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Effects of warmer winters due to climate change on chilling and dormancy release of sweet cherry

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Effects of warmer winters due to climate change on chilling and dormancy release of sweet cherry

The main objective was to contribute to dormancy research by one the one hand investigating the chilling and forcing requirements of sweet cherry trees in one of the major fruit growing regions in Germany and on the other hand to investigate the changes in carbohydrate levels and water relations of cherry buds to identify and distinguish the dormancy phases on a plant physiological base, and to determine transition points between dormancy stages. To quantify the requirements at an entire tree level under natural photoperiod rather than single buds or cut shoots in dark cold chambers, 160 potted sweet cherry trees of three varieties with a very wide range of chilling requirements (3-fold) were raised over two years to initiate uniform flower buds before applying 24 chilling regimes (8 regimes per variety) per year followed by forcing to determine the effect on flowering. This expense was needed to see real flower behaviour of the entire fruit tree. To assess future scenarios for fruit growing (e.g. global warming), potted trees where placed in an unheated greenhouse with an average of ca. 2°C temperature increase. To assess the carbohydrate fluxes ca. 10,000 flower buds from nine varieties and bi-weekly sampling over two years were sampled. The results can be summarized as follows:

1. When applying the three chilling models presented to calculate winter chilling in a cherry orchard at Klein-Altendorf, Meckenheim, Germany, to an unheated greenhouse (average temperature increase by ca. 2°C) to simulate global warming, in the unheated greenhouse, the available chilling increased by 12 % (Chill Units - CU), 15% (Chill Portions - CP) and 20% (Chilling Hours - CH), respectively. In climate change predictions for other locations, especially in the South of Europe or in the North of Africa, the warmer winter temperatures often induce lack of chilling. Options and limitations of countermeasures in terms of cultivation methods such as microclimate manipulation, rest breaking agents, change of orchard location to a higher altitude and breeding are options to counter possible lack of chilling.
2. The Meckenheim fruit growing region in Western Germany may be affected as a consequence of climate change and lack of chilling. The orchard temperatures in the warmer winter (6.0°C) exceeded those in the unheated greenhouse (4.7°C) in the cold winter. Maximum chill accumulation in very warm winters at this location is currently achieved, so that even warmer winters may reduce the available chill, but there will be still enough to grow high chilling varieties. Chilling computations for this region, with all three major chilling models, showed that cherry trees of low chill cultivar ‘6000CZ’ required 22.3-26.6 (CP), 465-684 (CU) or 402-483 (CH), medium chill cv. ‘Brooks’ about 37.9-54.4 CP, 819-1,267 CU, or 779-941 CH and the high chill cv. ’Schneiders späte Knorpelkirsche’ about 54.4-79.3 CP, 1,267-1,696 CU, 941-1,494 CH, respectively, for a natural flowering. Cherry trees of cv. ‘6000CZ’ receiving <300 CH, cv. ‘Brooks’ <500 CH and cv. ‘Schneiders späte Knorpelkirsche’ <700 CH were unable to flower, equivalent of 50% of the assumed chilling optimum of the respective cultivar. The beginning of leaf drop was identified as a suitable initiation point for computing chill accumulation.
3. Four transition points were proposed to clearly distinguish dormancy phases by relating carbohydrate and relative water content (RWC) in reproductive buds to concomitant chilling fulfilment. Further, two groups of cherry varieties were defined based on their different initial sorbitol and starch level in the autumn. The first separation between para- and (deep) d-endo-dormancy is characterized as a transition from a decrease (variety group 1) or a constant level (variety group 2) to a sharp increase in hexoses (glucose and fructose), sorbitol and a drop of starch content. The second transition point (d-endo- to f-endo-dormancy) is characterized as the changes in both hexoses (increase) and starch (decrease) terminate, ca. 650 Chilling Hours (CH), i.e. insufficient chilling as measured in the concomitant forcing experiment. This third transition point (f-endo- to eco-dormancy) was characterized by ca. 1,000 CH, the minimum chilling requirement and restrained flowering (cut branches). The fourth transition point (forcing initiation) marked an increase in water content at ca. 1,550 CH, optimum chilling for cherry and coincided with natural flowering. A ratio of hexoses to starch content (<2:1) appeared to be a potential indicator of the beginning of chilling (para-dormancy); a ratio of 14-20:1 typical for endo-dormancy, whereas the release from dormancy was associated with a decline to less than 10:1 at the end of winter (eco-dormancy).
4. The effects of more forcing due to diminishing available chill as a result of climate change the possibility of substitution of chilling by forcing were investigated. In the scenarios applied, *minimum* chill fulfilment ranged from 400 CH (Chilling Hours) in low chill, 550 CH in medium chill and 750 CH in the high chill variety with maximum forcing of ca. 11,000 Growing Degree Hours (GDH) for low, ca. 12,000 GDH for medium and ca. 13,000 GDH for high chill varieties for sufficient flowering. With *optimum* chill, the optimum forcing was ca. 8,000 GDH (>12°C), irrespective of variety, allowing upscaling of the results to possibly other varieties. Trees exposed to excess chilling (150%) required less forcing (ca. 4,000 GDH) to reach full bloom. Hence, chilling can compensate for up to half of the required forcing, i.e. ca 4,000 GDH. Ratios of forcing to chilling were computed for future comparisons, which ensure flowering in the orchard. Slightly negative temperatures (-5°C to 0°C), which are presently exempt in the common chilling models, contributed to chilling accumulation of the fruit trees. Overall, the results have shown that diminishing chilling as a result of climate change can be compensated for, in part (up to 50%), by a larger amount of forcing to obtain natural flowering in the orchard.

Effekte von warmen Wintern, verursacht durch den Klimawandel, im Bezug auf Dormanz Beginn und Ende bei Süßkirschen

Ziel der Arbeit war es, einen Beitrag zur Dormanz-Forschung zu leisten, indem erstens das Kälte (Chilling)- und Wärmebedürfnis von Süßkirschen in einer der wichtigsten Obstanbauregionen in Deutschland untersucht wurde und zweitens die Veränderungen der Kohlenhydrat - und Wasserhaushaltverhältnisse in Kirschknospen während der Dormanz zu bestimmen. Um die Kälte- und Wärmebedürfnisse an intakten Bäumen, unter natürlicher Photoperiode anstelle von einzelnen Knospen oder abgeschnittenen Ästen in dunklen Kühlkammern zu quantifizieren, wurden drei Sorten mit insgesamt 160 mindestens drei Jahre alten Süßkirschenbäumen in 35-L Containern, mit einem breit gefächertem Kältebedürfnis (3-fach) 24 Kälteszenarien (acht Szenarien pro Sorte) pro Jahr untersucht, gefolgt von einer Wärmeperiode zur Bestimmung des Blühverhaltens. Um Auswirkungen des Klimawandels auf den Obstbau zu simulieren, wurden zudem Containerbäume in ein ungeheiztes Gewächshaus mit einer um ca. 2°C erhöhten Durchschnittstemperatur gebracht. Zur Analyse der Kohlenhydratgehalte wurden ca. 10.000 Blütenknospen von neun Sorten alle zwei Wochen über zwei Jahre gesammelt. Die Ergebnisse können wie folgt zusammengefasst werden:

1. Unter Berücksichtigung der drei wichtigsten Kälte (Chilling)-Modelle zur Erfassung des winterlichen Kältebedürfnisses (Chilling) stieg das verfügbare Chilling um 12% (Chill Units- CU), 15% (Chill Portions- CP) bzw. 20% (Chilling Hours - CH) in dem unbeheizten Gewächshaus (durchschnittliche Temperaturerhöhung um ca. 2°C) im Vergleich zu der Obstanlage in Klein-Altendorf, Meckenheim. Bei Klimaprognosen für andere Standorte, insbesondere im Süden Europas oder im Norden Afrikas, führten die wärmeren Wintertemperaturen hingegen häufig zu einer Reduktion des verfügbaren Chillings. Möglichkeiten und Grenzen von Gegenmaßnahmen in Bezug auf Anbaumethoden wie Mikroklimamanipulation, Dormanz brechende Mittel, Etablierung einer Obstanlage in höherer Altitude und Züchtung sind Optionen, um einem möglichen Mangel an Chilling entgegen zu wirken.

2. Die Durchschnittstemperaturen in wärmeren Wintern (6,0°C) in der Obstplantage in Meckenheim überstiegen die Temperaturen im ungeheizten Gewächshaus (4,7°C) in kälteren Wintern. Die maximale Chilling-Verfügbarkeit an diesem Standort wird in sehr warmen Wintern erreicht, so dass noch wärmere Winter die verfügbare Kälte reduzieren können. Dennoch ist die Chilling-Verfügbarkeit voraussichtlich für den Anbau von High-Chill-Sorten ausreichend. Berechnungen für diese Region, mit den drei wichtigsten Chilling-Modellen, zeigten, dass Kirschbäume der Low-Chill-Sorte '6000CZ' 22,3-26,6 (CP), 465-684 (CU) oder 402-483 (CH), Medium-Chill-Sorte 'Brooks' etwa 37,9-54,4 CP, 819-1.267 CU oder 779-941 CH und die High-Chill-Sorte 'Schneiders späte Knorpelkirsche' etwa 54,4-79,3 CP, 1.267-1.696 CU, 941-1.494 CH für eine natürliche Blüte benötigen. Kirschbäume von cv. '6000CZ' mit <300 CH, cv. 'Brooks' mit <500 CH und cv. 'Schneiders späte Knorpelkirsche' mit <700 CH hatten keine natürliche Blüte, was ca. 50% des Chill-Optimums der jeweiligen Sorte entspricht. Der Beginn des Blattfalls wurde als geeigneter Ausgangspunkt für die Berechnung des Chillings identifiziert.

