments, with similar starch concentrations in irrigated and non-irrigated trees on all sampling dates (Fig. 5-5). We observed that flower primordia tissue stained lightly for starch at the end of January. Just a week later in early February, strong differences were observed among the flower primordia ($p < 0.05$). In mid-February, an intense dark reaction revealed that the flower primordia had significantly ($p < 0.05$) more starch compared to the previous sampling date (Fig. 5-5). Subsequently, flower primordia presented first signs of growth in March and the starch content was significantly reduced ($p < 0.001$) in comparison with the concentration observed on 13th February.

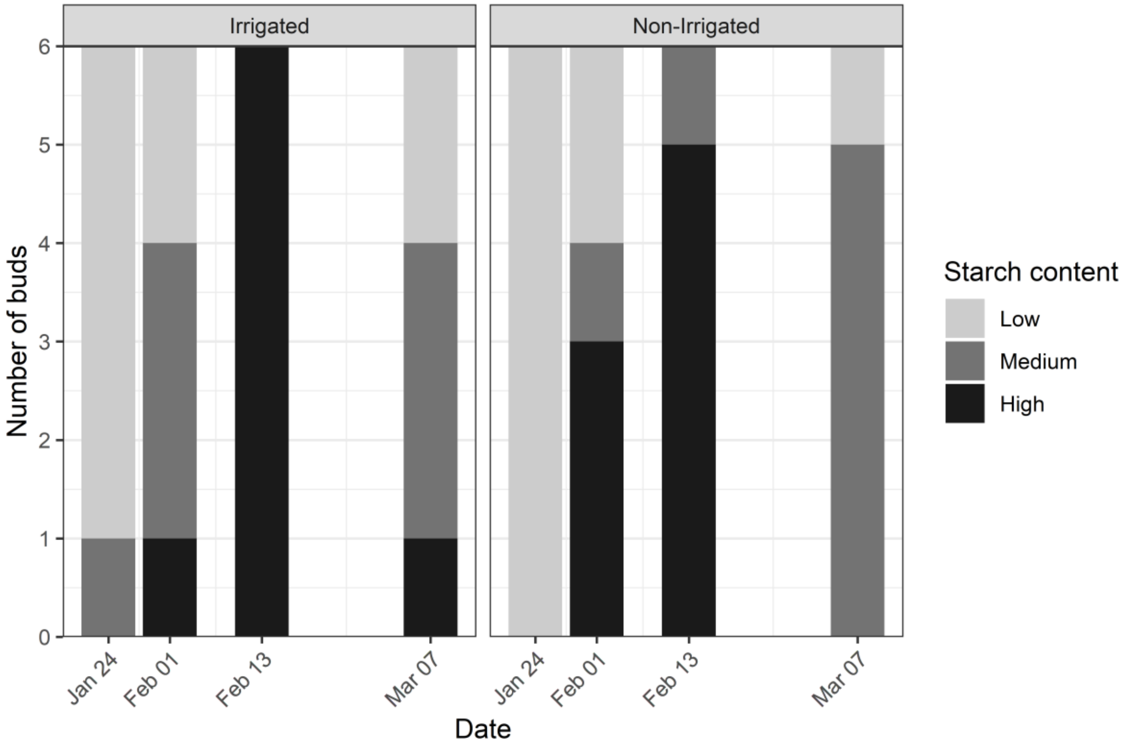

Figure 5-5. Frequency of buds in a given starch content class (low, medium, or high) according to qualitative observations on flower primordia tissue (see section Starch evaluation on king flower primordia in Materials and Methods). Samples were taken from shoots and spurs of apple trees cv. Elstar grown in Campus Klein-Altendorf exposed to two irrigation regimes (irrigated and non-irrigated panels) during the summer of 2018. For the figure, we used all analyzed buds (48) collected on four dates during the winter of 2019

Bud development on one-year-old shoots under greenhouse conditions

Only a small number of lateral buds on sampled shoots were able to reach bud burst or another more advanced phenological stage under greenhouse conditions. We therefore focused the analysis on apical buds on one-year-old shoots.

Apical bud development during this experiment was affected by both the irrigation treatment and the chill received in the field (Fig. 5-6). Using the first sampling date (after 24.9 CP had accumulated), the percentage of buds that had reached at least BBCH stage 51 at the end of the experiment was about 25% in shoots from irrigated trees, while about 50% in shoots from non-irrigated trees reached such stages. From 39.4 CP accumulated under field conditions, most of the buds reached at least BBCH stage 51 by the end of the experiment in both irrigated and non-irrigated treatments.

The amount of heat necessary to force bud burst decreased with higher levels of chill accumulated in the field. For samples taken at 24.9 CP, buds required 11,000–15,000 Growing Degree Hours (GDHs) to reach BBCH stages 51 and 52. After accumulating 29.7 CP by the subsequent sampling date, heat requirements for reaching the same BBCH stages had decreased from 6,000 to 9,400 GDH (Fig. 5-6). For samples taken in Week 9 (63.7 CP), the first bud burst (BBCH stages 51 and 52) was recorded after about 3,000 GDH for both irrigated and non-irrigated shoots. Along the same lines, the transition between BBCH states was faster when more chill had been accumulated by the buds under field conditions. For samples taken at 24.9 CP, about 12.5% of the buds remained at BBCH stages 51 and 52 at the end of the experiment (up to 16,000 GDH), while for samples taken at 55.5 CP all the buds had overcome this stage after about 7,500 GDH.

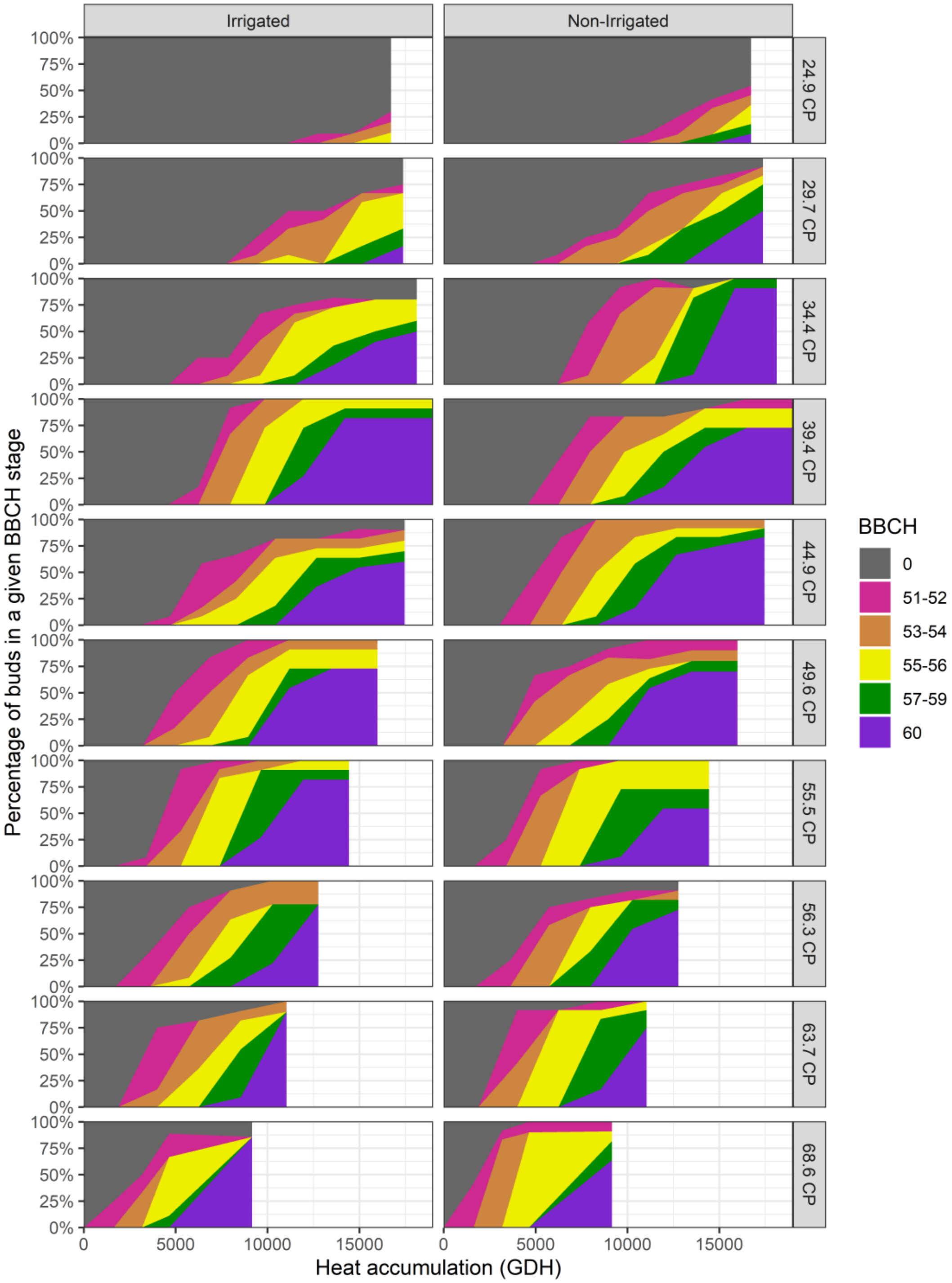

Figure 5-6. Apical bud development in one-year-old apple shoots (cv. Elstar) sampled during the winter season of 2018/2019 (Table 5-1). Shoots sampled from trees grown in Campus Klein-Altendorf were exposed to two water regimes (irrigated and non-irrigated panels) during the growing season of 2018. Each row of panels corresponds to a particular sampling date represented by the chill accumulated in the field (in Chill Portions–CP). The x-axis represents heat accumulation (in Growing Degree Hours–GDHs) under greenhouse conditions after the shoots had been sampled. The colors represent the sub-stages for the principal growth stages 5 (inflorescence emergence) and 6 (flowering) of reproductive buds, according to the BBCH scale. We monitored a total of 213 apical reproductive buds (27 apical vegetative buds were excluded)

The cumulative link model indicated both chill and heat, as well as the interaction between them, as highly significant predictors of the BBCH stages of buds under both irrigated and non-irrigated conditions ($p < 0.001$). Regarding the effects of the irrigation treatment on the transition points between BBCH stages, we determined that the estimates for the parameters significantly differed for the two first thresholds. From BBCH 0 to BBCH 51 and 52, the estimate was 0.69 in irrigated buds, whereas in the non-irrigated buds this value decreased to 0.07 ($p < 0.001$). Similarly, the difference between the estimates of the thresholds from BBCH 51 and 52 to BBCH 53 and 54 in irrigated versus non-irrigated buds was 0.59 ($p < 0.001$, Table 5-2). From the third transition point (e.g. BBCH 53 and 54) onwards, the estimates tended to show similar values among treatments, with only the fourth transition point indicated as significantly different between treatments.

Table 2. Differences between estimated thresholds for transition points between non-irrigated and irrigated buds through cumulative link modeling. Data in the confidence interval column reflects results on significance from Wald's test (z-value). *** indicates $p < 0.05$, while n.s. means that treatments were not significantly different at this confidence level

Transition point (from stage x to stage y)	Difference in threshold estimates (non-irrigated – irrigated)	Confidence interval for the difference (95%)
BBCH 0 to BBCH 51-52	-0.62	-0.90 – -0.34 ***
BBCH 51-52 to BBCH 53-54	-0.59	-0.88 – -0.30 ***
BBCH 53-54 to BBCH 55-56	-0.27	-0.57 – -0.05 n.s.
BBCH 55-56 to BBCH 57-59	-0.52	-0.91 – -0.13 ***
BBCH 57-59 to BBCH 60	-0.09	-0.61 – -0.42 n.s.

Phenology progression and flowering rates under field conditions

Based on the previous results, we concluded that endo-dormancy was overcome around the 11th of January in both treatments, after 50 CP had accumulated (Fig. 5-6). Under field conditions, buds from both the irrigated and non-irrigated treatments showed similar phenological patterns at bloom time (Fig. 5-7). The first changes in flower bud phenology were observed on the 7th of March, after 1,876 GDHs had accumulated in both treatments. Only minor differences were observed on subsequent dates. On the 11th of April, after 4,500 GDHs had accumulated, about 85% of the buds in the non-irrigated and 70% in the irrigated treatment presented some open flowers (BBCH 60 or higher). Full bloom (BBCH 65) occurred in a small proportion of the buds simultaneously in both orchards on the 18th of April, after at least 5,000 GDHs had accumulated.

According to the ordinal linear regression analysis, only heat and the interaction between chill and heat significantly ($p < 0.05$) affected the phenology of both irrigated and non-irrigated buds. After chill requirements had been met (which appeared to be the case for chill accumulation greater than 50 CP), additional chill (without heat) was not found to produce significant differences ($p = 0.25$). Regarding the effect of irrigation on the transition points, we detected that under field conditions, most of the transition thresholds did not significantly differ between treatments. The difference between only 2 out of 12 transition points were found statistically significant under field conditions at a p level of 0.05. These were from BBCH 52 to BBCH 53 ($p = 0.014$) and from BBCH 53 to BBCH 54 ($p = 0.039$), for which non-irrigated buds showed lower thresholds for the transition points compared to irrigated buds.

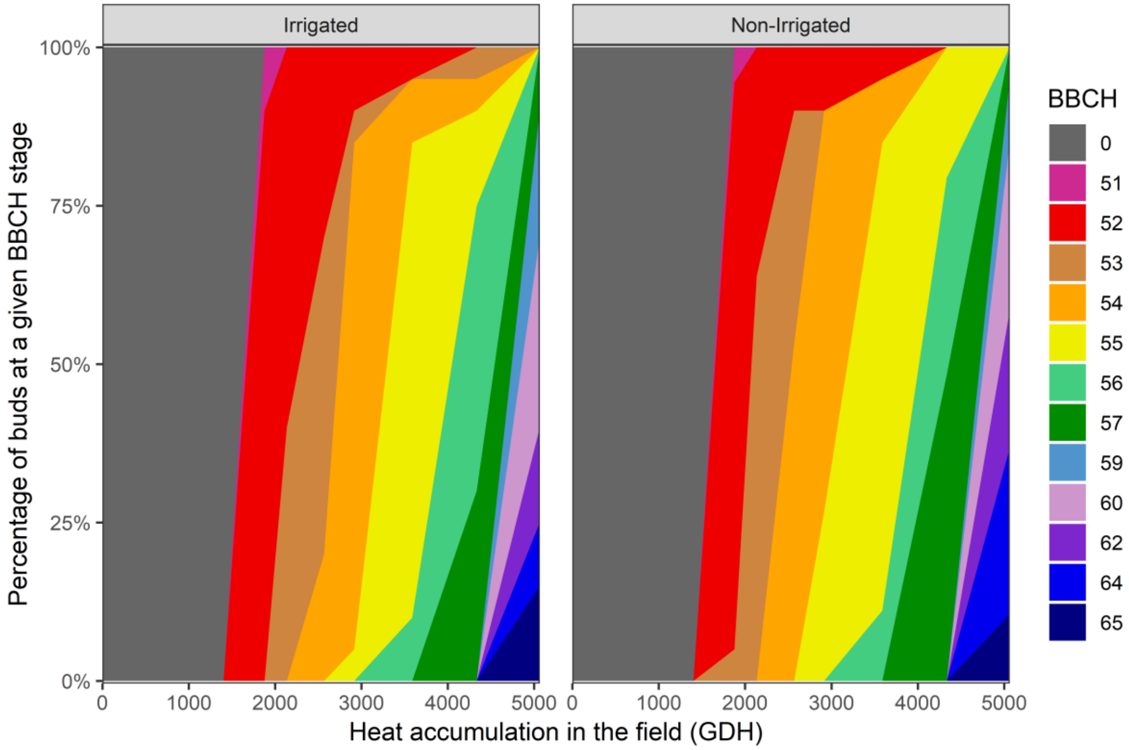

Figure 5-7. Phenology progression of the apical buds in apple trees cv. Elstar grown in Campus Klein-Altendorf exposed to two irrigation regimes (irrigated and non-irrigated panels) during the growing season of 2018. The x-axis represents the accumulation of heat (in Growing Degree Hours–GDHs) under field conditions after a minimum chill requirement of 50 CP had been fulfilled. The colors represent the sub-stages for the principal growth stages 5 (inflorescence emergence) and 6 (flowering) of reproductive buds, according to the BBCH scale. We monitored 208 apical buds in total

The probability of a bud generating a cyme was higher on buds that developed on shoots compared to buds from spurs in both irrigated ($p < 0.001$) and non-irrigated ($p = 0.07$) treatments. Furthermore, buds from non-irrigated trees presented a higher probability of bloom than those from irrigated trees (Fig. 5-8). This impression was maintained when the type of bud was also considered, with buds from non-irrigated shoots presenting a higher chance of flowering than buds from irrigated shoots ($p = 0.02$). The latter was also observed on buds located on spurs ($p < 0.001$).