3. Es wurden vier Übergangspunkte vorgeschlagen, um die Phasen der Dormanz zu unterscheiden, indem der Kohlenhydratgehalt und der relative Wassergehalt (RWC) in den Reproduktionsknospen in Bezug zur Chilling-Akkumulation gesetzt wurde. Zudem wurden zwei Gruppen von Kirschsorten basierend auf ihrem unterschiedlichen anfänglichen Sorbitol- und Stärkegehalt im Herbst definiert. Der Erste Übergangspunkt zwischen para- und d-Endo-Dormanz charakterisiert eine Abnahme (Sortengruppe 1) oder eine konstante Menge (Sortengruppe 2) zu einem starken Anstieg von Hexosen- und Sorbitol- und einem Abfall an Stärke-gehalt. Der zweite Übergangspunkt (d-Endo- zu f-Endo-Dormanz) liegt vor, wenn die Veränderungen in den beiden Hexosen (Zunahme) und Stärke (Abnahme) enden. Bis dahin wurden ca. 650 CH, d. h. unzureichendes Chilling für die Sorten, akkumuliert. Der dritte Übergangspunkt (f-Endo- zu Eco-Dormanz) wurde durch ca. 1.000 CH, Minimum Chilling Bedarf und zurückhaltendes Aufgehen der Knospen (an abgeschnittenen Ästen) definiert. Der vierte Übergangspunkt (Anfang Wärmeakkumulation) markierte einen Anstieg des Wassergehalts bei ca. 1.550 CH, optimales Chilling für diese Süßkirschsorten und fiel mit der natürlichen Blüte zusammen. Ein Verhältnis von Hexosen (Glukose plus Fruktose) zum Stärkegehalt (<2:1) schien ein möglicher Indikator für den Beginn der Chilling-Akkumulation zu sein (Para-Dormanz); ein Verhältnis von 14-20:1, wurde in der Endo-Dormanz erreicht, während das Aufwachen aus der Winterruhe (Eco-Dormanz) mit einem Rückgang auf weniger als 10:1 am Ende des Winters verbunden war.

4. Die Auswirkungen von mehr Wärmeakkumulation aufgrund von weniger verfügbarem Chilling als Folge des Klimawandels wurde untersucht. In den angewandten Szenarien lag das minimale Kältebedürfnis bei 400 CH (Chilling Hours) für die Low-Chill-, 550 CH bei der Medium-Chill- und 750 CH bei High-Chill-Sorte mit max. 11.000 GDH (Growing Degree Hours) für die Low-Chill- ca. 12.000 GDH für die Medium-Chill- und ca. 13.000 GDH für die High-Chill-Sorte für eine natürliche Blüte. Bei optimalem Chilling war das optimale Wärmebedürfnis ca. 8.000 GDH (>12°C), unabhängig von der Sorte. Bäume, die einem mehr als optimalen Chilling (150%) ausgesetzt waren, benötigten weniger Wärme (ca. 4.000 GDH), um die volle Blüte zu erreichen. Daraus resultierend kann bis zur Hälfte der erforderlichen Wärme kompensiert werden, d.h. etwa 4.000 GDH. Leicht negative Temperaturen (-5°C bis 0°C), trugen zur Chilling-Akkumulation der Obstbäume bei. Insgesamt haben die Ergebnisse gezeigt, dass sich die Kälteakkumulation zum Teil (bis zu 50%) durch eine größere Menge an Wärmeakkumulation ausgleichen lässt, um eine natürliche Blüte in den Obstplantagen zu erreichen.

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List of abbreviations

ca.	circa, around, about
CH	Chilling Hours
°C	degree Celsius
cm	centimeter
CP	Chill Portions
CU	Chill Units
cv.	cultivar
DM	dry mass
et al.	et alii (m.), et aliae (f.), and others
°F	degree Fahrenheit
FM	fresh mass
g	gram
g/L	gram per liter
GDH	Growing Degree Hours
h	hours
H20	water
HPLC	High pressure liquid chromatography
i.e.	id est, that is
M	Molar mass
max.	maximum
μl	microlitre
mg	milligram
min	minutes
min.	minimum
mL	millilitre

°N	degree North
pH	pondus Hydrogenii
%	percent
rpm	rounds per minute
RWC	relative water content
v/v	volume per volume
vs	versus
WC	water content

A Introduction

1. Sweet cherry in the context of globalization; species origin and climate adaption

1.1 Sweet cherry (*Prunus avium* L.); production acreage

Sweet cherry is an important species in horticultural production throughout the temperate climate zone. In 2012, 45,597 ha fruit trees were planted in Germany with almost 81 million trees. Thereof 2.5 million sweet cherry trees were planted on 5,258 ha (destatis, 2012). Until 2017, the area of fruit production continuously increased to 49,934 ha with 6,066 ha of sweet cherry (destatis, 2017). Therefore, due to its importance for consumers and industry, the growing area of sweet cherries constantly increased during recent years. This trend goes along with an increased urge for regional, qualitatively high value products in Germany.

In general, sweet cherry production and orchard management in Germany prefers regions with about 750mm precipitation per year on average soils. Dwarfing rootstocks like GiSelA 5 allow high density tree planting between 667 to 1,250 trees per hectare with trees between 2.5m and 4m height (Quero-García et al., 2017). Although GiSelA 5 is the standard rootstock in Germany, other rootstocks are also used, such as GiSelA 3 and 6, Krymsk 5 and 6, MaXma 14 and Mazzard (Long et al., 2014). Sweet cherry trees are mostly planted in single rows on a trellis system and trained as slender spindle (Balmer and Hilsendegen, 2017). This concept allows best access with a tractor and other machinery as well as light interception within the tree (Overbeck et al., 2018). The dwarfing rootstocks and smaller tree sizes also allow rain or hail covers (Quero-García et al., 2017).

1.2 Sweet cherry - origin, selection and adaption

In the 1950s, the breeding of sweet cherry cultivars became more popular (Quero-García et al., 2017). The demand for sweet cherry fruits is mostly determined by large buyers in the food retail sector, who usually expect large fruit size with high firmness and intense dark red colour and a good taste (Dekova et al., 2009). Therefore, breeders are looking for such properties as well as resistances or tolerances to pathogens in plants (Friedrich et al., 2000). Parents for use in breeding are collected in countries all over the world to generate the best possible breeding result.

However, this wide sourcing of germplasm may lead to poorly adapted cultivars, because the climates from which the various cultivars originate, differ greatly. Each tree species has adapted to growing conditions in its region of origin over centuries (Bateman et al., 1998). Adaptions range from suitability for prevailing soil conditions, levels of solar radiation to, arguable most importantly, the local seasonality of temperature and precipitation (Winter et al., 2002). Adaption to this seasonality is reflected by interspecific variation in the so called chilling requirements. The chilling requirement is a protection mechanism during a cold period in winter, when the tree goes dormant. Since the buds are already established in the fall, they need protection against frost temperatures (Westwood, 1993). Hereby, the plant senses cold temperatures to a certain extent before enhancing regrowth. As long as this cold temperature requirement is not fulfilled, bud break is inhibited or retarded even when warm periods during winter appear. Due to their high chilling requirement, most sweet cherry (*Prunus avium* L.) varieties are one of the most susceptible fruit trees to warmer winters (Kaufmann and Blanke, 2017a).

The breaking of dormancy may be disturbed by a change in climatic conditions, which can reduce tree productivity. On the one hand, varieties from e.g. the warm climate of California with very mild winters can be introduced, due to their good fruit characteristics, to Central or Northern Europe, where the winter is longer and harder. This can lead to substantially lower yield potential e.g. in sweet cherry (Millan et al., 2009), because the chilling need of warm winter cultivars is so low that plants flower too early in the year (Blanke and Kunz, 2011). This can increase the risk of frost damage, which can lead to a complete loss, if frost occurs during the open flower stage. On the other hand, cultivars with a high chilling requirement may, in a milder winter climate, receive insufficient chilling during the winter, which can lead to uneven budburst (Blanke und Kunz, 2009).

2. Dormancy and dormancy release of deciduous fruit trees

2.1 Chilling: requirements and quantification

Chilling is a requirement for perennial deciduous trees to accumulate cold temperatures during winter time, especially for fruit trees, which has a large influence on the performance of a uniform flowering behaviour in spring (Jackson, 2003).

The chilling phase is separated into three sections: The “early dormancy” (para-dormancy), the “true dormancy” (endo-dormancy) and the “late dormancy” (eco-dormancy) (Lang et al., 1987). Para-dormancy is the initial phase, when the tree prepares for winter, sheds its leaves and starts to accumulate chilling. High temperatures, pruning or Nitrogen fertilization can affect the length of para-dormancy (Lang et al., 1987). The endo-dormancy differes from the para-dormancy, which has its cause in the buds themselves and can be hardly influenced by manipulating the plant. Usually, it is not possible for shoots and branches, which are brought into a warm environment, to bloom as long as the endo-dormancy is not over yet. This phase can also be divided into two stages of d-(deep)-endo-dormancy and f-(facultative)-endo-dormancy (Hillmann et al., 2016; Kaufmann and Blanke, 2018b). The endo-dormancy is the result of a gradual development, where the plant goes into a deep rest with a gradual waking up. Since this development is not uniform throughout all the buds, the endo-dormancy and the rest breaking can differ between buds on one tree. This phenomenon is called “acrotony”, the dominance of the distally located proleptic lateral buds and shoots over the apical buds (Fig. 1). The fruit trees enter the point of deepest true winter dormancy around leaf fall (Friedrich et al., 2000; Kaufmann and Blanke, 2017a; Chmielewski et al., 2017). In the eco-dormancy phase, the tree has met its requirement for chilling and waits for warmer temperatures (forcing) and longer days (photoperiod) (Lang et al., 1987).

Figure 1. Sweet cherry tree with acrotony. Showing fruit next to open flowers.

The majority of older studies on chilling requirement of plants were performed with cut one-year-old single branches placed in cooling chambers with artificial lighting (Albuquerque et al., 2008; Mahmood et al., 2000b; Ramos et al., 2018). This kind of experiment allows relatively easy quantification of chill effects, but conclusions drawn from such artificial treatments are hardly applicable to whole trees under natural weather and light conditions (Mahmood et al., 2000a). There is even evidence that branches taken from different parts of the tree may differ in the way they break dormancy e.g. due to acrotony. Hence, it is important to carry out studies with whole trees, which are exposed to natural chilling conditions and experienced forcing in a heated greenhouse with natural lighting to exactly quantify the chilling and forcing requirements of a tree.

Also, the single node cutting test by Tabuenca (1964) is performed to evaluate dormancy breaking. An axillary bud is cut and placed in favorable conditions for about eight days at 20-25°C to resume growth (increase of fresh weight) (Champagnat, 1989). In some studies, the green tip stage (Campoy et al. 2011a; Guak and Neilsen 2013) was assessed whereas in others the point 20% budbreak (Cook and Jacobs, 2000) or of 50% open flowers (BBCH 65 after Meier et al., 1994, F2 after Baggiolini, 1952; Fleckinger, 1955) as point of end of chilling requirement was noted (Cesaraccio et al., 2004; Kaufmann and Blanke, 2017b, c) showing the inconsistency employed in this research.

2.2 Forcing: requirement and quantification

After the minimum chilling requirement is met, the tree enters the eco-dormancy phase, where the tree can still accumulate chilling, but warm temperatures start to be effective for regrowth and flowering in spring (Lang et al., 1987). Since cold and warm temperatures can be accumulated concommittantly it is not clear when which phase starts or ends. Kaufmann and Blanke (2018b) found up to 50% tradeoff between chilling and forcing temperatures requirements. Once the chilling and forcing requirement is fulfilled, the tree starts to flower. To quantify forcing requirements, buds (Tabuenca, 1964), sticks (Alburquerque et al., 2008) or entire potted trees (Kaufmann and Blanke, 2017b) are taken into forcing chambers with >16°C temperature to cease chilling and start accumulation of forcing.

3. The three chilling models

An orchard manager would benefit greatly from ex-ante projection of such yield losses (Millan et al., 2009) with models that are tailored to the region and the species grown. The three most commonly used chilling models all have their origin in warm climates such as South-Eastern United States or Israel. None of these models consider temperatures below 0°C as effective for chilling although latest research shows the effectiveness of slightly negative temperatures (Kaufmann and Blanke, 2018b, Mahmood et al., 2000a). Besides, all common models only include temperature and do not include other potentially important factors such as global radiation and photoperiod (Caffarra and Donelly, 2011). While chill models differ in their method of converting temperature to chill estimates, they are similar in that they are not well based on plant biology. It is unclear, when the plant actually starts accumulating chill. The range of suggested chilling dates start from the time of the shoot growth cessation to leaf fall (Cesaraccio et al., 2004; Chmielewski et al., 2017; Kaufmann and Blanke, 2017a) or a certain date in the year (Richardson et al., 1976).

There are several approaches to calculate chilling, but not every chilling model is valid for all species and regions, since the chilling needs even between varieties of the same fruit crop may differ greatly. Especially, if one combines projections of climate change with the currently available chilling models, the outcomes of how much chilling is available in between the models differ enormously (Luedeling et al., 2009a) (Fig. 2). Most chilling estimates have been made in Chilling Hours (CH), so cold requirements cannot easily be transferred to a new location other than where requirements were determined. (Luedeling and Brown, 2011a; Dennis Jr, 2003).

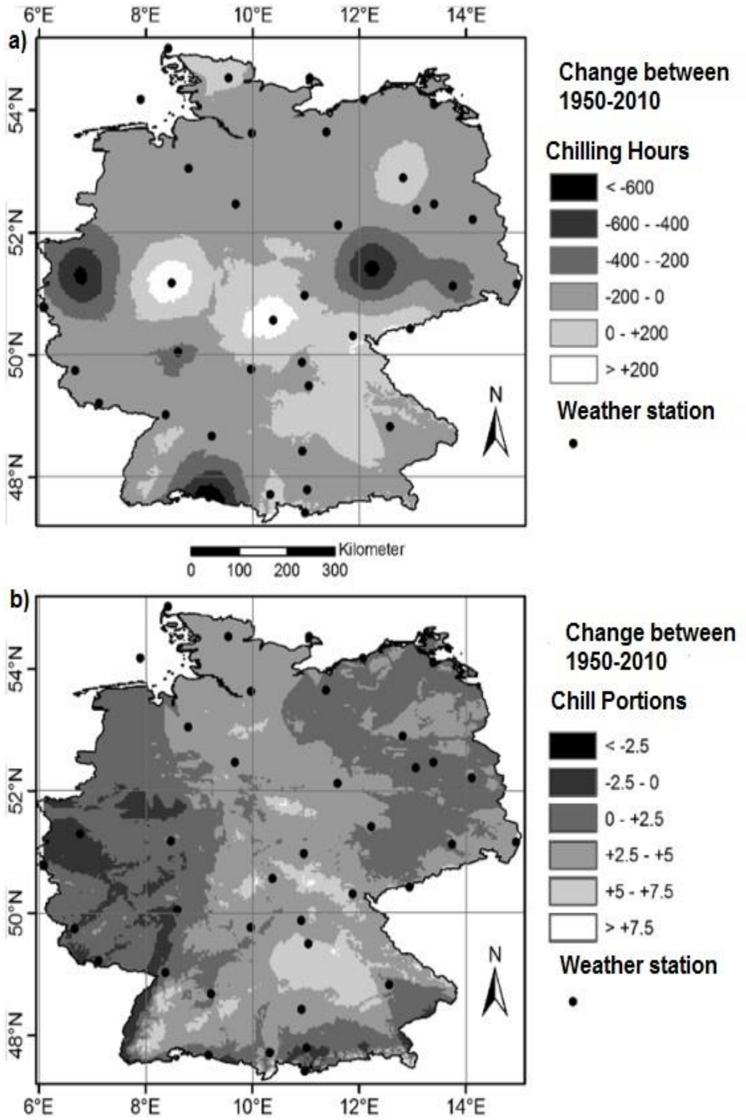

Figure 2 Expected chill effect in Germany quantified in Chill Hours (a) and Chill Portions (CP) (b) under the climatic conditions of 2010 (Luedeling et al., 2009a).

3.1 The Weinberger Chilling Hours Model

The oldest and most widespread model is the Weinberger Chilling Hours Model, which adds the number of hours with temperatures of 0 - 7.2 °C (Weinberger, 1950) (Table 1). Originally developed for peaches in Georgia (USA), it is now applied to many other types of fruit crops. In this model, a Chilling Hour (CH) is registered for each hour with temperatures of between 0 °C to 7.2 °C; the latter originates from the original US version of the model, where the upper limit is 45°F (degree Fahrenheit). Therefore, it is not as precise as it may first appear. On the one hand, this model is very simple, but also easy to apply. On the other hand, it can hardly be considered as biologically exact (Kaufmann and Blanke, 2017a).

3.2 The Utah Model

The second and also very popular chilling model is the Utah model developed for peaches in Utah (Richardson et al., 1974). It represents a further development of the Weinberger chilling model from 1950. Temperatures are weighted, and especially warm temperatures can nullify already accumulated Chill Units (CU). Effective chilling temperatures range from 1.4°C to 12.4°C. Slight modifications, such as the ‘positive Utah model’, are also used in the Western Cape (South Africa) (Lindsey-Noakes et al., 1995). Since the temperature range during winter rarely exceeds 10°C, the original version of the Utah Model is often used in Northern Europe. In the Utah model, temperatures above 15.9°C can overcome previous chilling effects. Since its publication 40 years ago, this model has been adjusted to varieties and locations to improve its validity on other fruit crops and locations (Lindsey-Noakes et al., 1995).

3.3 The Dynamic Model

The Dynamic Model (Fishman et al., 1987; Erez et al., 1990) was developed for peaches in Israel (Table 1). It divides the chilling in a two-phase process. In the first phase, cold winter temperatures lead to a precursor of the chilling accumulation. This precursor is “vulnerable” and can be destroyed by warm temperatures. When the precursor has been overcome, the actual chilling is taken into account. These so-called Chill Portions (CP) will then remain until the end of winter. The equations for this model are far more complicated than those in the other two models. This could also have contributed to the hesitant spread of this model. Physiologically, this model is based more on speculation rather than biologically facts, but in comparison to the other models it seems to be plausible.