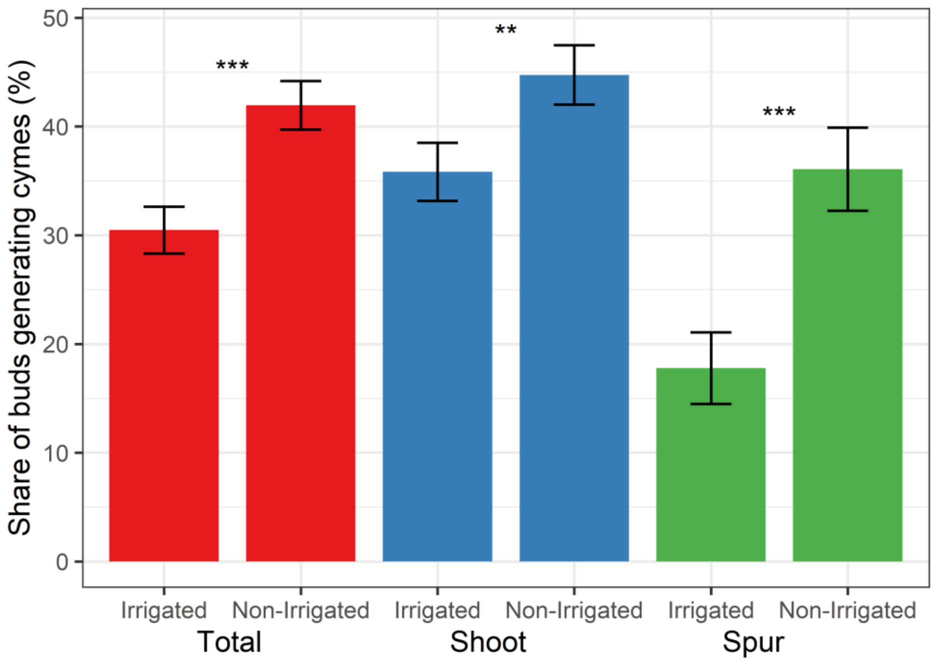

Figure 5-8. Share of apical flower buds generating cymes for apple trees cv. Elstar exposed to two irrigation regimes. Measurements were taken on the 22nd of April 2019 in Campus Klein-Altendorf. Bar colors indicate the structure on which the bud developed (one-year-old shoots versus spurs), with red bars illustrating results for all sampled buds. Error bars in each category show the standard error of the mean. In total, 602 buds were counted, out of which 212 (35%) were from spurs and 390 (65%) were from one-year-old shoots. *** represents $p < 0.001$ and ** means $p < 0.05$

Discussion

We evaluated the impacts of two irrigation regimes on apple trees grown under the unusual moisture conditions in the summer of 2018 at the experimental station at Campus Klein-Altendorf. Although we made use of what may be considered a natural experiment, we were able to document that the additional water supplied during summer increased vegetative growth (i.e. shoot length) during the growing season. Shoots on irrigated trees were significantly longer (by 39%) and thinner compared with trees grown without irrigation. However, this did not affect the apical buds at the top of the shoots, which developed a similar number (4–5) of flower primordia per cyme. Observations during the winter season showed that the flower primordia and the starch dynamics in bud tissue followed a similar pattern in both treatments. Our CR estimations resulted in about 50 CP for both non-irrigated and irrigated trees. The use of one-year-old shoots exposed to forcing conditions revealed that, after low exposure to chill (i.e. 24.9–34.4 CP), buds from non-irrigated trees developed significantly faster and at higher rates compared to buds from irrigated trees. However, these differences became less pronounced after longer exposure to chilling conditions. Under field conditions, differences in phenological development between treatments disappeared, but buds on non-irrigated trees presented a significantly higher probability of flowering (0.42) than buds on irrigated trees (0.30) during the following spring.

The difference we observed in total shoot length was explained by the length of the internodes rather than a different number of lateral buds. On average, additional irrigation of 133 mm during July and August of 2018 effected an increase of 39.6% in the internode length of one-year-old shoots. Similar results on shoot length have previously been observed on one-year-old M9 apple rootstocks exposed to a water deficit of 50% of tree water needs (Bolat et al. 2014). Chen et al. (2019) reported that water stress during the summer promoted the cessation of vegetative growth, probably due to an increase in the production of ABA in the roots (Sobeih et al. 2004). Moreover, the drought stress responses of other hormones regulating growth, development, and senescence (i.e. auxins and ethylene) have also been studied (reviewed in Ullah et al. 2018). Changes in the concentration of these hormones during the growing season may explain why shoots on irrigated trees were longer than shoots on non-irrigated trees.

Meristems inside the apical buds differentiated into cymes composed of a similar number of flower primordia (4–5), with the king flower presenting a similar state of development during winter in both treatments. This suggests that these traits were not greatly affected by this level of water stress. In agreement with previous studies on apple flower differentiation (Foster et al. 2003), the king flower did not present differentiated flower verticiles, but remained in an earlier development stage (i.e. round domes). This early flower developmental stage remained during endo- and beginning of early eco-dormancy. This observation indicates a less mature stage of development during dormancy in apples compared to *Prunus* spp., where flower verticiles are already distinguishable (sepals, petals, anthers, and pistil) during winter (Fadón et al. 2018b; Felker et al. 1983). The king flower started to accumulate starch at this early developmental stage in late January, showing peak starch reserves in mid-February. Later, the starch content was reduced and the king flower presented the first signs of growth. Starch dynamics have been reported in the flower primordia of sweet cherry during winter as a source of energy for subsequent flower development (Fadón et al. 2019, 2018a). In apple primordia, maximum starch content occurred when about 63.7 CP–68.6 CP had accumulated, which was after fulfillment of the estimated chill requirement (50 CP), but corresponded with a rapid growth response under forcing conditions. In sweet cherry, maximum starch accumulation has been shown to occur earlier in winter and to be closely associated with fulfillment of the chill requirement (Fadón et al. 2018a).

Compared to irrigated trees, apical buds from non-irrigated shoots showed a tendency towards lower thresholds for transition points between BBCH stages, indicating that they developed faster than buds from irrigated trees. This tendency was clearly observed under forcing conditions for low-chill sampling dates (i.e. 24.9–34.4 CP). Nonetheless, this difference was not observed for shoots that had been exposed to greater chill levels in the field, with shoots from both treatments showing the same bud burst pattern and similar thresholds between BBCH stages. This may suggest that water stress during summer may affect the CR, especially in trees grown in warm-winter locations. Under field conditions, buds from the non-irrigated treatment showed a significantly higher probability of flowering (0.42) in spring than buds from the irrigated block (0.30). An increase in flowering rates during the following season, after experiencing water deficit, has also been observed in other temperate fruit trees, such as pear and peach (Behboudian and Mills 1997). In young pomelo trees (*Citrus grandis*), the number of inflorescences, flower buds, and flowers that opened increased as the duration of water stress was extended (Nakajima et al. 1993). Our initial hypothesis to explain these differences hinged on disparities in the starch content of the flower primordia. In fact, one could expect less starch to accumulate in the irrigated treatment, as more energy might have been spent on shoot growth rather than storage during the previous growing season. Alternatively, less starch may have accumulated in the non-irrigated treatment because of a reduction in the photosynthetic rate due to water stress (Naschitz et al. 2010). However, in light of our results, this expectation was not confirmed, with both treatments showing a similar pattern regarding starch levels in the flower primordia during winter. Thus, differences in phenological development, as well as in flowering probability, may rely on changes in hormonal regulation (Sanyal and Bangerth 1998), different levels of reactive oxygen species (ROS) produced during the growing season (Beauvieux et al. 2018), or other biochemical changes inside the buds during bud initiation in summer or bud dormancy in winter. Further experimentation is still needed to clarify these findings.

Based on the results obtained from shoots exposed to forcing conditions (Fig. 5-6), we estimated that this variety overcame dormancy after 50 CP had accumulated in the field. Similar estimates have been reported for apples cv. Golden Delicious (50 CP in Erez 2000) and cv. Starkrimson Delicious (about 65 CP in Shaltout and Unrath 1983, using conversion factors for Chilling Hours to CP in North Carolina according to Luedeling and Brown 2011). However, the heat units accumulated until the first signs of bud burst differed between sampled shoots and buds on trees grown under field conditions. Buds from cuttings required about 5,000 GDHs, whereas buds from trees in the field required 2,000 GDHs. This difference can be explained by trees in the field accumulating more chill than 50 CP, which may have compensated for the lower heat accumulation outside the greenhouse. This dynamic relationship between chilling and heating phases during dormancy has been previously proposed by Harrington et al. (2010). Indeed, shoots sampled at 63.7 CP only required about 3,500 GDHs to reach bud burst. This may also suggest that our CR estimation corresponds to the minimum amount of chill needed to reach a homogeneous bud burst pattern after a given amount of heat and might not be valid for comparison with observations under field conditions, where trees can receive additional chill. Along the same lines, while winter chill is expected to decrease in many growing regions of the world due to rising winter temperatures (Luedeling et al. 2011a), heat accumulation during late winter and early spring is expected to increase. In this regard, an overlapping relationship between chill and heat phases may suggest that different chill and heat accumulation patterns may result in similar bloom dates. However, further experimentation on this relationship across a wide range of climates, as well as on the impact of water stress on CR at low chill levels, could greatly improve our knowledge on the dormancy of temperate fruit trees.

Conclusions

Mild water stress during the growing season impacts on the morphology of shoots in the same growth period and may possibly affect the phenology and bloom probability of apical buds in the following spring. Irrigated trees presented more vegetative growth compared to those that did not receive additional water. The implications of this phenomenon for orchard management are currently unclear. Based on morphological observations, we suggest that this level of water stress did not affect bud initiation, bud differentiation, or storage processes. Regarding phenological development, we observed that, after low chill exposure, apical buds from non-irrigated shoots developed faster and at higher rates than those from irrigated shoots. This suggests that summer drought may affect the chill requirement of apple buds. However, further validation and experimentation on the mechanisms behind this finding are needed. Similarly, further experimentation is required to validate our findings on flowering probability, as well as to test the effect of summer drought on subsequent phases, such as fruit set and fruit development. Climate change is widely expected to affect the cultivation of temperate deciduous fruit trees by increasing winter temperatures as well as modifying total precipitation and rainfall distribution. We hope that our findings will contribute to understanding the medium-term (i.e. subsequent seasons) effects of summer drought on apple cultivation, as well as provide useful guidance for future research on this topic.

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Conflict of interest

This research has been conducted in the absence of any relationship that can construct a possible conflict of interest.

Author contributions

EF (Erica Fadón) and EF (Eduardo Fernandez) designed the experiment with the assistance of EL and AK. DB and SVdV established the experiment and recorded the data with the guidance of AK, EF (Erica Fadón), and EF (Eduardo Fernandez). All the authors contributed to analyzing and discussing the results. EF (Eduardo Fernandez), EF (Erica Fadón), and EL wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

Alburquerque N, Garcia-Montiel F, Carrillo A, Burgos L (2008) Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. Environ Exp Bot 64 (2):162-170. doi:10.1016/j.envexpbot.2008.01.003

Alcaraz ML, Hormaza JI, Rodrigo J (2013) Pistil starch reserves at anthesis correlate with final flower fate in avocado (*Persea americana*). PLoS One 8 (10):12. doi:10.1371/journal.pone.0078467

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration. Guidelines for computing crop water requirements. Irrigation and Drainage Paper No. 56.:FAO, Rome, p 300

Anderson JL, Richardson EA, Kesner CD (1986) Validation of chill unit and flower bud phenology models for 'Montmorency' sour cherry. Acta Horticulturae (ISHS) 184:71-78. doi:10.17660/ActaHortic.1986.184.7

Beauvieux R, Wenden B, Dirlewanger E (2018) Bud dormancy in perennial fruit tree species: A pivotal role for oxidative cues. Front Plant Sci 9:13. doi:10.3389/fpls.2018.00657

Behboudian MH, Mills TM (1997) Deficit irrigation in deciduous orchards. Hortic Rev 21:105-131. doi:10.1002/9780470650660.ch4

Benmoussa H, Ghrab M, Ben Mimoun M, Luedeling E (2017) Chilling and heat requirements for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a warm Mediterranean location based on 30 years of phenology records. Agric For Meteorol 239:34-46. doi:10.1016/j.agrformet.2017.02.030

Berhe DF, Taxis K, Haaijer-Ruskamp FM, Mulugeta A, Mengistu YT, Burgerhof JGM, Mol PGM (2017) Impact of adverse drug events and treatment satisfaction on patient adherence with antihypertensive medication - A study in ambulatory patients. Br J Clin Pharmacol 83 (9):2107-2117. doi:10.1111/bcp.13312

Berman ME, DeJong TM (1996) Water stress and crop load effects on fruit fresh and dry weights in peach (*Prunus persica*). Tree Physiol 16 (10):859-864

Bolat I, Dikilitas M, Ercisli S, Ikinci A, Tonkaz T (2014) The effect of water stress on some morphological, physiological, and biochemical characteristics and bud success on apple and quince rootstocks. Sci World J 2014:1 - 8. doi:10.1155/2014/769732

Bonferroni C (1936) Teoria statistica delle classi e calcolo delle probabilita'. Pubbl R Ist Sup Sci Econ Commer Fir 8:3–62

Campoy JA, Ruiz D, Nortes MD, Egea J (2013) Temperature efficiency for dormancy release in apricot varies when applied at different amounts of chill accumulation. Plant Biol 15:28-35. doi:10.1111/j.1438-8677.2012.00636.x

Chai Q, Gan Y, Zhao C, Xu H, Waskom RM, Niu Y, Siddique KHM (2015) Regulated deficit irrigation for crop production under drought stress. A review. Agron Sustain Dev 36 (1):3. doi:10.1007/s13593-015-0338-6

Chen DY, Pallas B, Martinez S, Wang YK, Costes E (2019) Neoformation and summer arrest are common sources of tree plasticity in response to water stress of apple cultivars. Ann Bot 123 (5):877-890. doi:10.1093/aob/mcy224

Cooke JEK, Eriksson ME, Junttila O (2012) The dynamic nature of bud dormancy in trees: Environmental control and molecular mechanisms. Plant Cell Environ 35 (10):1707-1728. doi:10.1111/j.1365-3040.2012.02552.x

Cruz de Carvalho MH (2008) Drought stress and reactive oxygen species. Plant Signal Behav 3 (3):156-165. doi:10.4161/psb.3.3.5536