Table 1: Comparison of the three chilling models

Chilling - Model	Author/ Year	Place of origin	Fruit	Effective temperatures	Weighted temperatures
Weinberger Chilling Hours Model	Weinberger 1950	Georgia, USA	Peach	0 - 7.2°C	NO
Utah Model	Richardson et al. 1974	Utah, USA	Peach	1.4 - 12.4°C	YES
Dynamic Model	Fishman et al. 1987	Israel	Peach	0 - 13°C	YES

3.4 The Forcing Model

The forcing model is based on the concept of Growing Degree Hours (GDH) (Anderson et al., 1986). Like the previous mentioned chilling models, it uses a temperature range. The heat accumulation starts after chilling fulfillment at a base temperature and ends at a maximum temperature. Furthermore, a temperature point is set where the heat accumulation reaches its maximum efficiency. This point is located in between the start and the end of the temperature range from 4°C to 36°C with its maximum effective point at 25°C, as suggested for fruit trees by Andersen et al. (1986). Besides few adaptations for certain plants (Black et al., 2008 for *Rubes* species), the standard Growing Degree Hour Model is employed for most experiments.

4. Climate change effects on chilling and fruit growing

According to the IPCC report (IPCC, 2013), climate change is steadily progressing, and weather extremes are expected to increase worldwide. On the one hand, high temperatures throughout the year in combination with water shortage can cause great harm (Friedrich et al., 2000). On the other hand, temperatures are shifting. Early warm temperatures followed by cold temperatures at the end of the winter and beginning of spring can cause late frost damage. Furthermore, early frosts in autumn can cause serious damage to fruits, which are not yet harvested (Cannell and Smith, 1986). The temperature rise in early spring also advances the average bloom phase (Blanke and Kunz, 2011; Chmielewski and Rötzer, 2001; Cleland et al., 2007; Legave et al., 2013).

Besides transporting plants from all over the world for commercial uses, climate change is raising ambient temperatures more rapidly than during most -if not all- previous eras of the tree’s evolution, so that plants at their original locations no longer have sufficient time to adapt to the new conditions (Nicotra et al., 2010). Regions with Mediterranean climate such as South and North Africa, which are under a particularly strong influence of climate change, experience high yield losses, which threaten the profitability of the cultivation of current locally grown varieties (Blanke, 2013). Many fruit growing regions at temperate climate zone latitudes derive their characteristic features from the cultivation of fruit trees (Lorenzo et al., 2015). As an example, fruit growing regions in the Mediterranean climate are exposed to warmer winters, particularly on the North African coast, and a decreasing amount of precipitation, which is increasingly concentrated during the winter season (Midgley and Lötze, 2011).

In South Africa, climate change even led to orchards at lower elevation facing total losses in their production (Midgley and Lötze, 2011). Therefore, some studies (Alburquerque et al., 2008) evaluated the relationship between cherry flowering and higher altitudes. If a new cherry orchard is planted, it has to remain in production for two decades. Therefore, the choice of the variety needs to be taken in consideration of the potentially changing climatic conditions, especially milder winters that may compromise the trees’ ability to amass sufficient chill. Yet, chilling requirements of fruit trees are only roughly known. Models which reflect the duration and intensity of winter temperatures are not based on physiological facts, come from one certain area and are established for one certain cultivar. Chilling requirements can also differ greatly in between varieties (Kaufmann and Blanke, 2017a). It is crucial to gain more knowledge about the chilling needs and their correct approximation by chilling models to ensure a climatically sustainable fruit production.

Regions with warm winters (South and North Africa, Spain, Italy, Greece, Morocco and South of France) may be adversely affected in several ways (Campoy et al., 2011b; Rosegrant et al., 2009), including lack of chilling (Legave et al., 2009; Millan et al., 2009). The chilling predictions for these Mediterranean regions to the end of this century are 40-70 CP, which is equivalent to a loss of ca. half of their existing chilling availability (Luedeling et al., 2013). Their successful cultivation requires specific climatic conditions, and trees may quickly decline when their climatic requirements are not met (Blanke et al., 2012). Particular attention needs to be paid to availability of winter chill, produced by cool conditions during the winter season, which stimulates trees to break their dormancy and resume productive growth in spring. Environmental changes like warmer winters as a result of climate change (IPCC, 2013) require adaption strategies in agriculture (Bindi and Olesen, 2011), including those to overcome the lack of chilling, i.e. a period of cool temperature required to induce buds to flower e.g. in forest (Harrington et al., 2010; Heide, 1993) as well as in fruit trees (Faust et al., 1997; Lang et al., 1987; Luedeling, 2012). Chilling requirements are difficult to quantify due to uncertainty about a number of factors, including the start point of chill accumulation.

For the Meckenheim fruit growing regions, Blanke and Kunz (2009) reported a more pronounced temperature rise in January and February for Klein Altendorf, Germany, due to climate change, based on 60 years of climate and phenology data collection. Depending on the employed chilling model, a moderate up to a serious decline of chilling in different areas of the world can be observed (Fig. 3).

Mild winters with insufficient chilling led to an average of 32% yield loss of sweet cherries in France 2007 (Millan et al., 2009). Fruit production in Europe may be hampered by climate change in terms of warmer winters without sufficient chilling especially in Mediterranean climate (Luedeling et al., 2013). The last warm winters such as 2013/14 and 2015/16 in Europe, showed how climate change affects fruit tree phenology with earlier bloom and higher risk of early frost damage.

Since 0 – 7°C is the range of effective temperatures for chilling in the literature (Weinberger, 1950), the climate change can also affect the number of Chilling Hours in a positive way in places with temperatures previously just below 0°C. Due to global warming, temperatures rise above 0°C, which now increases the number of chilling hours in that area. Based on this 0 – 7°C temperature basis, no significant changes are expected in the number of Chilling Hours in Germany despite climate change (Luedeling et al., 2009a).

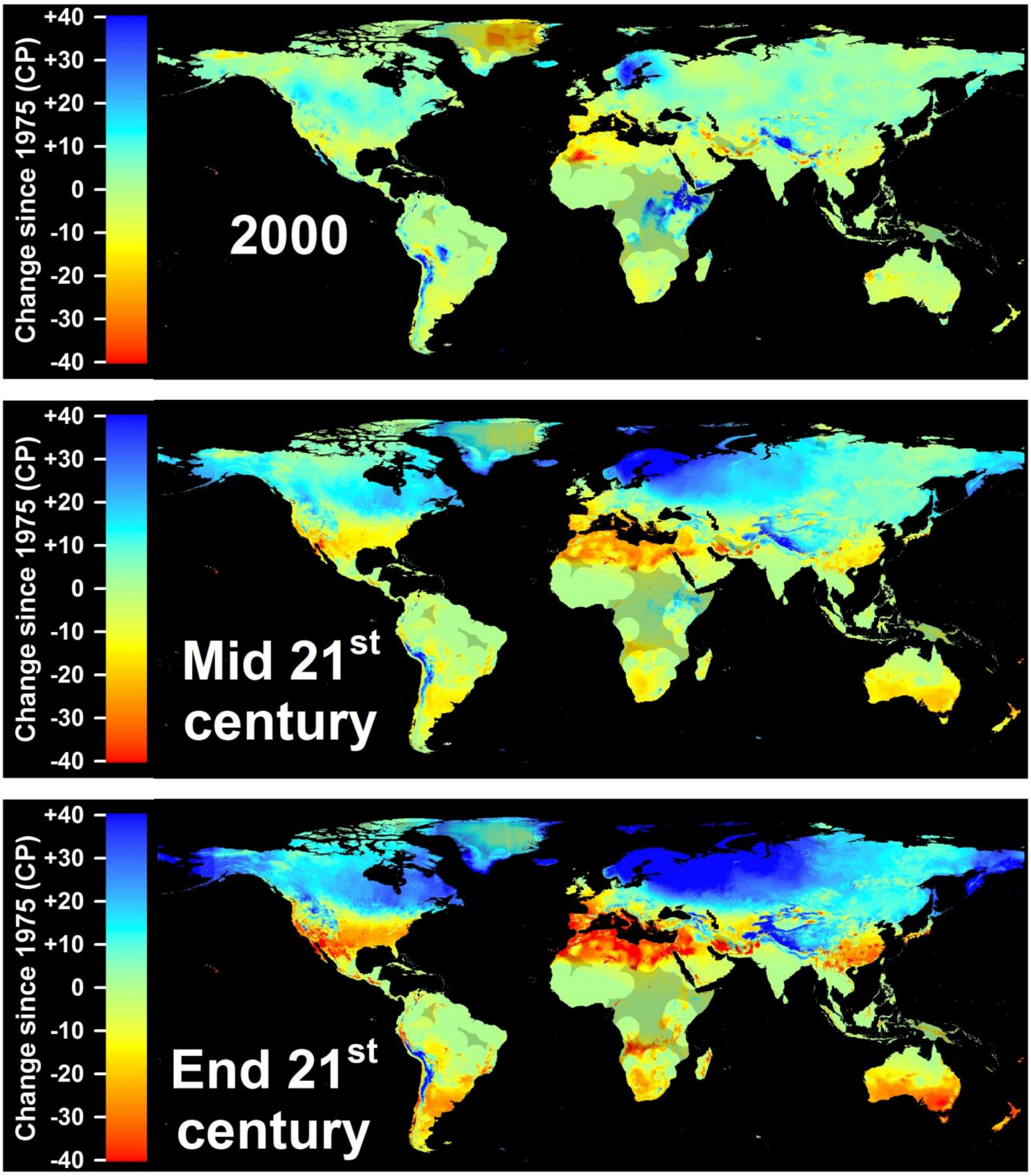

Figure 3 Modeled and projected losses in Safe Winter Chill compared to 1975 for the year 2000 (top), the middle of the 21st century (middle), and the end of the 21st century (bottom). The calculations with the Dynamic-model are in Chill Portions-CP (Luedeling et al., 2011b)

Flowering phenology of apple trees in the local Meckenheim fruit growing area has been accelerated by up to 10 days in the last 50 years (Blanke and Kunz 2009; Legave et al., 2013). Changes in flowering synchronization in different varieties of a given fruit tree species could arise from interactions between regional changes in temperature and varietal differences in temperature requirements (Spiegel-Roy and Alston, 1979). This can affect the cross-pollination phase that determines the annual yield of many fruit tree varieties, especially in sweet cherry cultivation. Diverging flowering times may therefore modify insect pollinator resources or impact interspecific gene flow (Menzel et al., 2006; Miller-Rushing et al., 2007).

In some areas of the world, chilling hours decrease rapidly during the winter. This problem is monitored in med-climate regions such as Israel or Spain and the Southern states of the U.S. (e.g. Georgia, California), Oman (Luedeling et al., 2009c), North (Marocco) and South Africa (Midgley and Lötze, 2011), where apple and cherry production in the Western Cape is limited by lack of Chilling Hours. The resident fruit growers renounce their previous grown fruits and switch, if possible, to other varieties and even different fruit species (Fig. 4).

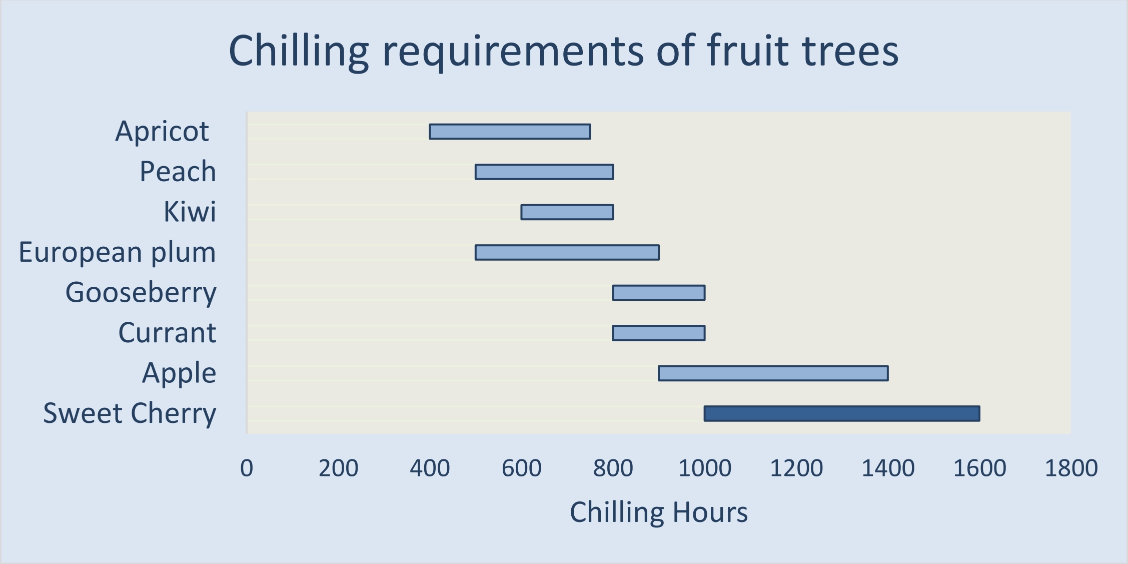

Figure 4. Chilling requirement of selected fruit (in Chilling Hours – CH) (Kaufmann and Blanke, 2018a).

Adaptation strategies include the introduction of lower chill cultivars (Labuschagne et al., 2002) and orchard cooling technologies such as evaporative cooling, shading and kaolin-based particle film during autumn to boost early accumulation of chill units.

If the chilling need, especially in the hot climates of the earth, is not satisfied, the initiation of the bud burst can be delayed and therefore be uneven throughout the plant; next to still-closed flowers, hazelnut sized fruits hang side by side at the tree. For example, in Israel or in southern China large apples are harvested next to pygmy fruit (Jones et al., 2012).

This phenomenon can either be handled by breeding climate-adapted varieties or by spraying rest-breaking agents such as DNOC, oil, TBU or cyanamide at the end of winter (Friedrich et al., 2000, Kaufmann and Blanke, 2017a), the latter can kill the tree, if used inappropriately, as seen in South Africa (Blanke, 2013).

5. Bio-indicators and molecular markers

During dormancy, the plant phenology does not change. The tree remains dormant without leaves and closed buds. Hence, research has to be done within the tree. So called bio-indicators, such as carbohydrate levels (Chmielewski et al., 2014; Keller and Loescher, 1989), water content (Kaufmann and Blanke, 2017c), dehydrins (Erez et al., 1998), plant hormone changes (Chmielewski et al., 2017; Rinne et al., 1994) or gene regulators (Howarth et al., 2009) are key factors to understand dormancy processes. Carbohydrates like sucrose, fructose and glucose as well as starch and the sugar transport alcohol in *Rosaceae* sorbitol (Leite et al., 2004; Whetter and Taper, 1963) in buds undergo certain fluxes during dormancy initiation and release (Bonhomme et al., 2005; Gonzalez-Rossia et al., 2008; Marquard et al.,1998). Although carbohydrates are also associated with frost protection during temperature drops in winter (Bonhomme et al., 2005; Hamman et al., 1996), when starch is hydrolyzed into sugar, they play a major role in the control of bud growth and development during dormancy and dormancy release (Bonhomme et al., 2005; Cottignies, 1986; Hillmann et al., 2016; Marquat et al., 1999).

In addition, changes in water content are associated with different dormancy phases. Certain patterns in water content where recorded from Chmielewski et al. (2017a) and Kaufmann and Blanke (2017c), where water content declines during dormancy and recovers at dormancy release in flower buds of sweet cherry trees. During cold temperatures in winter, water is bound by hydrophilic proteins, dehydrins, in the cells in the bud (Erez et al., 1998). These proteins are activated by cold temperatures and short days and prevent growth of the bud e.g. beginning of dormancy. At dormancy release, bound water returns to free water to initiate growth, when temperatures are back in a favorable range (Faust et al., 1991).

Other bioindicators for dormancy phases are plant phytohormones like gibberellic acid (GA) and abscisic acid (ABA). Altman and Goren (1974) showed that GA_4 can induce bud swelling, when absorbed by the buds. Therefore, it can help to overcome dormancy (Dennis Jr, 1994). During bud dormancy, ABA levels rise in the flower bud at the beginning of dormancy and stagnate at highest level before decreasing with the release of dormancy (Chmielewski et al., 2014; Leida et al., 2012; Rinne et al., 1994).

Besides carbohydrate, water and phytohormone fluxes recent experiments were carried out to evaluate the epigenetic regulations during dormancy processes. Specific gene expressions have consequences on cell regulations, sensitivity to light or plant hormone production or degradation as well as on stress reactions (Rios et al., 2014). Genes associated with dormancy are called DAM-genes, which are associated with the MADS-Box. Several DAM genes were found to either react to short (DAM 1 and DAM 4) or long (DAM 2) days (Castede et al., 2015; Leida et al., 2012; Li et al., 2009). The DAM 3 gene was unaffected by either short or long days, but suppressed by constant colder temperatures during winter e.g. the dormancy phase (Li et al., 2009).

6. Hypothesis and Objectives

The aim of this PhD thesis was to

A: i) present the three commonly used chilling models, ii) compare the chilling requirement of Mediterranean zone cultivars with other temperate zone fruits, iii) explore countermeasures to improve the microclimate in an orchard to gain chilling, and iv) present the first results from a chilling experiment at Klein Altendorf (Bonn, Germany).

B: i) determine the chilling requirements of three sweet cherry cultivars using three different chilling models. It was tested, if one chilling model is superior to the others for cherry in a temperate zone winter. ii) elucidate in what respects models were deficient and what changes, such as inclusion of mild frost temperatures, should be made to improve model predictions. iii) clarify the effects of insufficient chilling during the winter can have on the flowering behaviour of the whole cherry tree under natural orchard condition (including photoperiod) instead of cut single branches and to determine the initiation point of chilling for the tree. IV) examine the effects of simulated climate change with a 2°C temperature increase (IPCC, 2013) on chilling availability in the temperate climate zone (50°N), the major fruit growing belt in Europe.

C: investigate the underlying regulatory mechanisms of the carbohydrate dynamics and water relations of cherry buds to identify and distinguish the dormancy phases (para-, endo, eco-dormancy) on a plant physiological base, rather than statistical relations between observed flowering and temperature records, and to determine transition points between dormancy stages using ca. 10,000 flower buds from nine varieties and bi-weekly sampling over two years. The synthesis of carbohydrate dynamics, changes in relative water content (RWC) and chilling status are used to calculate carbohydrate ratios, and to elaborate four transition points as thresholds and representatives of current dormancy stage and integrated in a schematic of the underlying mechanisms that drive dormancy and dormancy release.

D: i) elaborate if chilling and forcing can be substituted by each other to a certain extent and one can compensate for the other, irrespective of variety, and if negative temperatures may have a positive effect on chilling accumulation. ii) investigate the effect of diminishing available chill as a result of environmental change on forcing accumulation. iii) to elaborate thresholds for minimum chilling fulfilment and its interaction with forcing accumulation. iv) cater for possible effects of slightly negative temperatures (0 °C to -5 °C) and the effects of simulated climate change with a 2 °C global temperature increase (IPCC, 2013) on chilling availability in the temperate climate zone (50 °N), the environment for the major pome and stone fruit growing belt in Europe using climate sensitive sweet cherry as model crop.