Deutsche Wetterdienst (2019) Zeitreihen und Trends. https://www.dwd.de/DE/leistungen/zeitreihen/zeitreihen.html#buehneTop. Accessed 17 November 2019

Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R (2014) Nonstructural carbon in woody plants. In: Merchant SS (ed) Annual Review of Plant Biology, Vol 65. Annual Review of Plant Biology. Annual Reviews, Palo Alto, pp 667-687. doi:10.1146/annurev-arplant-050213-040054

Ebel RC, Proebsting EL, Evans RG (1995) Deficit irrigation to control vegetative growth in apple and monitoring fruit growth to schedule irrigation. HortScience 30 (6):1229-1232. doi:10.21273/HORTSCI.30.6.1229

Ebel RC, Proebsting EL, Patterson ME (1993) Regulated deficit irrigation may alter apple maturity, quality, and storage life. HortScience 28 (2):141-143

Erez A (2000) Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics. In: Erez A (ed) Temperate Fruit Crops in Warm Climates. Springer Netherlands, Dordrecht, pp 17-48. doi:10.1007/978-94-017-3215-4_2

Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. Acta Hort 276:165-174

Fadón E, Herrero M, Rodrigo J (2018a) Dormant flower buds actively accumulate starch over winter in sweet cherry. Front Plant Sci 9:10. doi:10.3389/fpls.2018.00171

Fadón E, Herrero M, Rodrigo J (2019) Anther and pollen development in sweet cherry (*Prunus avium* L.) in relation to winter dormancy. Protoplasma 256 (3):733-744. doi:10.1007/s00709-018-01332-4

Fadón E, Rodrigo J (2018) Unveiling winter dormancy through empirical experiments. Environ Exp Bot 152:28-36. doi:10.1016/j.envexpbot.2017.11.006

Fadón E, Rodrigo J, Herrero M (2018b) Is there a specific stage to rest? Morphological changes in flower primordia in relation to endodormancy in sweet cherry (*Prunus avium* L.). Trees-Struct Funct 32 (6):1583-1594. doi:10.1007/s00468-018-1735-7

Failla O, Zocchi G, Treccani C, Cocucci S (1992) Growth, development and mineral-content of apple fruit in different water status conditions. J Hortic Sci 67 (2):265-271. doi:10.1080/00221589.1992.11516247

Faust M, Erez A, Rowland LJ, Wang SY, Norman HA (1997) Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance, and release. HortScience 32 (4):623-629

Felker FC, Robitaille HA, Hess FD (1983) Morphological and ultrastructural development and starch accumulation during chilling of sour cherry flower buds. Am J Bot 70 (3):376-386. doi:10.2307/2443246

Fernandez E, Baird G, Farias D, Oyanedel E, Olaeta JA, Brown P, Zwieniecki M, Tixier A, Saa S (2018) Fruit load in almond spurs define starch and total soluble carbohydrate concentration and therefore their survival and bloom probabilities in the next season. Sci Hortic 237:269-276. doi:10.1016/j.scienta.2018.04.030

Fernandez E, Cuneo IF, Luedeling E, Alvarado L, Farias D, Saa S (2019) Starch and hexoses concentrations as physiological markers in dormancy progression of sweet cherry twigs. Trees-Struct Funct 33 (4):1187-1201. doi:10.1007/s00468-019-01855-0

Fishman S, Erez A, Couvillon GA (1987a) The temperature-dependence of dormancy breaking in plants - Computer-simulation of processes studied under controlled temperatures. J Theor Biol 126 (3):309-321. doi:10.1016/s0022-5193(87)80237-0

Fishman S, Erez A, Couvillon GA (1987b) The temperature-dependence of dormancy breaking in plants - Mathematical-analysis of a 2-step model involving a cooperative transition. J Theor Biol 124 (4):473-483. doi:10.1016/s0022-5193(87)80221-7

Foster T, Johnston R, Seleznyova A (2003) A morphological and quantitative characterization of early floral development in apple (*Malus* x *domestica* Borkh.). Ann Bot 92 (2):199-206. doi:10.1093/aob/mcg120

Guo L, Dai JH, Wang MC, Xu JC, Luedeling E (2015) Responses of spring phenology in temperate zone trees to climate warming: A case study of apricot flowering in China. Agric For Meteorol 201:1-7. doi:10.1016/j.agrformet.2014.10.016

Harrington CA, Gould PJ, St Clair JB (2010) Modeling the effects of winter environment on dormancy release of Douglas-fir. For Ecol Manage 259 (4):798-808. doi:10.1016/j.foreco.2009.06.018

Hedhly A, Hormaza JI, Herrero M (2009) Global warming and sexual plant reproduction. Trends Plant Sci 14 (1):30-36. doi:10.1016/j.tplants.2008.11.001

Hedhly A, Vogler H, Schmid M, Pazmino D, Gagliardini V, Santelia D, Grossniklaus U (2016) Starch turnover and metabolism during flower and early embryo development. Plant Physiol 172:2388–2402. doi:10.1104/pp.16.00916

Herr JM (1971) A new clearing-squash technique for the study of ovule development in Angiosperms. Am J Bot 58 (8):785-790. doi:10.2307/2441475

Herr JM (1972) Applications of a new clearing technique for the investigation of vascular plant morphology. J Elisha Mitchell Sci Soc 88 (3):137-143

Herrero M, Dickinson HG (1979) Pollen-pistil incompatibility in *Petunia hybrida*: Changes in the pistil following compatible and incompatible intraspecific crosses. J Cell Sci 36:1-18

Hollander M, A. Wolfe D, Chicken E (2015) Ranked Set Sampling. In: Hollander M, A. Wolfe D, Chicken E (eds) Nonparametric Statistical Methods. Wiley Series in Probability and Statistics, Third edition edn. John Wiley & Sons, Inc. , Hoboken, New Jersey, pp 676-743. doi:doi:10.1002/9781119196037.ch15

Iglesias DJ, Tadeo FR, Primo-Millo E, Talon M (2003) Fruit set dependence on carbohydrate availability in citrus trees. Tree Physiol 23 (3):199-204

Jacob D, Kottmeier C, Petersen J, Rechid D, Teichmann C (2017) Regionale klimamodellierung. In: Brasseur GP, Jacob D, Schuck-Zöller S (eds) Klimawandel in Deutschland: Entwicklung, Folgen, Risiken und Perspektiven. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 27-35. doi:10.1007/978-3-662-50397-3_4

Kramer PJ (1983) 2 - Cell Water Relations. In: Kramer PJ (ed) Water Relations of Plants. Academic Press, pp 23-56. doi:10.1016/B978-0-12-425040-6.50005-9

Kurokura T, Mimida N, Battey NH, Hytönen T (2013) The regulation of seasonal flowering in the Rosaceae. J Exp Bot 64 (14):4131-4141. doi:10.1093/jxb/ert233

Lang GA (1987) Dormancy - A new universal terminology. HortScience 22 (5):817-820

Luedeling E (2012) Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci Hortic 144:218-229. doi:10.1016/j.scienta.2012.07.011

Luedeling E (2019) chillR: Statistical methods for phenology analysis in temperate fruit trees. R package version 0.70.21

Luedeling E, Brown PH (2011) A global analysis of the comparability of winter chill models for fruit and nut trees. Int J Biometeorol 55 (3):411-421. doi:10.1007/s00484-010-0352-y

Luedeling E, Girvetz EH, Semenov MA, Brown PH (2011a) Climate change affects winter chill for temperate fruit and nut trees. PLoS One 6 (5):13. doi:10.1371/journal.pone.0020155

Luedeling E, Kunz A, Blanke M (2011b) Mehr Chilling für Obstbäume in wärmeren Wintern? Erwerbs-Obstbau 53 (4):145-155. doi:10.1007/s10341-011-0148-1

Luedeling E, Kunz A, Blanke MM (2013) Identification of chilling and heat requirements of cherry trees - A statistical approach. Int J Biometeorol 57 (5):679-689. doi:10.1007/s00484-012-0594-y

Martinez R, Legua P, Martinez-Nicolas JJ, Melgarejo P (2019) Phenological growth stages of 'Pero de Cehegin' (*Malus domestica* Borkh): Codification and description according to the BBCH scale. Sci Hortic 246:826-834. doi:10.1016/j.scienta.2018.11.067

Meier U (2001) Growth stages of mono-and dicotyledonous plants. BBCH Monograph, Second edn. Federal Biological Research Centre for Agriculture and Forestry, Oxford

Nakajima Y, Susanto S, Hasegawa K (1993) Influence of water-stress in autumn on flower induction and fruiting in young pomelo trees (*Citrus grandis* (L.) Osbeck). J Jpn Soc Hortic Sci 62 (1):15-20

Naor A, Naschitz S, Peres M, Gal Y (2008) Responses of apple fruit size to tree water status and crop load. Tree Physiol 28 (8):1255-1261. doi:10.1093/treephys/28.8.1255

Naschitz S, Naor A, Genish S, Wolf S, Goldschmidt EE (2010) Internal management of non-structural carbohydrate resources in apple leaves and branch wood under a broad range of sink and source manipulations. Tree Physiol 30 (6):715-727. doi:10.1093/treephys/tpq028

Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Koppen-Geiger climate classification. Hydrol Earth Syst Sci 11 (5):1633-1644. doi:10.5194/hess-11-1633-2007

Pimienta E, Polito VS (1982) Ovule abortion in 'Nonpareil' almond (*Prunus dulcis* [Mill.] D. A. Webb). Am J Bot 69 (6):913-920. doi:10.2307/2442888

Pope KS, Da Silva D, Brown PH, DeJong TM (2014) A biologically based approach to modeling spring phenology in temperate deciduous trees. Agric For Meteorol 198:15-23. doi:10.1016/j.agrformet.2014.07.009

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Version 3.5.3. Vienna, Austria

Rodrigo J, Herrero M (1998) Influence of intraovular reserves on ovule fate in apricot (*Prunus armeniaca* L.). Sex Plant Reprod 11:86-93. doi:10.1007/s004970050124

Rodrigo J, Herrero M, Hormaza JI (2009) Pistil traits and flower fate in apricot (*Prunus armeniaca*). Ann Appl Biol 154 (3):365-375. doi:10.1111/j.1744-7348.2008.00305.x

Rodrigo J, Hormaza JI, Herrero M (2000) Ovary starch reserves and flower development in apricot (*Prunus armeniaca*). Physiol Plant 108 (1):35-41. doi:10.1034/j.1399-3054.2000.108001035.x

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends Plant Sci 12 (5):217-223. doi:10.1016/j.tplants.2007.03.012

Ruiz R, García-Luis A, Monerri C, Guardiola JL (2001) Carbohydrate availability in relation to fruitlet abscission in Citrus. Ann Bot 87 (6):805-812. doi:10.1006/anbo.2001.1415

Sanyal D, Bangerth F (1998) Stress induced ethylene evolution and its possible relationship to auxin-transport, cytokinin levels, and flower bud induction in shoots of apple seedlings and bearing apple trees. Plant Growth Regul 24 (2):127-134. doi:10.1023/a:1005948918382

Shaltout AD, Unrath CR (1983) Rest completion prediction model for 'Starkrimson Delicious' apples. J Am Soc Hort Sci 108 (6):957-961

Sobeih WY, Dodd IC, Bacon MA, Grierson D, Davies WJ (2004) Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial root-zone drying. J Exp Bot 55 (407):2353-2363. doi:10.1093/jxb/erh204

Statistisches Bundesamt (2019) Areas and quantities harvested (production for the market). https://www.destatis.de/EN/Themes/Economic-Sectors-Enterprises/Agriculture-Forestry-Fisheries/Fruit-Vegetables-Horticulture/Tables/2-4-areas-quantities-harvested.html. Accessed 08 November 2019

Tukey JW (1970) Exploratory Data Analysis: Limited Preliminary Ed. Addison-Wesley Publishing Company,

Ullah A, Manghwar H, Shaban M, Khan AH, Akbar A, Ali U, Ali E, Fahad S (2018) Phytohormones enhanced drought tolerance in plants: A coping strategy. Environ Sci Pollut Res 25 (33):33103-33118. doi:10.1007/s11356-018-3364-5

Van Oldenborgh GJ, Collins M, Arblaster J, Christensen J, Marotzke J, Power S, Rummukainen M, Zhou T (2013) Annex I: Atlas of global and regional climate projections. In: Stocker TF, Qin D, Plattner G-K et al. (eds) Climate Change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Wilkie JD, Sedgley M, Olesen T (2008) Regulation of floral initiation in horticultural trees. J Exp Bot 59 (12):3215-3228. doi:10.1093/jxb/ern188

Zhang JL, Taylor C (2011) The Dynamic Model provides the best description of the chill process on 'Sirora' pistachio trees in Australia. HortScience 46 (3):420-425

Chapter 6

Topic-related collaborations

During my studies, I collaborated with national and international researchers in studying the physiology and architecture of walnut trees, as well as reviewing the current literature regarding the dormancy phase in temperate tree species. Additionally, I contributed to assessing the possible impacts of global warming on the cultivation of deciduous fruit trees in southern South America and mountain oases in northern Oman.

In the first study, I collaborated with researchers from the Pontificia Universidad Católica de Valparaíso in Chile and the Department of Plant Sciences of the University of California in Davis, USA. My co-authors and I evaluated the branching pattern of walnut trees in relation to light availability inside the canopy, as well as the relationship between parent wood and the formation of new shoots. Results showed that poor light environments in adult walnut trees increase the number of catkins on a shoot, reducing the probability of bearing fruits.

An internal collaboration with Dr. Erica Fadón and co-authors at the Horticultural Department of the University of Bonn yielded a review article published in 2020 in the journal Agronomy (MDPI). In this study, we proposed a conceptual framework covering four main physiological processes occurring during the progression of dormancy. These processes are internal transport, phytohormone dynamics, genetic and epigenetic regulation and carbohydrate dynamic. We linked such processes to changes in temperature and photoperiod during late autumn, winter and early spring.

Finally, I contributed to two research articles evaluating the possible impacts of climate change on the availability of winter chill under future scenarios in northern Patagonia, Argentina and mountain oases in northern Oman. For each site, my co-authors and I applied the methodology presented in chapters 3 and 4 of this thesis to generate historic and future temperature scenarios. We then estimated climate-related agricultural metrics such as chill (in Chill Portions) and heat (in Growing Degree Hours) availability, and the number of hours with freezing temperature (below 0 °C) in early spring. Based on the results and the current literature about temperate trees, we provide insights regarding risks and opportunities for the cultivation of deciduous species in southern South America and Omani oases.

In the following chapter, I reproduce the abstracts of these collaborations.

A network of shoots: effects of ontogeny and light availability on growth units in Chandler walnuts

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Abstract

Tree architecture results from the functioning of populations of meristems across consecutive periods of extension or growing cycles. The study of growth units (GUs) and the type of their axillary meristem lead to a better understanding of tree growth and allow the prediction of tree development under different environmental conditions. To study the effect of position inside the canopy on branch development and the relationship between parent wood and new shoots, 348 GUs from 14 five to six-year-old limbs (7 from upper/exposed and 7 from lower/shaded zones within the canopy) were analyzed. Each node was classified according to its axillary meristem type as: 0 (bud, dormant bud, bud scar and dead bud), 1 (fruit and fruit peduncle scar), 2 (male inflorescence or catkin, catkin scar and dead catkin) and 3 (branch and branch scar). Data were analyzed through a multinomial logistic model to summarize the distribution of axillary meristem type as a function of explanatory variables. Length of GUs and the number of catkins were modeled through a generalized linear mixed model, whereas the probability of finding fruit on apical nodes was analyzed through generalized additive models. Results show that axillary meristem type is affected by zone, node rank and wood age. On GUs of wood age two or younger, apical buds from upper/exposed zones showed higher probabilities of producing fruits compared with apical buds from lower/shaded zones. The latter was not observed for older wood. In upper/exposed zones, the slope of the relationship between the length of GUs and the length of parent wood increased with wood age. The number of catkins was higher in the lower/shaded than in the upper/exposed zone. The results of this study improve our understanding about walnut tree growth as affected by factors like ontogenetic changes and different light conditions within the tree canopy and might be useful to address tree managements such as training and pruning systems.

Keywords Axillary meristems · Branching pattern · Multinomial models · Canopy · Shade · Tree development · Walnut

A conceptual framework for winter dormancy in deciduous trees

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Abstract

The perennial life strategy of temperate trees relies on establishing a dormant stage during winter to survive unfavorable conditions. To overcome this dormant stage, trees require cool (i.e. chilling) temperatures as an environmental cue. Numerous approaches have tried to decipher the physiology of dormancy, but these efforts have usually remained relatively narrowly focused on particular regulatory or metabolic processes, recently integrated and linked by transcriptomic studies. This work aimed to synthesize existing knowledge on dormancy into a general conceptual framework to enhance dormancy comprehension. The proposed conceptual framework covers four physiological processes involved in dormancy progression: (i) transport at both whole-plant and cellular level, (ii) phytohormone dynamics, (iii) genetic and epigenetic regulation, and (iv) dynamics of nonstructural carbohydrates. We merged the regulatory levels into a seasonal framework integrating the environmental signals (i.e. temperature and photoperiod) that trigger each dormancy phase.

Keywords Phenology · Chilling · Cell-to-cell communication · Genetics · Carbohydrates · Phytohormones · Vascular transport · *DAM* genes

Climate change impacts on agriculture's southern frontier – Perspectives for farming in North Patagonia

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Abstract

Winter chill is expected to decrease in many of the suitable growing regions for deciduous trees. Argentinean North Patagonia hosts extensive fruit tree cultivation, which provides an important contribution to both local and global food security. Using historic records from 11 weather stations from North Patagonia, we evaluate the possible impacts of climate change on fruit tree cultivation. We assess winter chill and seasonal heat availability, and the risk of spring frost events based on outputs from 15 Global Climate Models (GCMs) for two Representative Concentration Pathway (RCP) scenarios and two future time periods (represented by central years 2050 and 2085). Metrics were estimated for 47 years of records from the weather stations, as well as typical conditions for 10 past scenarios and 60 future GCM and RCP projections. Scenarios consisted of 100 plausible annual temperature records produced by a weather generator. Results suggest that fruit tree dormancy in Argentinean North Patagonia will not be strongly affected by climate change. Compared to the past, winter chill may only decrease by 9% in the RCP4.5 scenario by 2050 in the northeastern and eastern subregion, while in the central-south and west the reduction seems unlikely to exceed 6% by the same RCP scenario and year. Our models project stable high growing season heat in the northeastern and eastern regions, and major increases in the south by 2085 in both RCP scenarios. Projections of spring frost events varied between 0 and about 25 hours below 0 °C depending on the site. Increasing heat availability may create opportunities for fruit and nut growers to introduce new species and cultivars to the region. Our results provide a basis for planning such introductions and for enabling growers to exploit new opportunities for producing temperate orchard crops beyond their traditional ranges.

Keywords Chill models · Chill requirement · Heat requirement · *Prunus* sp. · Spring frost risk · temperate trees

Revisiting climate change effects on winter chill in mountain oases of northern Oman

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Abstract

For centuries, traditional high-altitude oases in Oman have depended on the cultivation of deciduous fruit trees. This study explores the effects of climate change on winter chill (estimated as Chilling Hours—CH and Chill Portions—CP), a prerequisite to overcoming dormancy and initiating flowering, in three Omani oases. The results are compared with findings from an earlier study which reported a decrease in the numbers of CH in high-elevation oases by an average of 1.2–9.5 CH year^{-1} between 1983 and 2008. Location-specific weather data were obtained by merging 15 years of in situ recordings with 28 years of observations from an official weather station near the top of the investigated watershed. Between 1991 and 2018, scenarios of the past few decades show chill reductions by 75, 35 and 18% when estimated in CP at the oases of Masayrat ar Ruwajah (1030 m a.s.l.), Qasha' (1640 m a.s.l.), and Al 'Ayn (1900 m a.s.l.), respectively. Over the course of the twenty-first century, the lowest-elevation oasis at Masayrat ar Ruwajah is projected to lose virtually all winter chill, whereas, despite significant chill losses, conditions are expected to remain viable for some of the currently grown species in the higher-elevation oases. These projected changes will compromise the cultivation of temperate fruit trees in the near future, affecting the sustainability of Omani oases. Our methods support results from earlier work performed at these locations and provide an updated procedure for assessing climate change effects on temperature-dependent systems.

Keywords Arabia · Arid environments · Chill requirements · Fruit production · Global warming · Warm winters

Chapter 7

Conclusions

The main objective of the work that I have presented in these chapters was to study the dormancy phase of temperate fruit trees and assess the possible impacts of climate change on the cultivation of these species in temperate and Mediterranean climate areas. To this end, my co-authors and I developed a number of studies and collaborations based on field experiments as well as historic weather records in a three-year project.

The results of the works that I have presented in this thesis suggest that the cultivation of deciduous fruit species is likely to face major challenges regarding the dormancy phase in the near future, especially in Mediterranean climate areas. In these regions, global warming will decrease the availability of winter chill, a key factor determining the success of temperate fruit orchards. Decreasing winter chill in Mediterranean climate areas, such as the major production zone of Chile, might represent moderate to severe losses for the temperate fruit industry. Under such scenarios, farmers are likely to observe a reduction in marketable yield due to irregular and dispersed budburst as well as decreasing budburst rates. In places where the current levels of winter chill are adequate to promote dormancy release, strategies to mitigate the impacts of warm winters may become critical to ensure acceptable yields. In regions where the temperature to date is challenging the production of temperate fruit, the development and use of new cultivars that require very low exposure to chill conditions during winter to overcome dormancy can offer a strategy to farmers to adapt to the impacts of future warming.

Together with decreasing winter chill in some regions of the world, changes in climate are likely to produce rising temperatures during critical periods of tree development, generating hazardous scenarios. For instance, warmer conditions during late winter or early spring in cool temperate climates, may lead to advances in bloom dates, increasing the risk of spring frost events. Along the same lines, heat waves occurring during the period of bud initiation and differentiation are likely to affect the development of several deciduous fruit species. However, warmer conditions during spring and summer due to changes in climate may open opportunities for the cultivation of temperate species in places where the current conditions are unfavorable for tree development. When using current likely dates of bloom for sweet cherry trees, the analysis that my co-authors and I performed for a temperate region of Chile suggests an important decrease in the risk of spring frost events. Nonetheless, further research is still needed to clarify the possible impacts of warming during spring on bloom dates of temperate trees across the climatic gradient where deciduous trees are grown. This research is likely to improve our understanding on the risks associated with warmer temperatures during late winter and early spring.

Changes in climate are likely to affect other agricultural parameters together with winter chill and heat availability. Drought periods, which are rarely observed in places where the normal precipitation is currently capable to support the production of temperate fruits, may become more frequent in the future and therefore motivate farmers to adopt strategies to prevent negative impacts on fruit and tree development. Such impacts have been widely studied in the past regarding immediate effects (i.e. in the same growing season). However, the implications of drought periods during summer on bud development in the medium term (i.e. next spring) remains mostly unknown. The results of this thesis might indicate an under-studied risk of the impacts of unusual drought events on dormancy and subsequent bud phenology. Further research, however, is still required to validate these results, as well as to understand the mechanisms involved in such a relationship. Nonetheless, accounting for the impacts of dry spells during summer as well as warm winters may be necessary for accurately predicting the phenology of temperate trees under climate change.

To accurately predict the impacts of climate change on tree and bud phenology, scientists, farmers and practitioners must use mathematical approximations describing the relationship between the environment and the behavior of buds. In this regard, most models have focused on describing the processes of chill and heat accumulation, as well as bud phenology. The results of the studies presented in this thesis suggest that dormancy model development must be a key factor in the development of the temperate fruit industry as well as the strategies to adapt orchards to new climatic conditions. Currently available options for estimating the accumulation of chill during dormancy only rely on temperature as input without including any biological parameters, despite the evidence generated in the last 30 years of dormancy-related research. A process-based model might greatly improve our estimations of chill accumulation as well as accurately forecast future chill levels.

Without major advances in dormancy modelling, farmers and orchard managers are likely to continue using approaches that were developed long ago. This is worrying since the comparative study I reported on in chapter 4 revealed that most of the variation observed when estimating chill availability is generated by the chill model, regardless of the site and future scenario. This highlights the need for a concerted effort to generate an updated model as well as the importance of chill model selection for estimating chill availability for managing present orchards and planning for future plantings. The Dynamic model was among the least sensitive in Mediterranean regions and projected only minor chill increases in temperate regions. Although this model is widely considered the most credible currently available, it may also present some important drawbacks. On the one hand, its use among farmers is still restricted due to the complex model structure. On the other hand, the parameters currently used by the Dynamic model correspond to those reported by the authors in the original publication after implementing dormancy-related experiments on peach trees in Israel. Short-term efforts might focus on generating user-friendly technologies to facilitate the use of the Dynamic model in orchard planning and management as well as on a species- and probably site-specific re-parameterization. Improving the Dynamic model as well as other approaches might represent an advance in dormancy research, although the main effort must focus on taking a process-based approach to develop new model structures.

Dormancy is a phase of tree development that is characterized by the absence of any visible growth in a bud or in other structures of the plant. This trait makes dormancy modelling a challenging and complex task, especially under climate change. While current available modelling approaches only use temperature as input, renewed efforts must include key biological aspects occurring during the entire phase. The results of the work I have presented in this thesis reveal that carbohydrate dynamics are closely related to chill accumulation. However, given the experimental setup, it is difficult to decipher whether variations in starch and hexoses concentrations are a response to cold tolerance or a specific signaling/response pathway of dormancy. This may partially explain the inconsistencies that we observed across varieties when carbohydrates were used as predictors of budburst time. Future research may test this on other molecules or processes already described during dormancy progression. Additionally, considering possible interactions between the diverse set of biological aspects involved in tree dormancy might be useful in developing biologically valid dormancy models. An integrative approach is likely to improve our ability to describe the entire process, and therefore help practitioners face the effects of climate change on the dormancy of temperate trees. To achieve this, a collaborative research effort, with a diverse group of scientists working on different aspects of dormancy, is required.

Overall, the work that I have presented in this thesis improves the collective knowledge on some of the key biological mechanisms that occur during dormancy progression. The results demonstrate that dormancy is a complex process that is likely to be affected by a number of co-occurring sub-processes. Additionally, results demonstrated the possible impacts of climate change (i.e. warm winters and unusual summer drought) on winter chill availability as well as on apple bud phenology, and highlighted the importance of chill model selection. The results and methods developed here have also been useful for guiding additional experiments and studies, to continue improving the state of knowledge on this topic. Among these are the studies projecting climate-related metrics under future climate scenarios in Argentina and Oman, as well as a review of the current literature on dormancy in collaboration with Dr. Erica Fadón and co-authors. I expect the results of this thesis to be helpful for scientists studying the dormancy phase, plant breeders developing new cultivars, and farmers and orchard managers cultivating temperate trees. Together, these studies provide new insights on methods for forecasting climate-related metrics, the possible risks of climate change, and the cultivation of temperate fruit species in temperate and Mediterranean climate areas.

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Annex

Supplementary material for Chapter 4

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This document provides additional information for chapter 4 ‘The importance of chill model selection – A multi-site analysis.’

Additional information on the filling procedure

We used auxiliary weather stations to fill the gaps in the main sources of weather information. This procedure requires computation of quality check metrics including the average difference for minimum and maximum temperatures between the primary and secondary weather stations. Similarly, it requires computing the difference across weather records in the standard deviation of minimum and maximum temperature observations. The following table (supplementary table 4-1) shows a summary for those variables as well as for the mean distance and elevation difference relative to the main weather station.

Supplementary table 4-1. Quality check information used to fill the gaps in the main sources of weather information for all primary and secondary weather stations

Country[+]	Site	Variable	Temperature bias (°C)*		Mean distance (km)†	Mean elevation difference (m)††
			Mean	Sd		
Chile	Quillota	Tmax	0.43	3.92	53	-352
		Tmin	-1.15	2.90		
	Curicó	Tmax	-0.95	2.00	9	5
		Tmin	-0.54	1.20		
	Chillán	Tmax	-1.18	2.28	12	-82
		Tmin	0.41	1.52		
Tunisia	Ben Arous	Tmax	0.67	2.34	106	-127
		Tmin	0.71	2.19		
	Sfax	Tmax	-0.01	2.97	153	-125
		Tmin	-0.16	2.66		
	Mellita	Tmax	-0.01	3.02	179	-157
		Tmin	1.52	2.77		

[+] Not applicable for sites in Germany since complete minimum and maximum temperature records were available for the whole period

* Temperature bias indicates the mean and standard deviation for minimum (Tmin) and maximum (Tmax) temperatures

† Mean distance shows the mean distance between auxiliary sources of data relative to the main weather station

†† Mean elevation difference column represents the mean elevation difference of auxiliary sources of data relative to the main weather station

Additional information about the chill models used in this study

All models which are not contained within the chillR package are available in a public repository at https://github.com/EduardoFernandezC/dormancyR

Dynamic model

The Dynamic model (Erez et al. 1990; Fishman et al. 1987a, b) has emerged as the most plausible model from several comparisons. Its advantage is that it posses a more physiological approach as compared to earlier models. This model was developed for use in warm winter climates such as Israel and South Africa. It postulates that chill is accumulated in a two-step process in which cold temperatures lead to the formation of an intermediate product. Once a certain amount of this product has been accumulated, it can be transformed into a Chill Portion (CP), by a process that requires relatively warm temperatures. The equations to compute Chill Portions from a dataset of hourly temperatures are more complex than those of the other models. Here we present a summary of functions adapted from Luedeling et al. (2009).

$$x_i = \frac{e^{slp*tetmlt*\left(\frac{T_k - tetmlt}{T_k}\right)}}{1 + e^{slp*tetmlt*\left(\frac{T_k - tetmlt}{T_k}\right)}}$$

$$x_s = \frac{a_0}{a_1} * e^{\frac{(e_1 - e_0)}{T_k}}$$

$$ak_1 = a_1 * e^{-\left(\frac{e_1}{T_k}\right)}$$

$$inter_E = x_s - (x_s - inter_s) * e^{-ak_1}$$

$$inter_S = \begin{cases} 0 & if\ t = t_0 \\ inter_{E_{t-1}} & if\ t > t_0 \wedge inter_{E_{t-1}} < 1 \\ inter_{E_{t-1}} * (1 - x_i) & if\ t > t_0 \wedge inter_{E_{t-1}} \geq 1 \end{cases}$$

$$delt = \begin{cases} 0 & if\ t = t_0 \\ 0 & if\ t > t_0 \wedge inter_E < 1 \\ x_i * inter_E & if\ t > t_0 \wedge inter_E \geq 1 \end{cases}$$

$$chill\ portions_t = \begin{cases} delt & if\ t = t_0 \\ delt + chill\ portions_{t-1} & if\ t \geq t_0 \end{cases}$$

Where:
slp = 1.6
tetmlt = 277
a_0 = 139,500
$a_1 = 2.567 \times 10^{18}$
e_0 = 12,888.8
e_1 = 4153.5
T_k = temperature in Kelvin
t = time during the season in hours
t_0 = starting point for chill accumulation