Some of the previous chilling experiments were based on cut branches in moist paper in the dark at a constant temperature in a cold chamber (Albuquerque et al., 2008; Mahmood et al., 2000b,). This kind of experiment allows relatively easy quantification of chill effects, but conclusions drawn from such artificial environments are difficult to apply to whole trees under natural weather and light conditions (photoperiod) (Mahmood et al., 2000a). To achieve these goals, 160 potted sweet cherry trees of three varieties with a wide range of chilling requirements were raised over two years to initiate uniform flower buds before applying 24 chilling regimes (eight regimes per variety) per year followed by forcing to determine the effect on flowering. The results include climate change simulations using containerized potted cherry trees in an unheated greenhouse. For carbohydrate and water analyses we also collected ca. 10,000 flower buds from nine different cherry varieties during winter from the orchard at Campus Klein Altendorf, research station of the University of Bonn.

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B Chilling in cherry – principles and projection – a brief introduction[1]

1. Introduction

Cherry production in Europe may be hampered by climate change in terms of warmer winters without sufficient chilling (Luedeling et al., 2013). After the last warm winters in Europe, climate change seems to affect fruit phenology. Regions with warm winters (South Africa, Spain, Morocco and South of France) are affected in that sweet cherry suffers from lack of chilling. The objective of this contribution is to i) present and explain the three commonly used chilling models, ii) compare the chilling requirement of cherry cultivars with other fruit and, iii) explore countermeasures to improve the microclimate in an orchard to gain chilling and present the first results from a chilling experiment at Klein Altendorf, Bonn, Germany. The results include climate change simulations using containerized potted cherry trees in an unheated greenhouse.

2. Chilling requirements of fruit trees

Fruit trees require chilling, a cold period during the winter for synchronized flowering. Insufficient chilling can induce uneven, irregular and delayed flowering with a high rate of flower abortion. This may result in an asynchronous pollination and fertilization, resulting in reduced fruit set and yield losses. Reports from the South of France indicate up to 35% yield losses in sweet cherries due to insufficient chilling (Millan et al., 2009).

Fruit trees differ in their chilling requirement from pomegranate (100-200 CH), kaki (100-400 CH), fig (100-500 CH), apricot (400-800 CH), peach (500-800 CH), European plum (500-900 CH), and to apple (up to 1,400 CH) and cherry (up to 1,600 CH; Fig. 1).

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[1] Kaufmann H, Blanke MM (2016) Chilling in cherry – principles and projection – a brief introduction. Proc COST cherry FA1104 Workshop WG 2 (‘Cherry phenology and climate change’). ISHS Acta Hort 1162:39-44

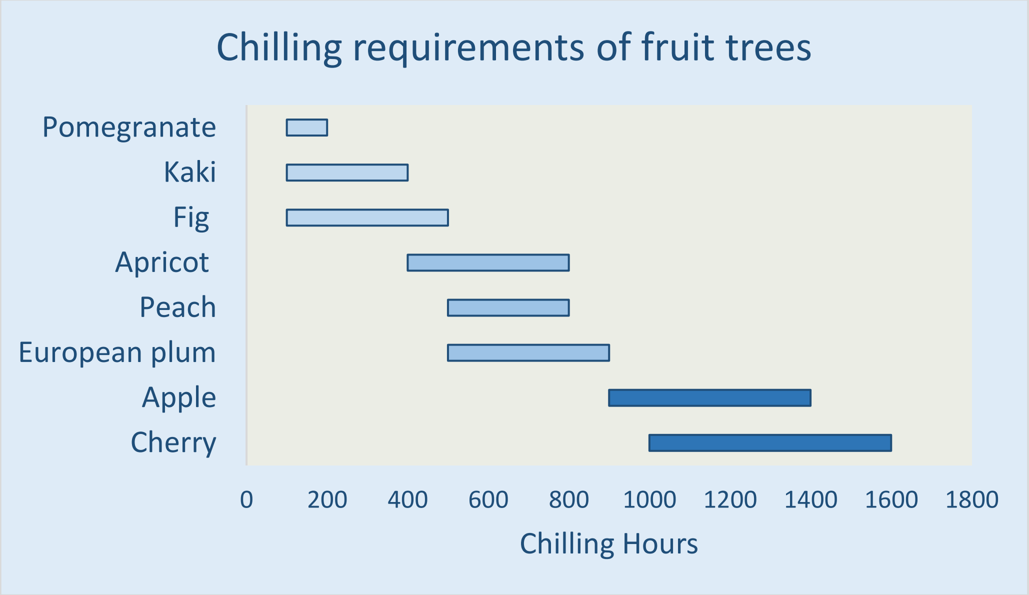

Figure 1. Chilling requirement of selected fruit (in Chilling Hours – CH)

3. Chilling requirements of sweet cherry

Sweet cherry with up to 1,600 Chilling Hours (CH) scores very highly in terms of chilling requirement during winter among perennial fruit crops (Fig 1.). The cultivar ‘Schneiders späte Knorpelkirsche‘, also known worldwide under many synonyms such as ‘Ziraat 0900’, ‘Ferrovia’, ‘Germersdorfer’, ‘Belge’, ‘Napoleon’ or ‘Zing’ requires one of highest chilling of all sweet cherry cultivars with ca. 1,000-1,500 Chilling Hours (CH) (Kaufmann and Blanke 2017). This is followed by cv. ‘Brooks‘ and ‘Sweetheart‘ with ca. 800-1,100 Chilling Hours (Guak and Neilsen, 2013; Kaufmann and Blanke 2017). In the Mediterranean climate the sweet cherry cultivars ‘Burlat‘ and ‘Lapins‘ require ca. 550-750 Chilling Hours for a natural flowering (Fig. 2)(Alburquerque et al., 2008).

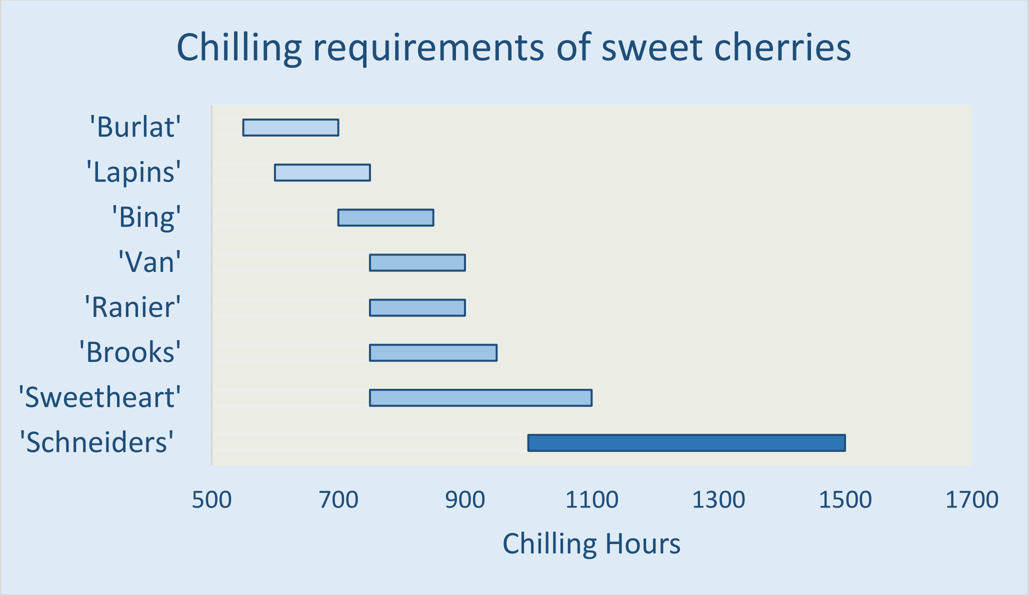

Figure 2. Chilling requirements of selected sweet cherry varieties reported in the literature (in Chilling Hours – CH)

4. The three most common chilling models

The oldest and most common model is the Chilling Hours model from Weinberger (1950), which counts every hour between 0 and 7.2 °C as one Chilling Hour (CH). The second approach, the Utah model (Richardson et al., 1974) uses Chill Units (CU) and the third one, the Dynamic model from Fishman et al., 1987 (Chill Portions – CP) are often used to calculate winter chilling (table 1); in this model by Fishman et al. 1987, optimum chilling occurs in a temperature range from 6-8°C. In addition to the Weinberger Chilling Hours model (0 - 7.2 °C), the temperature ranges were expanded [(-2)-12°C] and the temperatures weighted. This accommodates the different effectiveness of certain chilling temperatures; in the Utah model, one hour at 2°C represents 0.5 CU, whereas one hour at 5°C counts as 1 CU (Chilling Unit). In both models (CU and CP), warm temperatures above ca. 16°C in the winter time can nullify previously accumulated chilling (Erez et al 1990).

However, the three models are not based on physiological findings, but on observations when peach flowered and on hypothetical assumptions on metabolic processes in the plant (e.g. Fishman et al., 1987, Erez et al 1988). For a better understanding for the underlying mechanisms, bioindicators are assessed as to their suitability to show the status of dormancy phases i.e. progress of chilling accumulation (Hillmann et al. 2016). All three models originate from Mediterranean climate (California, Utah, USA and Israel; table 1) with warm winters and were developed on peach. Hence, a transfer to a) a different fruit species such as cherry and b) a different climate zone with considerably colder winter is difficult. This is also supported by the temperature ranges, which do not consider temperatures below 0°C, most likely, because these temperatures are uncommon in the original country of the respective model.