Chilling hours model

The Chilling Hours model, reported by Bennett (1949) and Weinberger (1950), gained prominence when it was used to study the dormancy period of peaches in Fort Valley, Georgia, USA. This model considers that all temperatures between 0 and 7.2 °C are effective to overcome dormancy. Specifically, and using hourly records, this model computes the total number of hours in which temperatures (T) fall within the range of interest. The metric used by this model is Chilling Hours (CH).

$$CH = \begin{cases} 1 & if\ \ 0\,°C < T < 7.2\,°C \\ 0 & if\ \ T < 0\,°C\ or\ T > 7.2\,°C \end{cases}$$

Utah model

The Utah model (Richardson et al. 1974) uses a concept to describe chill accumulation similar to that in the Chilling Hours model. The difference is that this model assigns varying effectiveness weights (Chill Units–CU per hour) depending on observed temperature. Another important trait of this model is the addition of negative contributions to chill accumulation for warm temperatures. This model was developed to study the dormancy period of Redhaven and Elberta peach trees. The function requires hourly temperature records as input (T).

$$CU = \begin{cases} 0 & if \quad T \leq 1.4\,°C \\ 0.5 & if \quad 1.4\,°C < T \leq 2.4\,°C \\ 1 & if \quad 2.4\,°C < T \leq 9.1\,°C \\ 0.5 & if \quad 9.1\,°C < T \leq 12.4\,°C \\ 0 & if \quad 12.4\,°C < T \leq 15.9\,°C \\ -0.5 & if \quad 15.9\,°C < T \leq 18.0\,°C \\ -1 & if \quad T > 18.0\,°C \end{cases}$$

Positive Utah model

The Positive Utah model (Linsley-Noakes et al. 1994) was developed to obtain a user friendly way to estimate winter chill and to improve the accuracy of the Utah model when used in warm winter zones of the South African fruit growing areas. This model takes into account concepts of the Dynamic model, avoiding the necessity of complex programming skills to estimate Chill Portions. Unlike the Utah model, in which temperature variations (negating high temperatures) have a carry-over effect on chill from one day to the next, the Positive Utah model considers only positive Chill Units (PCU). The Positive Utah model uses hourly temperature data as input (T).

$$PCU = \begin{cases} 0 & if\ T \leq 1.4\,°C \\ 0.5 & if\ 1.4\,°C < T \leq 2.4\,°C \\ 1 & if\ 2.4\,°C < T \leq 9.1\,°C \\ 0.5 & if\ 9.1\,°C < T \leq 12.4\,°C \\ 0 & if\ T > 12.4\,°C \end{cases}$$

North Carolina model

The North Carolina model (Shaltout and Unrath 1983) uses the same structure and concepts as the Utah model. It assigns chill contribution to temperatures below 0 °C and considers greater chill negation (up to a chill effectiveness weight of -2 CU per hour) for temperatures above 20.7 °C. These traits have possibly reduced the applicability in warm winter areas. This model, which uses hourly temperature records as input (T), was developed to study dormancy on apple trees in North Carolina, USA.

$$CU = \begin{cases} 0 & if \quad T \leq -1.1\,°C \\ 0.5 & if \quad -1.1\,°C < T \leq 1.6\,°C \\ 1.0 & if \quad 1.6\,°C < T \leq 7.2\,°C \\ 0.5 & if \quad 7.2\,°C < T \leq 13.0\,°C \\ 0 & if \quad 13.0\,°C < T \leq 16.5\,°C \\ -0.5 & if \quad 16.5\,°C < T \leq 19.0\,°C \\ -1.0 & if \quad 19.0\,°C < T \leq 20.7\,°C \\ -1.5 & if \quad 20.7\,°C < T \leq 22.1\,°C \\ -2.0 & if \quad T > 22.1\,°C \end{cases}$$

Modified Utah model

The Modified Utah model was developed by Linvill (1990) working on peach trees in Pontiac, South Carolina. Unlike the original approach, this model uses a continuous function instead of hard thresholds between steps. Chill Units (CU) are estimated from hourly temperatures (T), following a sinusoidal shape between two cut points (0 and 21 °C). This implies that positive values (≤1) are obtained for temperatures between 0 and 14 °C, reaching a maximum accumulation at 7 °C. Negative contributions (> -1) are assigned for temperatures between 14 and 21°C.

$$CU = \begin{cases} 0 & if\ T \leq 0\,°C \\ \sin\left(\frac{2\pi T}{28}\right) & if\ 0\,°C < T \leq 21\,°C \\ -1 & if\ T > 21\,°C \end{cases}$$

Low Chill model

The Low Chill model (Gilreath and Buchanan 1981) was developed to predict bud burst for 1-year-old rooted cuttings of Sungold nectarine plants. This model uses the same structure and concept of chill negation for warm temperatures as the original Utah model. The most significant modifications are the shift of the optimal temperatures for chill accumulation to between 7.9 and 13.9 °C and the shift for the threshold of chill negation to 19.4 °C. Chill Units (CU) are computed from hourly temperature records (T).

$$CU = \begin{cases} 0 & if \quad T \leq -1.0\,°C \\ 0.5 & if \quad -1.0\,°C < T \leq 1.8\,°C \\ 1 & if \quad 1.8\,°C < T \leq 8.0\,°C \\ 0.5 & if \quad 8.0\,°C < T \leq 14.0\,°C \\ 0 & if \quad 14.0\,°C < T \leq 17.0\,°C \\ -0.5 & if \quad 17.0\,°C < T \leq 19.5\,°C \\ -1 & if \quad T > 19.5\,°C \end{cases}$$

Chill Days model

The Chill Days model (Cesaraccio et al. 2004) was developed to estimate chill requirements and predict bud burst in temperate forest and fruit tree species in Italy. This sequential model uses the accumulation of Chill Days (CD) to break rest and the accumulation of Anti-Chill Days (CA) to overcome quiescence (defined as a separate stage during dormancy). Chill and Anti-Chill days depend on the use of a temperature threshold and a chill requirement. Both are specific for each species and cultivar. This model uses daily minimum and maximum temperature data as input. Originally, this model computes chill accumulation as a negative value. We instead used the absolute value in order to clarify and compare results between models.

$$CD = \begin{cases} 0 & if \;\; 0\,°C \le T_c \le T_n \le T_x \\ \left[(T_M - T_n) - \dfrac{(T_x - T_c)^2}{2(T_x - T_n)}\right] & if \;\; 0\,°C \le T_n \le T_c < T_x \\ (T_M - T_n) & if \;\; 0\,°C \le T_n \le T_x \le T_c \\ \left[\dfrac{T_x^2}{2(T_x - T_n)}\right] & if \;\; T_n < 0\,°C \le T_x \le T_c \\ \left[\dfrac{T_x^2}{2(T_x - T_n)}\right] - \left[\dfrac{(T_x - T_c)^2}{2(T_x - T_n)}\right] & if \;\; T_n < 0\,°C < T_c < T_x \end{cases}$$

Where:
T_c = temperature threshold
T_n = minimum daily temperature
T_x = maximum daily temperature
T_M = mean daily temperature

We used the mean thresholds for all fruit species and varieties reported in the original paper. These species and varieties were pears (cv. Butirra, cv. Coscia, cv. Precoce, cv. S. Maria), kiwifruit (cv. Hayward) and cherry (cv. Burlat, cv. Moreau, cv. D. Osini, cv. Comune, cv. Forli, cv. Ferrovia, cv. Marracocca). The respective thresholds were 7.0, 6.8, 6.9 and 7.0 °C for pears, 7.9 °C for kiwifruit and 7.5, 7.0, 7.0, 7.3, 7.1, 7.1 and 7.2 °C for cherries.

Chilling Rate function

This model is part of a larger set of models used to predict the beginning of apple blossom in Germany (Chmielewski et al. 2011). Specifically, this chill function is used in a sequential model, which considers both the state of chilling and the state of forcing. The Rate of Chilling (RC) is computed according to equations proposed by Hänninen (1990), with some modifications. In this model, the thresholds were modified to 0.0 °C for Tmin and 10.0 °C for Tmax. As inputs, the model requires the use of daily mean temperature (T) and the base temperature for chilling T_B (T_B = 4.2 °C, as was reported by the authors in the original document).

$$RC = \begin{cases} 0 & if \;\; T \le 0.0\,°C \; or \; T \ge 10.0\,°C \\ \dfrac{T}{T_B} & if \;\; 0.0\,°C < T < T_B \\ \dfrac{T - 10.0}{T_B - 10.0} & if \;\; T_B < T < 10.0\,°C \end{cases}$$

Triangular and Exponential Chill functions from Legave et al. (2013)

These functions, proposed in Legave et al. (2013), are part of a wide range of functions used to compute chill and to predict flowering time of apple trees in Europe. In the phenological model, all functions are coupled with a heat sub-model in a sequential structure. We selected those chill sub-models that were identified by the authors as the best options for predicting F1 phenological stage dates in apple trees. These chill sub-models were the Triangular Chill function (TCFL) and the Exponential Chill function (ECF). Following recommendations by the authors, we set the parameters of the Exponential Chill function to T_C (specific temperature) = 15 °C while for the Triangular Chill function the parameters T_C (optimal temperature) and I_C (temperature interval of efficiency around T_C) were set to 1 and 24 °C, respectively. In this version of the models, ECF uses maximum daily records as input whereas TCFL uses mean daily records as source of data.

$$CF = \begin{cases} 0 & if \quad T_C - I_C \geq T_{mean} \geq T_C + I_C \\ 1 - \left(\dfrac{|T_{mean} - T_C|}{I_C}\right) & if \quad T_C - I_C < T_{mean} < T_C + I_C \end{cases}$$

$$CF = \exp\left(-\frac{T_{max}}{T_C}\right)$$

Chilling Units function

Some efforts on modeling dormancy release in Douglas-fir plants have been made by Harrington et al. (2010). These authors used chilling and forcing functions to predict bud burst dates of forest plants. Chilling units (Cu) are computed by the following function:

$$Cu = \begin{cases} 0 & if \quad T < -4.66\,°C \; or \; T > 16\,°C \\ 3.13 * \left(\dfrac{T + 4.66}{10.93}\right)^{2.10} * e^{-\left(\frac{T+4.66}{10.93}\right)^{3.10}} & if \quad 4.66\,°C \leq T \leq 16\,°C \\ 1 & if \quad Cu > 1 \end{cases}$$

Where T is the hourly temperature and e is the base of the natural logarithm.

Triangular Chill Function from Hänninen (1990)

Another important effort for modeling bud burst dates of forest trees was reported by Hänninen (1990). We present his function to compute the rate of chill (Rc) developed for Finnish forest tree species. This function uses daily mean records and expresses daily chill accumulation.

$$Rc = \begin{cases} 0 & if \quad T \leq -3.4\,°C \\ 0.159 * T + 0.506 & if \quad -3.4\,°C < T \leq 3.5\,°C \\ -0.159 * T + 1.621 & if \quad 3.5\,°C < T \leq 10.4\,°C \\ 0 & if \quad T > 10.4\,°C \end{cases}$$

Analysis of variance on the dataset

We performed an ANOVA to determine the effect size among the factors site, scenario, chill model and climate model (supplementary table 4-2). Results showed that most of the variation (i.e. sum of squares among factors) is explained by site and chill model (46.5 and 52.3%). On the contrary, the factors scenario and climate model represented 0.7 and 0.5% of the sum of squares among factors.

Supplementary table 4-2. Summary of the analysis of variance applied to the dataset generated by this study

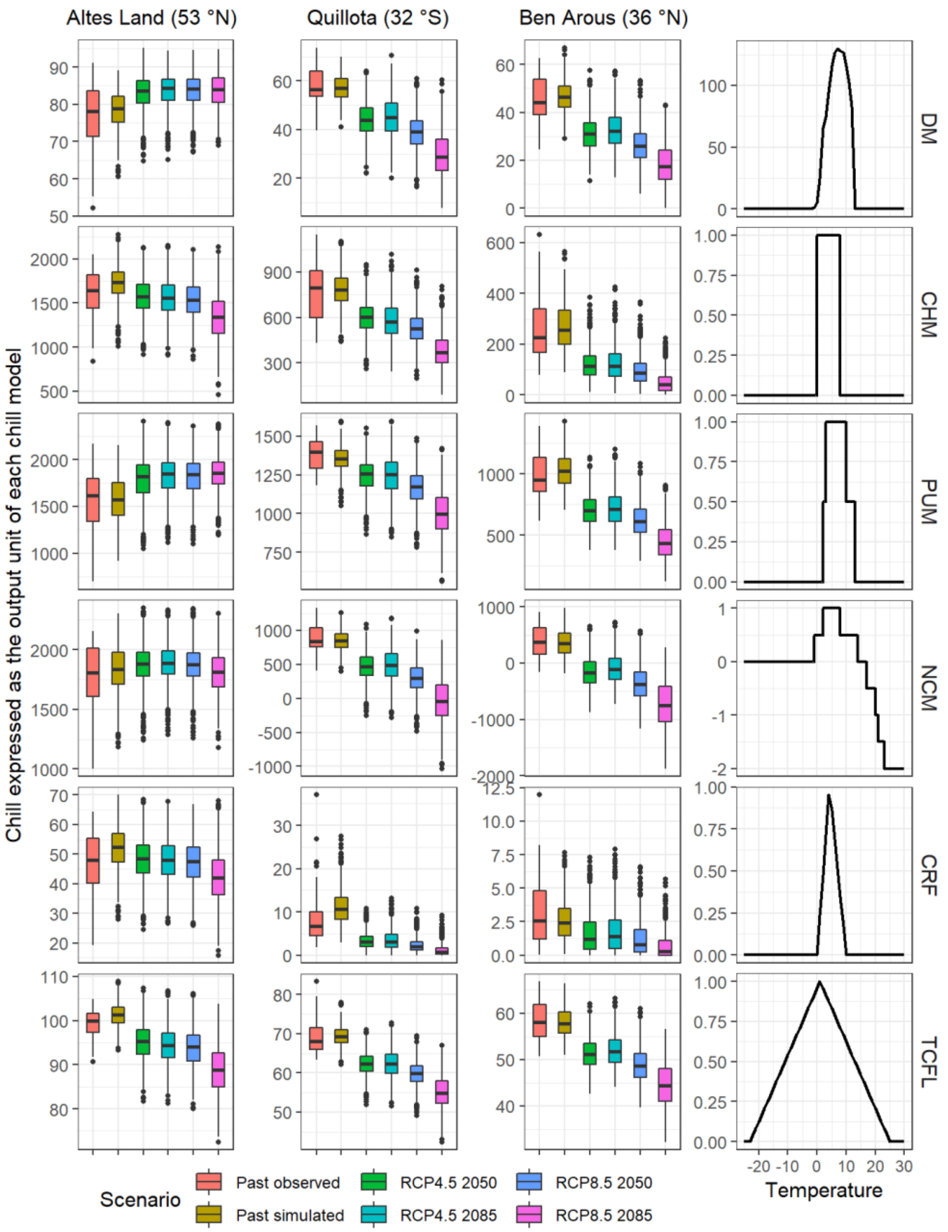
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	8	228274	28534	87478.8	<2e-16
Scenario	3	3404	1135	3478.4	<2e-16
Climate Model	14	2151	154	471.1	<2e-16
Chill Model	12	256637	21386	65565.3	<2e-16
Residuals	704302	229733	229733	0	

Additional figures

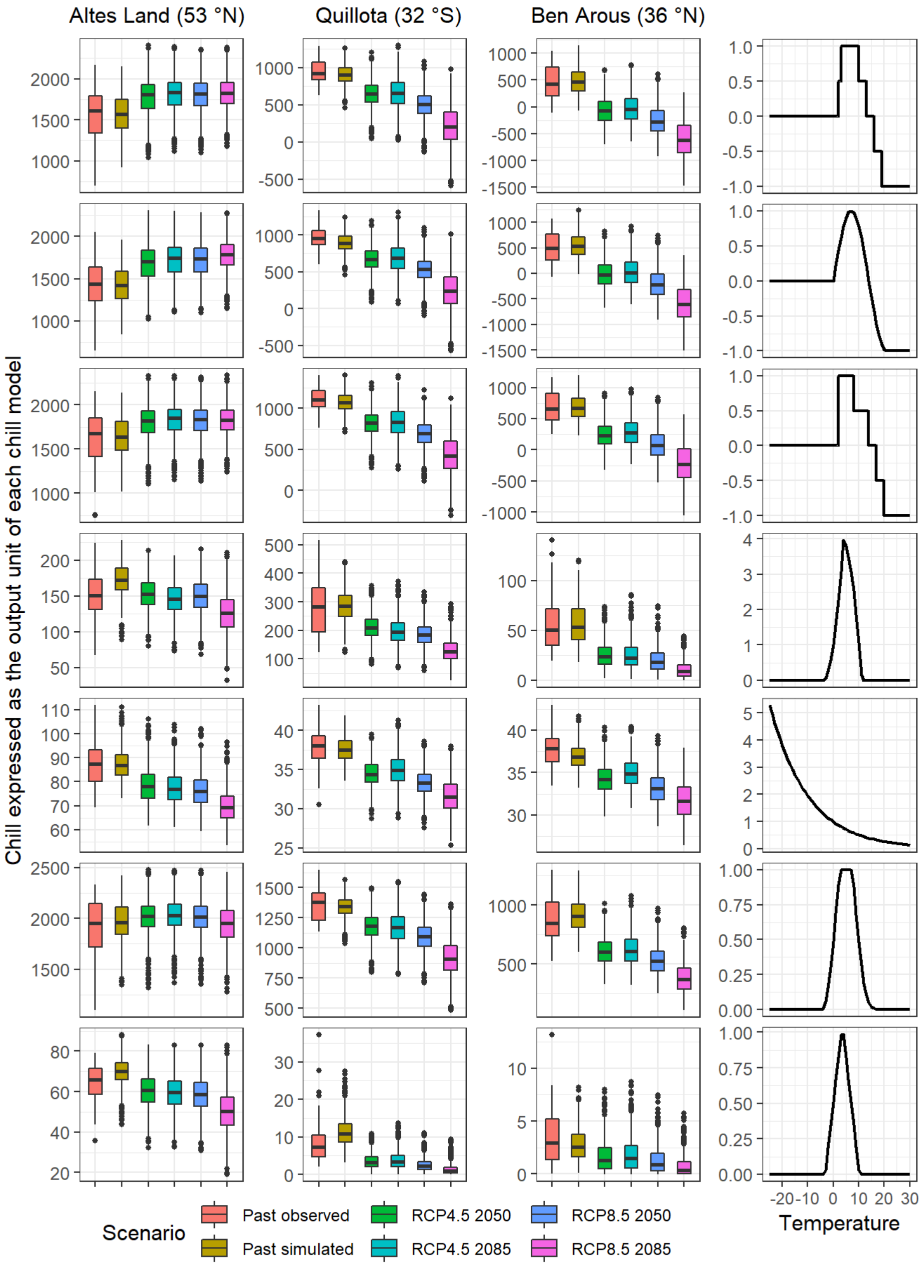
Chill model comparison for remaining sites

As we showed in the main chapter, the remaining Mediterranean climate sites (Quillota, Chillán, Ben Arous and Sfax) are consistently projected to experience chill decrease in future scenarios for all chill models (supplementary fig. 4-1 – 4-4). Conversely, for sites in Germany (Altes Land and Rhineland) our future projections varied according to the chill model used. Dynamic, Utah, Modified Utah, Positive Utah and Low Chill models tended to predict more chill by 2085 in the RCP4.5 scenario compared to the past in Altes Land and Rhineland. The North Carolina and Chilling function (CFH) models projected stable chill in future scenarios while the rest of the models tended to forecast varying degrees of chill reduction in the warmest evaluated scenario (RPC8.5 by 2085).

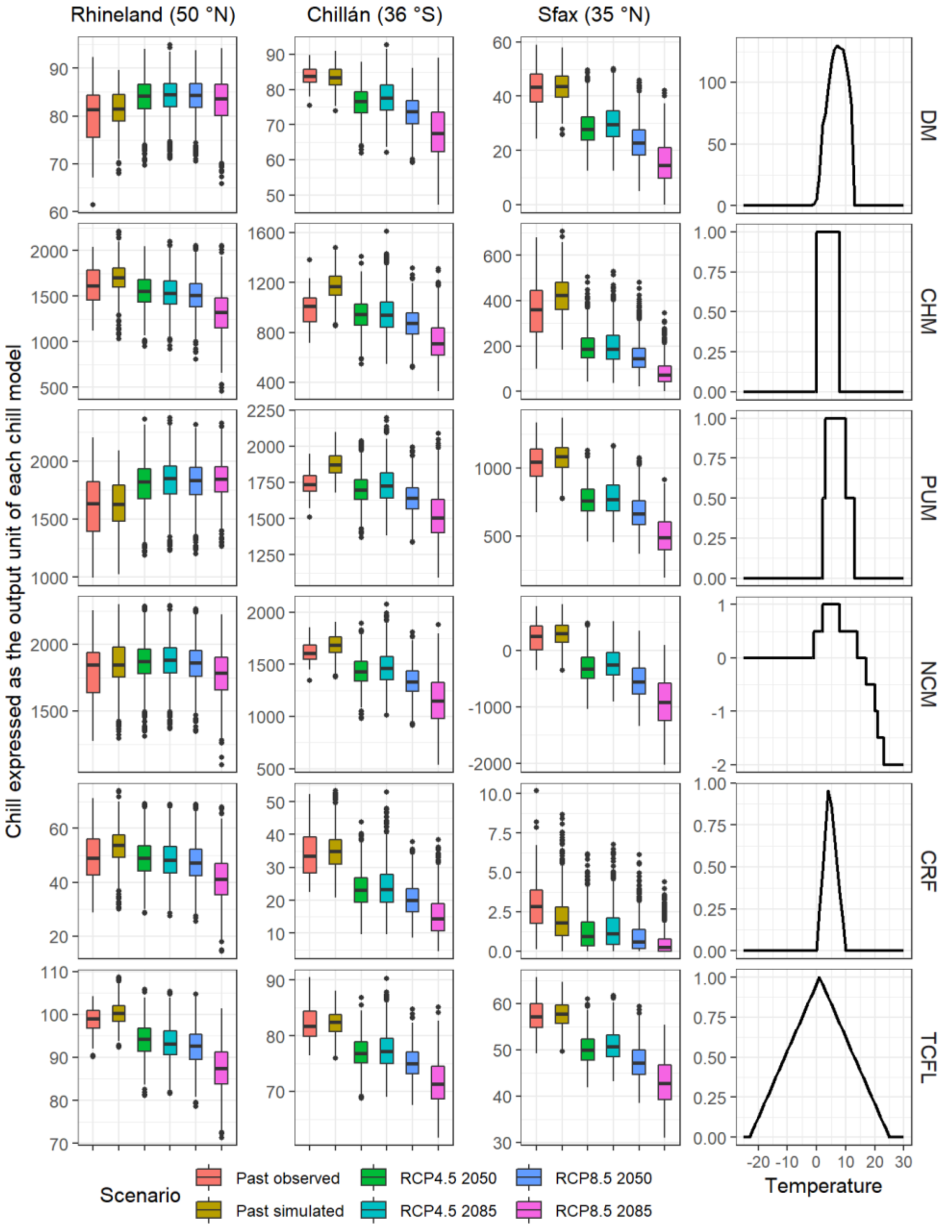
Models considering the chill negation effect due to warm temperatures estimated negative future chill levels in some sites of Chile and Tunisia. Whereas in Tunisia (Ben Arous and Sfax) and one site in Chile (Quillota) NCM, UM and MUM computed negative values for past scenarios, no model showed levels of chill below zero in Chillán.



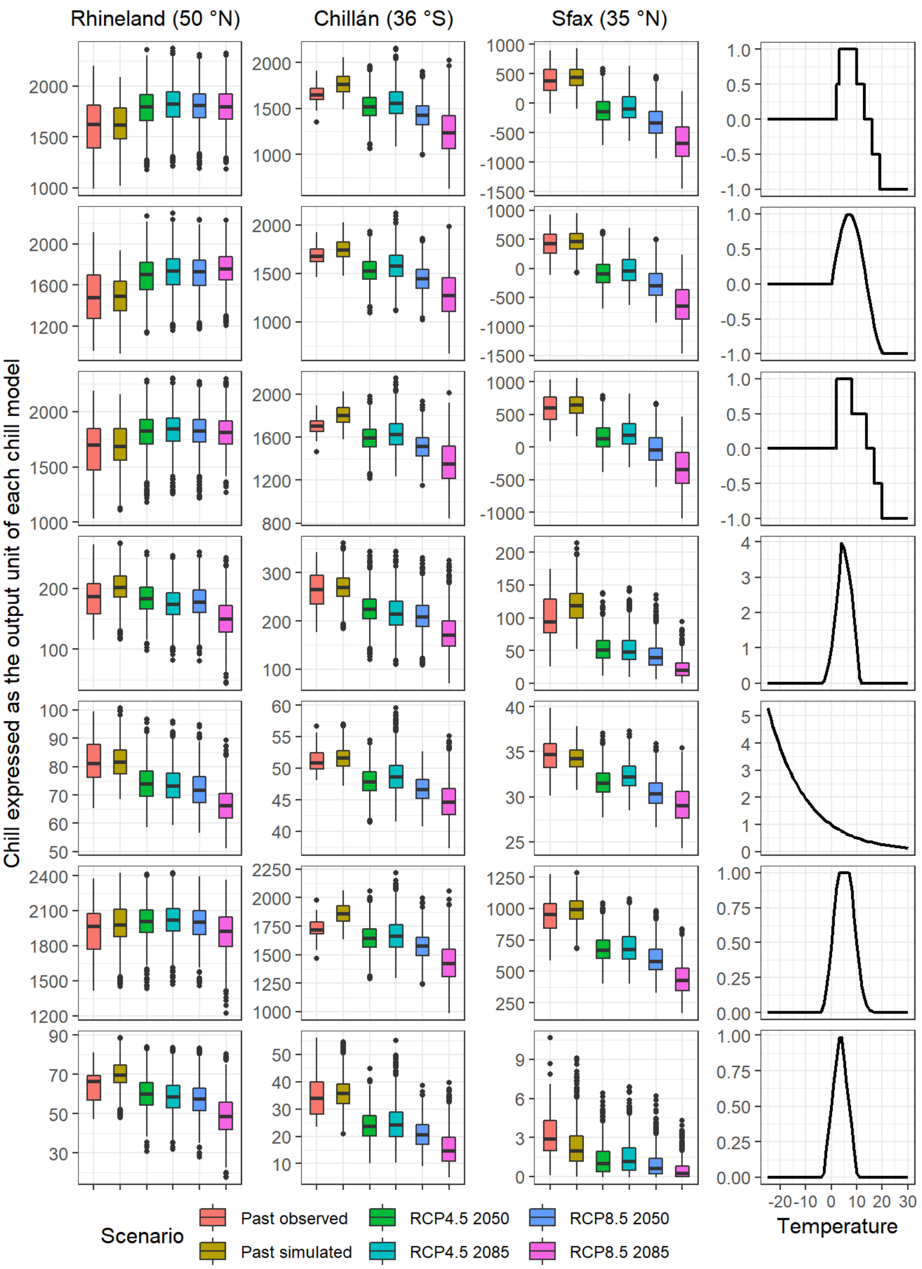
Supplementary figure 4-1. Winter chill estimations from six chill models (rows) at Altes Land (Germany), Quillota (Chile) and Ben Arous (Tunisia) (columns) for six past and future scenarios (boxplots). Chill models were: DM = Dynamic model, CHM = Chilling Hours model, PUM = Positive Utah model, NCM = North Carolina model, CRF = Chilling Rate function and TCFL = Triangular Chill function. Among the scenarios, past observed corresponds to the actual estimations of chill computed from the weather data (n = 45). Past simulated corresponds to chill calculations from the data generated as described in section Historic weather scenarios of chapter 4 (n = 1,000). RCP4.5 and RCP8.5 correspond to the possible future scenarios of total radiative forcing according to IPCC (2014), while 2050 and 2085 represent the year used as central year for each RCP scenario (n = 1,500 each). The column to the right shows chill effectiveness of chill models (y) for a range of temperatures (x). CHM, PUM and NCM use a step function while the remaining models use a continuous function. Effectiveness is expressed as the output unit of each model. In TCFL and CRF, temperature corresponds to the daily mean, whereas for DM, CHM, PUM and NCM, temperature corresponds to the hourly temperature. The DM does not contain a simple weight function. For this model, the effectiveness curve shows chill accumulated after 100 continuous hours at the specified temperature



Supplementary figure 4-2. Winter chill estimations from seven chill models (rows) at Altes Land (Germany), Quillota (Chile) and Ben Arous (Tunisia) (columns) for six past and future scenarios (boxplots). Chill models were: UM = Utah model, MUM = Modified Utah model, LCM = Low Chill model, CDM = Chill Days model, ECF = Exponential Chill function, CFH = Chilling function and TCFH = Triangular Chill function. Among the scenarios, past observed corresponds to the actual estimations of chill computed from the weather data (n = 45). Past simulated corresponds to chill calculations from the data generated as described in section Historic weather scenario of chapter 4 (n = 1,000). RCP4.5 and RCP8.5 correspond to the possible future scenarios of total radiative forcing according to IPCC (2014), while 2050 and 2085 represent the year used as central year for each RCP scenario (n = 1,500 each). The column to the right shows chill effectiveness of chill models (y) for a range of temperatures (x). UM and LCM use a step function while the remaining models use a continuous function. Effectiveness is expressed as the output unit of each model. In CDM, ECF and TCFH, temperature corresponds to daily records (mean, maximum and mean, respectively), whereas for UM, MUM, LCM and CFH, temperature corresponds to the hourly temperature



Supplementary figure 4-3. Winter chill estimations from six chill models (rows) at Rhineland (Germany), Chillán (Chile) and Sfax (Tunisia) (columns) for six past and future scenarios (boxplots). Chill models were: DM = Dynamic model, CHM = Chilling Hours model, PUM = Positive Utah model, NCM = North Carolina model, CRF = Chilling Rate function and TCFL = Triangular Chill function. Among the scenarios, past observed corresponds to the actual estimations of chill computed from the weather data (n = 45). Past simulated corresponds to chill calculations from the data generated as described in section Historic weather scenarios of chapter 4 (n = 1,000). RCP4.5 and RCP8.5 correspond to the possible future scenarios of total radiative forcing according to IPCC (2014), while 2050 and 2085 represent the year used as central year for each RCP scenario (n = 1,500 each). The column to the right shows chill effectiveness of chill models (y) for a range of temperatures (x). CHM, PUM and NCM use a step function while the remaining models use a continuous function. Effectiveness is expressed as the output unit of each model. In TCFL and CRF, temperature corresponds to the daily mean, whereas for DM, CHM, PUM and NCM, temperature corresponds to the hourly temperature. The DM does not contain a simple weight function. For this model, the effectiveness curve shows chill accumulated after 100 continuous hours at the specified temperature