Table 1. The three chilling models to calculate winter chilling

Name	Unit	Author	Effective temperature range	Weighting	Origin	Fruit tree
Chilling Hours Model	Chilling Hours – CH	Weinberger 1950	0 – 7.2 °C	No	California, USA	Peach
Utah Model	Chilling Units – CU	Richardson et al 1974	1.4 – 12.4 °C	Yes	Utah, USA	Peach
Dynamic Model	Chill Portions – CP	Fishman et al 1987	(-2) – 12 °C	Yes	Israel	Peach

5. Effects of climate change on available winter chilling

The critical global warming of 2°C (ante 2100 relative to 1900) was an outcome of the IPPC (2013). The majority of European fruit and cherry growing regions are affected by warmer winter (Blanke and Kunz, 2009). In cherry regions north of 50°N latitude, this may surprisingly result in an initial increase in available chilling (Luedeling et al., 2011).

Table 2. Countermeasures against insufficient chilling

Countermeasure in orchard planning	**Explanation**
Breeding	Select low chill varieties
Higher elevations	Higher altitudes provide more winter chilling
Countermeasure in the orchard	
Defoliation	Induce dormancy of cherry trees in the autumn
Shading	Microclimatic manipulation during the daytime; coating the trees with kaolinitic clays to reflect heat
Overhead sprinkling	Microclimatic manipulation - evaporative cooling
Rest breaking agents	Release cherry trees from dormancy

The phenomenon of an initial increase in available winter chilling north of 50°N latitude is based on the transition of winters with negative temperatures into those within the lower range of positive, chilling effective temperatures, e.g. from -4°C to +2°C, thereby contributing to the chilling requirement. Anyway, a further increase in global temperature will decrease available winter chilling in most cherry growing regions (Kaufmann and Blanke 2017). Effects of negation of previous accumulated chilling (Erez et al. 1979) or using the positive Utah model (Linsley-Noakes et al. 1995) are negligible due to mainly cooler winter temperatures in temperate climate zones where cherries are grown.

6. Countermeasures against lack of winter chilling

Options to improve the microclimate in the orchard to gain chilling for the cherry trees in warm winter periods, include evaporative cooling using overhead sprinklers and shading using e.g. existing hail or shade nets (table 2). Defoliation in autumn may assist the tree to enter dormancy. The application of rest breaking agents ca. 30 days before anticipated bud break can support or enhance the development of flower buds. Planning a new cherry orchard considers trends in local chilling availability and cultivars adapted to these winter climates to come. Moving to higher altitudes may increase available winter chilling for the cherry trees, when planning a new orchard.

7. Chilling computation for a cherry orchard in comparison to an unheated greenhouse (simulated global change)

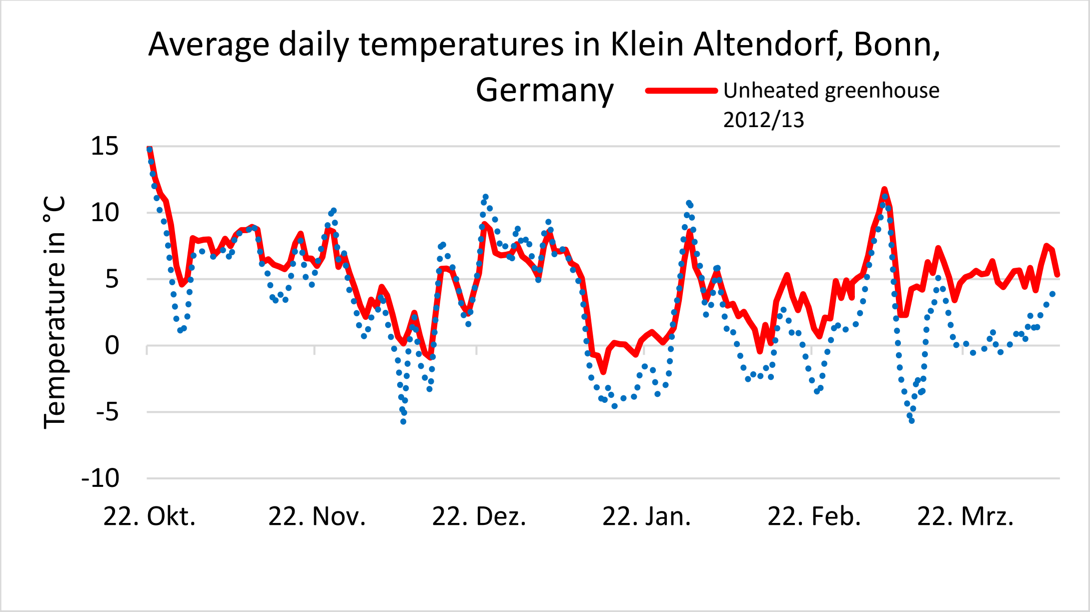

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Figure 3. Temperature courses over the winter 2012/13 in the orchard and in the unheated greenhouse (simulation of global warming) at campus Klein Altendorf, University of Bonn, Germany.

In a chilling experiment at the University of Bonn, three-year-old potted sweet cherry trees on GiSelA 5 rootstocks were exposed to either natural orchard conditions or in an unheated greenhouse (Fig 4) nearby to simulate global warming of ca. 2°C. Figure 3 shows the temperature records in both locations recorded by on site data loggers (Datahog 2, Skye, Wales, England). Chilling was calculated using the three chilling models described in table 1.

Table 3 shows the chilling values expressed as Chilling Hours, Chill Units and Chilling Portions. A comparison of the two locations (orchard and unheated greenhouse) indicates a first increase in available chilling with increasing winter temperatures at 50°N; this interpretation relates to a relatively cold winter by our standards.

Table 3. Winter chilling of 2012/13 (22 October till 27 February) calculated using the three chilling models (Weinberger 1950, CH; Richardson et al. 1974, CU; Fishman et al. 1987, CP)

Location	Chilling Hours (CH)	Chill Units (CU)	Chilling Portions (CP)
Orchard	1,466 CH	1,685 CU	80 CP
Unheated greenhouse	1,823 CH	1,903 CU	92 CP

Table 3 shows a 12 % (CU) to 20 % (CH) increase in available chilling in the unheated greenhouse (simulating global warming of ca. 2°C) irrespective of chilling model employed in this relatively cold winter.

Figure 4. Chilling experiment with container trees in an unheated greenhouse at campus Klein-Altendorf, Bonn, Germany and temperature logger on site recording of Chilling Hours

8. Conclusion

Two of the three chilling models ignore negative temperatures below zero due to the fact that they are derived from warm winter climates and may require adaptation for temperate zone climate. In warm winter with temperatures in excess of seven degree Celsius result in over proportionally higher calculated chilling values in the Utah and Dynamic Model whereas the results of all three chilling models show little differences in cooler winters with predominating temperatures from zero to seven degree Celsius. In the majority of temperate zone climate, where cherry is grown, temperatures in excess of 16 °C are scarce, which would otherwise negate previously acquired chilling.

9. Outlook

The first result indicates an initial increase in chilling with warmer winter temperatures and sufficient chilling for the majority of sweet cherry cultivars growing in the Meckenheim fruit growing region (50°N) with global warming <2°C.

10. Acknowledgement

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C Performance of three numerical models to assess winter chill for fruit trees – a case study using cherry as model crop in Germany[2]

1. Introduction

Many regions at temperate latitudes derive their characteristic features from the cultivation of fruit trees (Lorenzo et al. 2015). Their successful cultivation requires specific climatic conditions, and trees may quickly decline, when their climatic requirements are not met. Particular attention needs to be paid to availability of winter chill, i.e. a period of cool temperature during the winter season required to induce buds to flower in forest (Heide 1993) as well as in fruit trees in spring (Lang et al. 1987; Luedeling 2012). Environmental changes like warmer winters as a result of climate change (IPCC 2013) require adaption strategies in agriculture (Bindi and Olesen 2011), including those to overcome the lack of chilling.

Sweet cherry (*Prunus avium* L.) was chosen as model crop, because of its great chilling requirement of up to 1,500 Chilling Hours and is one of the most affected tree crops by environmental change and consequent temperature rises, particularly in warmer winters (Luedeling et al. 2013). In France, mild winters with insufficient chilling led to an average of 32% yield loss of sweet cherries (Millan et al. 2009). Chilling requirements are difficult to quantify due to uncertainty about a number of factors, including the start point of chill accumulation. Past chilling experiments with cut branches in moist paper in the dark at a constant temperature in a cold chamber (Mahmood et al. 2000a; Albuquerque et al. 2008) allows relatively easy quantification of chill effects, but conclusions drawn from such artificial environments are difficult to apply to whole trees under natural weather and light conditions (photoperiod) (Mahmood et al. 2000b). To our knowledge, no study has been carried out with in-situ observations on entire intact cherry trees of varieties with a broad range of chilling requirement and exposed to natural winter temperature regimes.

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[2] Kaufmann, H., Blanke, M.M., 2017. Performance of three numerical models to assess winter chill for fruit trees- a case study with cherry in Germany. Reg. Environ. Change 17, (3): 715-723

Hence, the aim of the interdisciplinary project between horticulture and agroforestry was to examine the changes in chilling availability as a consequence of regional environmental change; climate sensitive sweet cherry was used as model crop. The objective of this work was a) to determine the initiation point of the chilling for the tree, b) to compute/model the chilling requirement with all three major chilling models, and c) to examine the effects of simulated climate change with a 2°C global temperature increase (IPCC 2013) on chilling availability in the temperate climate zone (50°N), the major pome and stone fruit growing belt in Europe. To achieve these goals, 160 potted sweet cherry trees of three varieties with a very wide range of chilling requirements were raised over two years to initiate uniform flower buds before applying 24 chilling regimes (8 regimes per variety) per year followed by forcing to determine the effect on flowering.