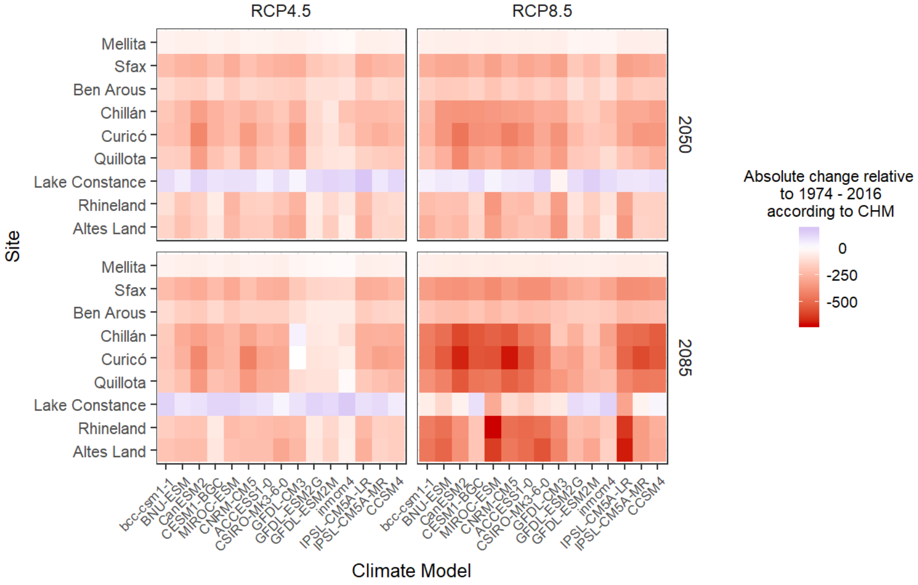


Supplementary figure 4-4. Winter chill estimations from seven chill models (rows) at Rhineland (Germany), Chillán (Chile) and Sfax (Tunisia) (columns) for six past and future scenarios (boxplots). Chill models were: UM = Utah model, MUM = Modified Utah model, LCM = Low Chill model, CDM = Chill Days model, ECF = Exponential Chill function, CFH = Chilling function and TCFH = Triangular Chill function. Among the scenarios, past observed corresponds to the actual estimations of chill computed from the weather data (n = 45). Past simulated corresponds to chill calculations from the data generated as described in section Historic weather scenario of chapter 4 (n = 1,000). RCP4.5 and RCP8.5 correspond to the possible future scenarios of total radiative forcing according to IPCC (2014), while 2050 and 2085 represent the year used as central year for each RCP scenario (n = 1,500 each). The column to the right shows chill effectiveness of chill models (y) for a range of temperatures (x). UM and LCM use a step function while the remaining models use a continuous function. Effectiveness is expressed as the output unit of each model. In CDM, ECF and TCFH, temperature corresponds to daily records (mean, maximum and mean, respectively), whereas for UM, MUM, LCM and CFH, temperature corresponds to the hourly temperature

Absolute change for remaining chill models

Chilling hours model

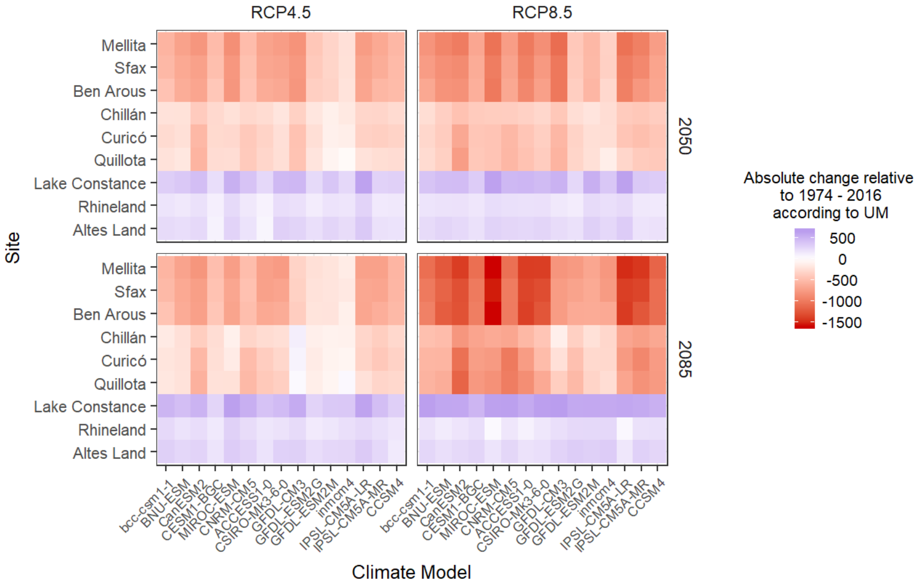
The Chilling Hours model (CHM) projected absolute chill decreases in most of the sites evaluated and for most of the climate models used (supplementary fig. 4-5). Only at Lake Constance, this chill model estimated increases by 2085 in the RCP4.5 scenario. Sites in Chile are expected to lose between 250 to 700 CH by 2085 in the RCP8.5 scenario. In Mellita and Ben Arous (Tunisia), by the same RCP scenario and time horizon, the Chilling Hours model forecasted only minor absolute chill reductions.



Supplementary figure 4-5. Absolute chill changes (in Chilling Hours) relative to the past period (1974-2016) estimated with the Chilling Hours model (CHM) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

Utah model

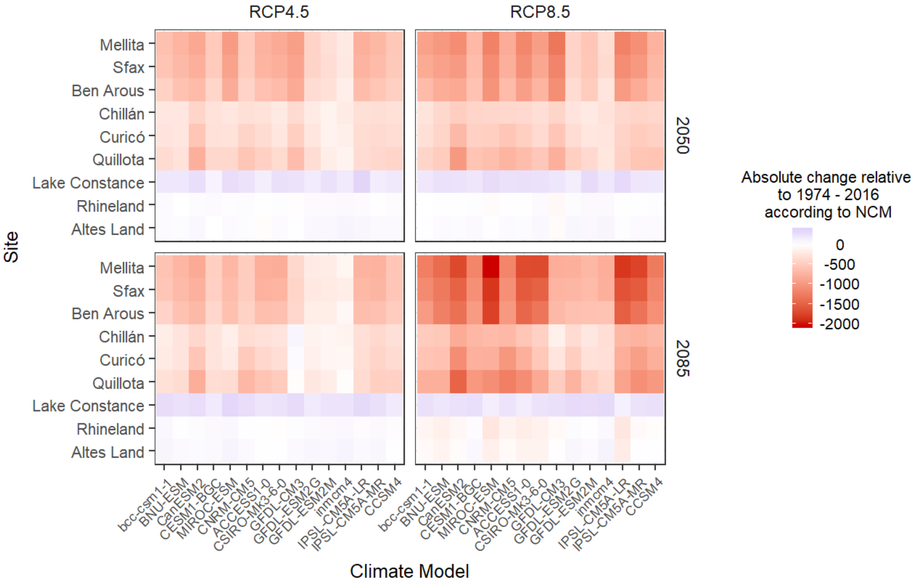
The Utah model (UM) estimated substantial chill increase for sites in Germany (about 400 CU), which varied according to the climate model used (supplementary fig. 4-6). The Lake Constance growing region is expected to gain the greatest amount of chill by 2085 in the RCP8.5 scenario. Mediterranean climate sites (i.e. Tunisian sites) show reductions up to 1,500 CU in the warmest future scenario.



Supplementary figure 4-6. Absolute chill changes (in Chill Units) relative to the past period (1974-2016) estimated with the Utah model (UM) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

North Carolina model

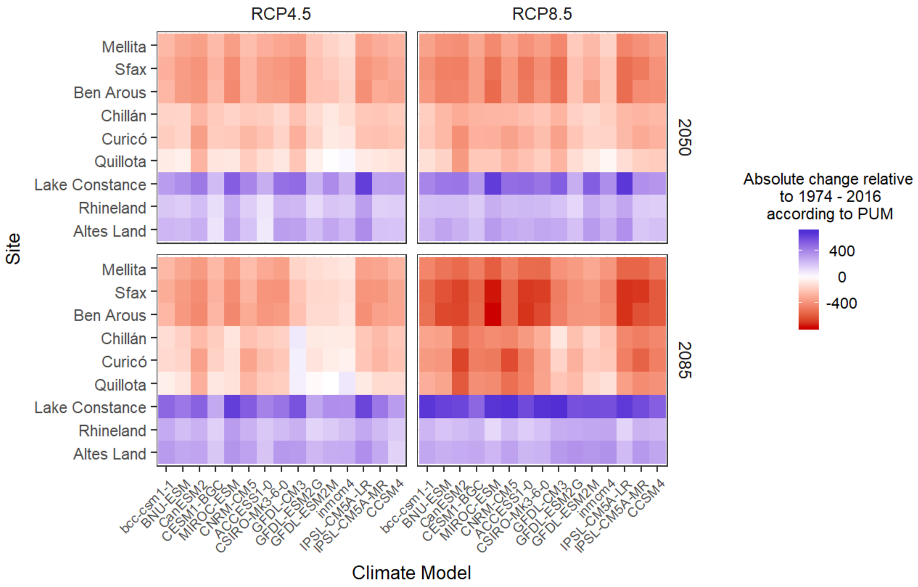
At Lake Constance (Germany), the North Carolina model (NCM) forecasted minor absolute chill increases compared to the past while in the remaining sites in Germany (Rhineland and Altes Land) the projection shows changes near zero in chill levels for future scenarios (supplementary fig. 4-7). Greatest absolute reductions were projected in Mediterranean climate sites such as those in central Chile and Tunisia reaching values up to 2,000 CU.



Supplementary figure 4-7. Absolute chill changes (in Chill Units) relative to the past period (1974-2016) estimated with the North Carolina model (NCM) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

Positive Utah model

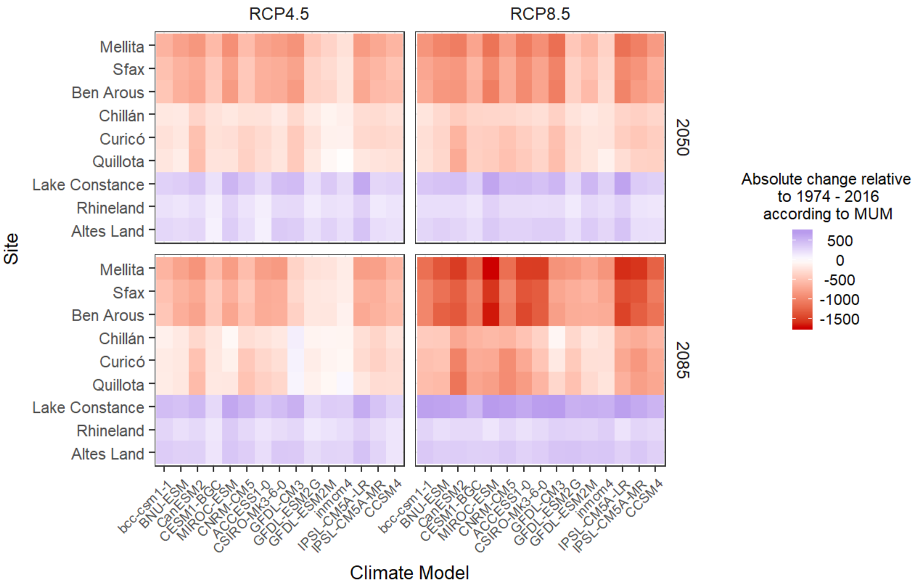
Similar to results observed in other models that are based on the Utah Model, the Positive Utah Model (PUM) shows that sites in Germany are expected to gain chill in the warmest future scenario evaluated. At Lake Constance changes in winter chill expected by 2085 in the RCP8.5 scenario reached values up to 400 PCU (supplementary fig. 4-8). Consistently, Tunisian and Chilean sites showed the greatest negative change. However, in Quillota by 2050 in the RCP4.5 scenario, expected changes are less severe than those forecasted for other sites.



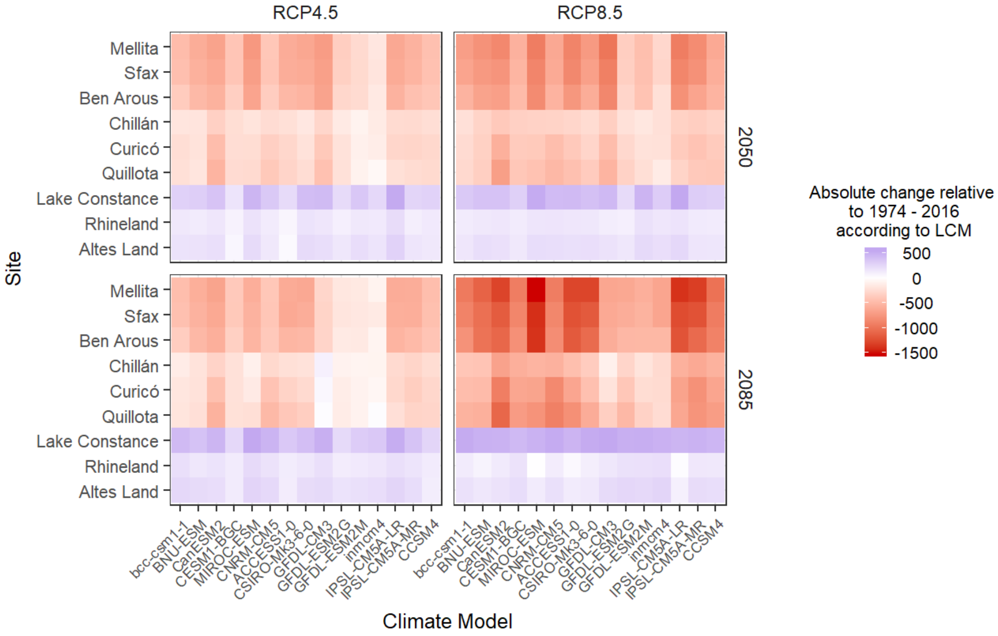
Supplementary figure 4-8. Absolute chill changes (in Positive Chill Units) relative to the past period (1974-2016) estimated with the Positive Utah model (PUM) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

Modified Utah model and Low Chill model

The Modified Utah model (MUM) and Low Chill model (LCM) projected similar amounts of chill among sites (supplementary fig. 4-9 – 4-10). Sites in Germany are expected to experience an increase in chill for future scenarios while the remaining sites are projected to reach substantial chill reduction in the near future (by 2050 in the RCP4.5 and RCP8.5 scenario). Sites in Tunisia show greater reductions compared to sites in Chile.



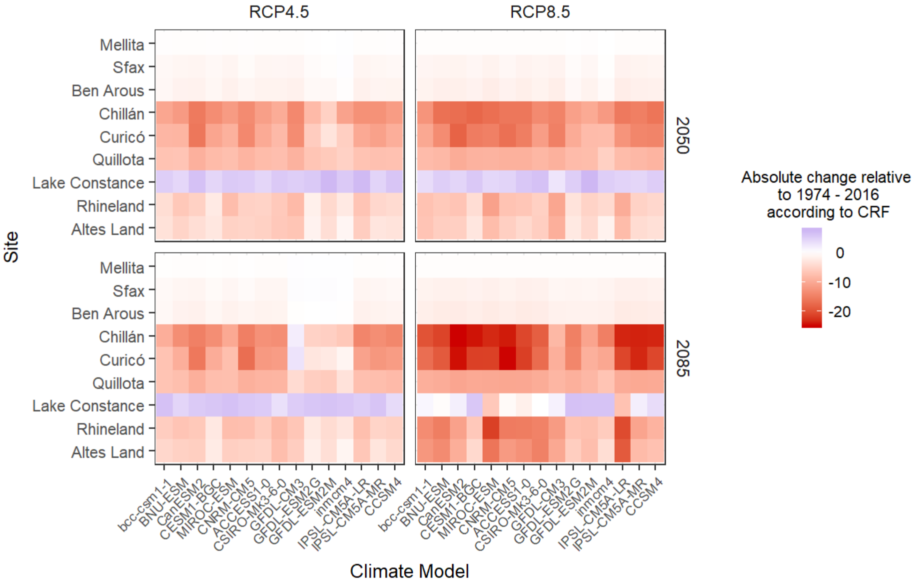
Supplementary figure 4-9. Absolute chill changes (in Chill Units) relative to the past period (1974-2016) estimated with the Modified Utah model (MUM) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models



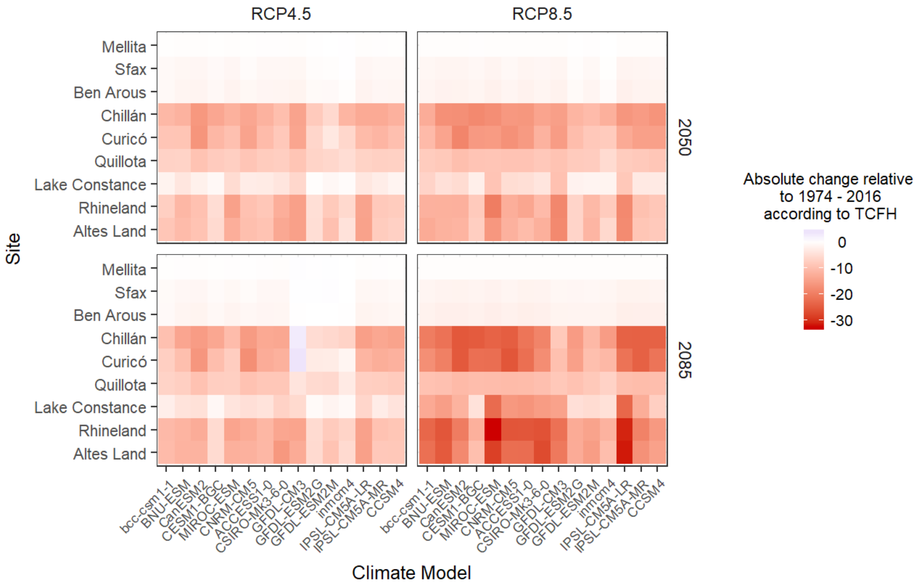
Supplementary figure 4-10. Absolute chill changes (in Chill Units) relative to the past period (1974-2016) estimated with the Low Chill model (LCM) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

Chilling rate function and Triangular chill function

The Chilling rate function (CRF) and Triangular chill function (TCFH) models forecasted changes near zero for sites in Tunisia (supplementary fig. 4-11 – 4-12). That situation might be explained by the extremely low values (about 0) computed by these models for past and future scenarios. Only in the Lake Constance growing area and when using the Chilling Rate function, estimated change represented a minor increase in chill accumulation for most of the possible future scenarios. TCFH estimated chill reduction in all sites of Chile and Germany.



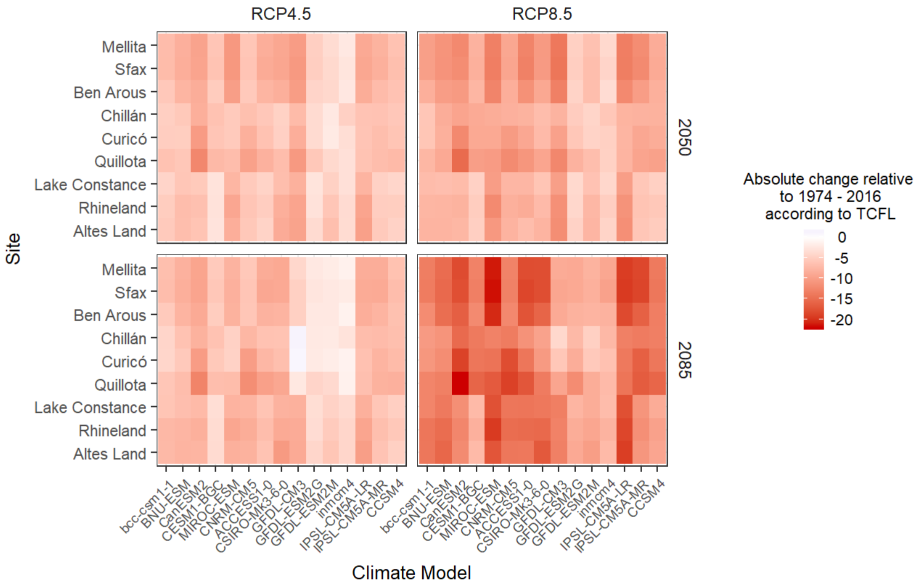
Supplementary figure 4-11. Absolute chill changes (in Rate of Chilling) relative to the past period (1974-2016) estimated with the Chilling Rate function (CRF) model for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models



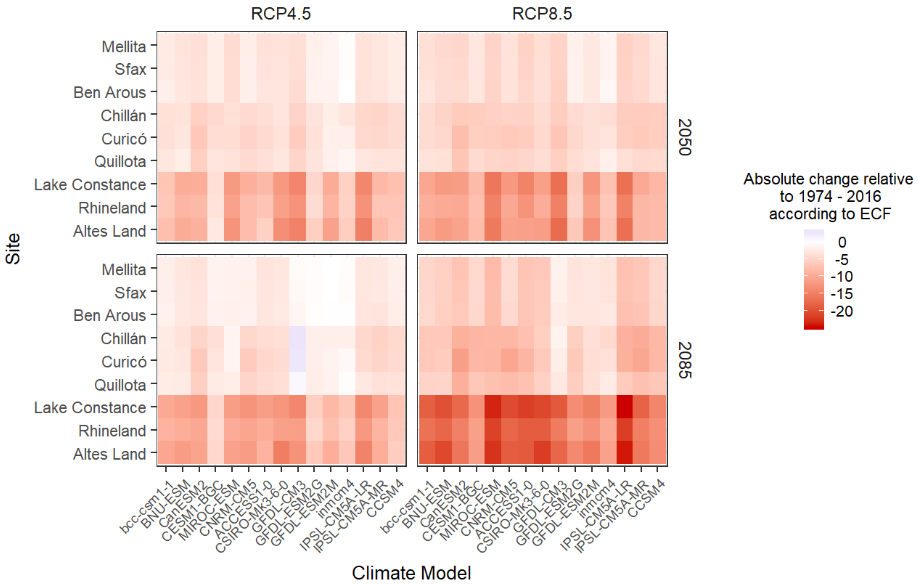
Supplementary figure 4-12. Absolute chill changes (in Rate of Chill) relative to the past period (1974-2016) estimated with the Triangular chill function model (TCFH) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

Triangular chill function and Exponential chill function

The Triangular chill function (TCFL) and Exponential chill function (ECF) consistently estimated lower chill levels for future scenarios in all places evaluated compared to the past (supplementary fig. 13 – 14). The TCFL projected similar reductions across sites while the ECF forecasted the greatest decreases for Altes Land, Rhineland and Lake Constance. Results from these sites reached changes up to -25 Chill Function units by 2085 in the RCP8.5 scenario.



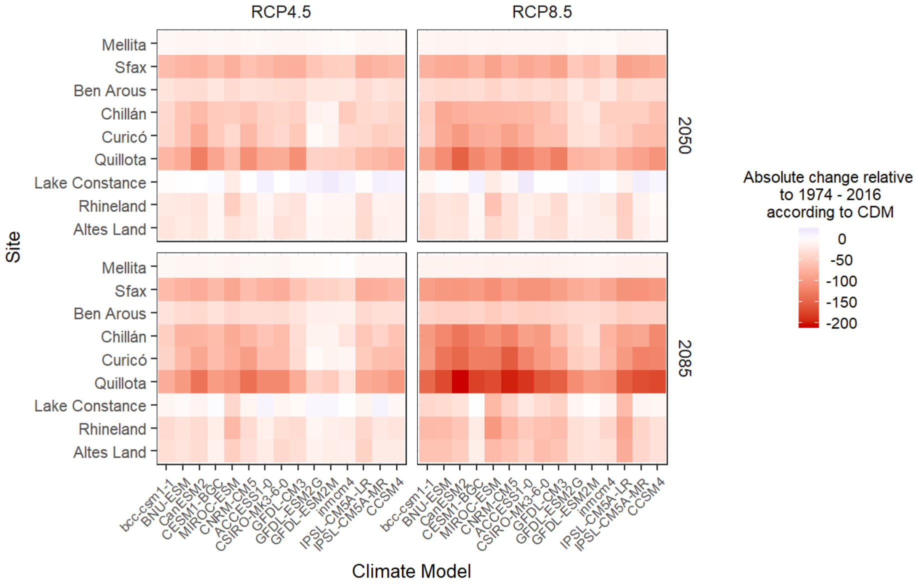
Supplementary figure 4-13. Absolute chill changes (in Chill Function units) relative to the past period (1974-2016) estimated with the Triangular Chill function model (TCFL) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models



Supplementary figure 4-14. Absolute chill changes (in Chill Function units) relative to the past period (1974-2016) estimated with the Exponential Chill function model (ECF) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

Chill Days model

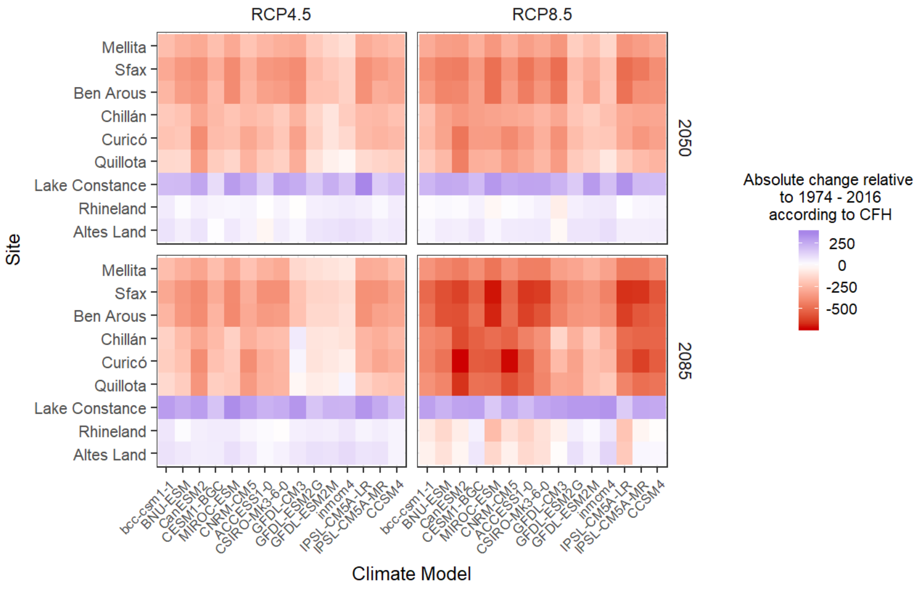
Similar absolute changes were computed with the Chill Days model (CDM) in Mellita, Ben Arous and Lake Constance among future scenarios (supplementary fig. 4-15). Expected changes reached between 0 and -50 Chill Days by 2085 in the RCP8.5 scenario. However, minor increases were forecasted at Lake Constance for the near future (2050) by some climate models. The greatest reduction was projected in Quillota (Chile) and Sfax (Tunisia).



Supplementary figure 4-15. Absolute chill changes (in Chill Days) relative to the past period (1974-2016) estimated with the Chill Days model (CDM) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

Chilling function

Substantial reductions were forecasted by the Chilling function (CFH) in Mediterranean climate sites such as those in Chile and Tunisia for any possible future scenario (supplementary fig. 4-16). Reduction in Sfax is expected to reach about 250 Chilling units. The greatest increase was projected in Lake Constance for the RCP8.5 scenario. The remaining sites in Germany showed an increase for the RCP4.5 scenario by 2050 and 2085 and for the RCP8.5 scenario by 2050. By 2085 in the RCP8.5 scenario those sites showed a future chill reduction between 0 to 200 Chilling units.



Supplementary figure 4-16. Absolute chill changes (in Chilling units) relative to the past period (1974-2016) estimated with the Chilling function model (CFH) for sites in Tunisia, Chile and Germany for two RCP scenarios, two time horizons (2050 and 2085) and 15 climate models

References

Bennett JP (1949) Temperature and bud rest period. Calif Agric 3 (11):9-12

Cesaraccio C, Spano D, Snyder RL, Duce P (2004) Chilling and forcing model to predict bud-burst of crop and forest species. Agric For Meteorol 126 (1-2):1-13. doi:10.1016/j.agrformet.2004.03.002

Chmielewski FM, Blumel K, Henniges Y, Blanke M, Weber RWS, Zoth M (2011) Phenological models for the beginning of apple blossom in Germany. Meteorol Z 20 (5):487-496. doi:10.1127/0941-2948/2011/0258

Erez A, Fishman S, Linsley-Noakes GC, Allan P (1990) The dynamic model for rest completion in peach buds. Acta Hort 276:165-174

Fishman S, Erez A, Couvillon GA (1987a) The temperature-dependence of dormancy breaking in plants - Computer-simulation of processes studied under controlled temperatures. J Theor Biol 126 (3):309-321. doi:10.1016/s0022-5193(87)80237-0

Fishman S, Erez A, Couvillon GA (1987b) The temperature-dependence of dormancy breaking in plants - Mathematical-analysis of a 2-step model involving a cooperative transition. J Theor Biol 124 (4):473-483. doi:10.1016/s0022-5193(87)80221-7

Gilreath PR, Buchanan DW (1981) Rest prediction model for low-chilling 'Sungold' nectarine. J Am Soc Hort Sci 106 (4):426-429

Hänninen H (1990) Modelling bud dormancy release in trees from cool and temperate regions. Acta For Fenn 213:1 - 47. doi:10.14214/aff.7660

Harrington CA, Gould PJ, St Clair JB (2010) Modeling the effects of winter environment on dormancy release of Douglas-fir. For Ecol Manage 259 (4):798-808. doi:10.1016/j.foreco.2009.06.018

IPCC (2014) Climate Change 2014: Synthesis report. Contributions of working groups I, II and III to the fifth assesment report of the Intergovernmental Panel on Climate Change IPCC, Geneva, Switzerland

Legave JM, Blanke M, Christen D, Giovannini D, Mathieu V, Oger R (2013) A comprehensive overview of the spatial and temporal variability of apple bud dormancy release and blooming phenology in Western Europe. Int J Biometeorol 57 (2):317-331. doi:10.1007/s00484-012-0551-9

Linsley-Noakes G, Allan P, Matthee G (1994) Modification of rest completion prediction models for improved accuracy in South African stone fruit orchards. J S Afr Soc Hort Sci 4:13-15

Linvill DE (1990) Calculating chilling hours and chill units from daily maximum and minimum temperature observations. HortScience 25 (1):14-16

Luedeling E, Zhang MH, Luedeling V, Girvetz EH (2009) Sensitivity of winter chill models for fruit and nut trees to climatic changes expected in California's Central Valley. Agric Ecosyst Environ 133 (1-2):23-31. doi:10.1016/j.agee.2009.04.016

Richardson EA, Seeley SD, Walker DR (1974) A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. HortScience 1:331-332

Shaltout AD, Unrath CR (1983) Rest completion prediction model for 'Starkrimson Delicious' apples. J Am Soc Hort Sci 108 (6):957-961

Weinberger JH (1950) Chilling requirements of peach varieties. Proc Am Soc Hortic Sci 56:122-128