2. Materials and methods

2.1 Sweet cherry

The 160 sweet cherry (*Prunus avium* L.) trees (Figure 3a) on dwarfing GiSelA 5 rootstock were planted in 35 litre pots on 24 March 2011 in order to initiate uniform flower buds two years before chilling treatments commenced in October 2012. The sweet cherry varieties were chosen to cover their widest possible range in chilling needs, a high chill variety ‘Schneiders späte Knorpelkirsche’, ‘Brooks’ as a medium chill and ‘6000CZ’ as a low chill variety (Gratacós and Cortés 2007; Luedeling et al. 2013). The cv. ‘Schneiders späte Knorpelkirsche’ is an old variety widely distributed all over the world and first archived in 1850 in Europe, while both cvs ‘Brooks’ and ‘6000CZ’ are from California, the latter specifically bred for cultivation in low-chill environments (Table 1).

Chilling was recorded and computed in four winters. Two contrasting winters 2012/13 and 2013/14 were selected at Campus Klein-Altendorf of the University of Bonn (6° 59’ E, 50° 37’ N, 180m asl) in the Meckenheim fruit growing area with a mean annual temperature of 9.8°C (Blanke and Kunz 2009). For the purpose of this paper, i.e. the Meckenheim fruit growing region is defined trough human activity and the climate along the 50°N latitude.

2.2 Experimental layout

Potted cherry trees of each variety were divided into eight groups of four trees each. Trees of the first three groups of each variety were placed in an unheated greenhouse in the autumn, while those of the second three groups were left outside in a cherry orchard to acquire chilling. For each group (orchard and unheated greenhouse), a control group with four trees of each variety was set up to establish tree responses under two environments. To assess the validity of the three chilling models, one group of cherry trees was exposed to i) 30% less chilling of the estimated optimum, ii) one to the assumed optimum value and iii) one to 30% additional chilling on top of the chilling optimum, as shown in table 1, to cater for all possible weather extremes, possibly associated with climate change. After the targeted chill accumulation, the trees were transported to a heated greenhouse with the natural photoperiod and diurnal temperature fluctuation (> 12°C) to prevent any further chilling and induce flowering. In the heated greenhouse, flower buds were counted on each tree. Full bloom was assessed every other day and is defined, when the cherry tree reached 50% open flowers of all flower buds each tree (BBCH 65; Meier 1994 equivalent to F2; Fleckinger 1955).

Table 1: Alleged chilling requirements and observed leaf drop dates of the three sweet cherry varieties under investigation and chilling applied in the experiment

Variety	Origin (country)	Winter climate at origin	Alleged chilling hours (CH)	Chilling applied in experiment	Begin leaf fall in 2012/2013
‘6000 CZ’	California, USA	Mild maritime Mediterranean	ca. 500 CH	300 CH, 500 CH, 700 CH	30 October 2012/ 25 October 2013
‘Brooks’	California, USA	Mild maritime Mediterranean	411 CH*, 720 CH**	500 CH, 800 CH, 1,100 CH	14 October 2012/ 16 October 2013
‘Schneiders späte Knorpelkirsche’	Germany	Cold Continental	ca. 1,400 CH***	1,000 CH, 1,400 CH, 1,700 CH	20 October 2012/ 23 October 2013

*Alburquerque et al. 2008, **Gratacós & Cortés 2007, ***Luedeling et al. 2013

2.3 Chilling metric calculation

Three models are commonly used to describe chilling of trees, e.g. climate change: The Weinberger Chilling Hours Model (Weinberger 1950), the Utah Model (Richardson et al. 1974) and the Dynamic Model (Fishman et al. 1987; Erez et al. 1990). Chilling Hours, Chill Units and Chill Portions were computed in collaboration with the agroforestry unit in Nairobi (Kenia) using chillR package (Luedeling et al. 2013) for the R programming language (R Development Core Team, 2015); hourly temperature data was obtained from our on-site temperature loggers (Datahog 2, Skye Ltd., Pontys, Wales, UK) 2 m above ground before the major drop of autumn temperatures on 22 October 2012 and on 30 October 2013 (Fig. 1).

3. Results

3.1 Chilling availablility in the orchard and unheated greenhouse in a warm and in a cold winter

Regional environmental changes in the Meckenheim fruit growing region include warmer winters as a result of global warming (Blanke and Kunz 2009). Eight climate scenarios for each variety and year were employed. Two of the four winters proved ideal for the scope of the investigation, because they differed in temperature by over 10 °C on a daily average at the beginning of December, in mid-January and the end of February (red dotted line in Fig. 1). On average, the winter of 2012/13 was 2.6 °C colder than the winter of 2013/14 (Table 2). This is very uncommon for two consecutive winters in the temperate climate zone, but an ideal situation for the present study.

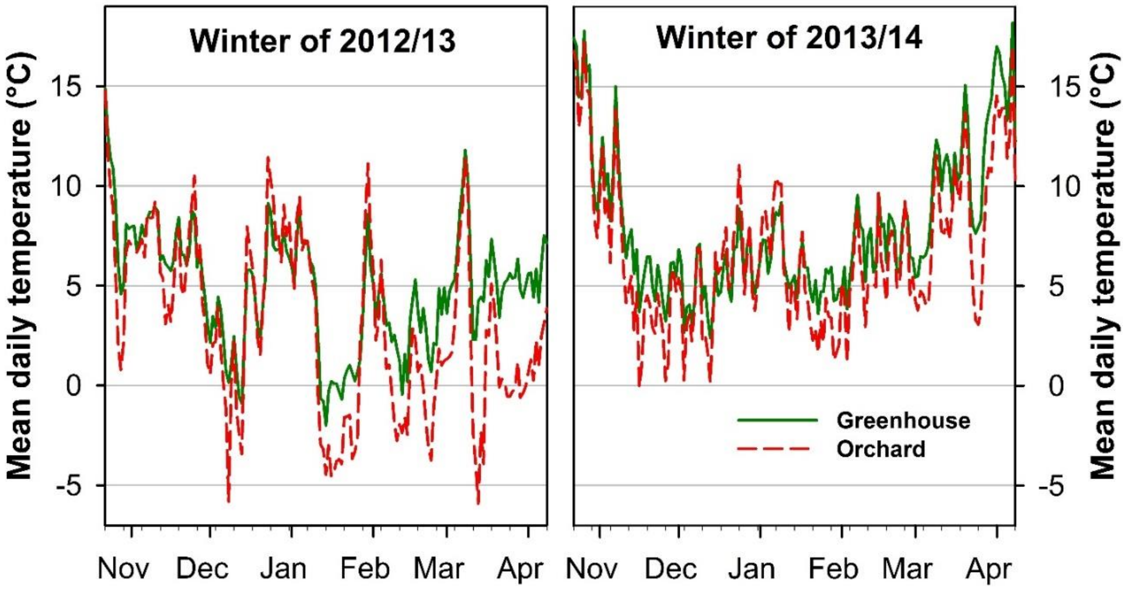

Fig. 1 Daily average temperatures in a cold (2012/13) and in a warm winter (2013/14), respectively in an unheated greenhouse (simulating global warming) and in the orchard near Bonn, Germany (50.5°N).

Table 2: Chilling in the orchard and the unheated greenhouse during winter 2012/13 and 2013/14

Date	Climate scenario	Chilling Hours	Chill Units	Chill Portions	Avg. Temperature in °C* (Oct - Feb)
winter 2012/13	Orchard	1,489	1,688	81	3.4
winter 2013/14	Orchard	1,997	2,281	94	6.0
winter 2012/13	Unheated Greenhouse	1,843	1,921	92	4.7
winter 2013/14	Unheated Greenhouse	1,852	2,148	88	7.0

*chilling period as proposed from beginning of leaf fall (22 October till 28 February)

Unexpectedly, the cold winter (relative to the average winter of 4.0°C) in the present fruit growing region (2012/13) provided ca. 25% **less** chilling (ca. 1,500 CH vs 2,000 CH) than the warmer winter (2013/14) in the orchard in all three chilling models (CH, CU, CP; Table 2). This is, because temperatures in the cold winter were often below the temperature range indicated as physiological effective for chill accumulation even for high chill cherry trees such as cv. ‘Schneiders späte Knorpelkirsche’.

This interpretation was confirmed by the results from the unheated greenhouse, used to simulate climate change, where the number of Chilling Hours (CH) in the cold winter resembled that in the warm winter (ca. 1,850 CH, Table 2). However, the Chill Units (CU) in the warm winter in the unheated greenhouse exceeded those in the cold winter. The situation reversed, when the Dynamic Model was used and resulted in fewer Chill Portions (CP) in the warm winter. To our knowledge, such a discrepancy between larger CU and lower CP values in the warm vs cold winter (in the unheated greenhouse) has not been reported before and maybe attributed to regional speciality with frequent daytime temperatures of up to 12°C, which are differently weighted in the Utah- (ca. 9-12°C= factor 1.0) and in the Dynamic Model (ca. 6-8°C= factor 1.0).

3.2 Regional environmental change: Reversal of chilling availability?

In the cold winter (2012/13), chilling computation resulted in ca. 1,500 CH, 1,700 CU or 81 CP in the orchard. However, chilling availability increased to ca. 2,000 CH, 2,300 CU or 94 CP in the winter 2013/14 (Fig. 2), the warmest winter in the Meckenheim fruit growing region. In contrast to an expected increase in chilling in the unheated greenhouse with the 1°C temperature increment over (7°C vs 6°C; Table 2), our experiment showed a decline in chilling availability in all the chilling models (Fig. 2). In years with up to 6°C average winter temperature (22 October 2013 till 28 February 2014) in our fruit growing region, maximum chilling becomes available, whereas this situation reversed in years with average winter temperatures above 7°C (Table 2).

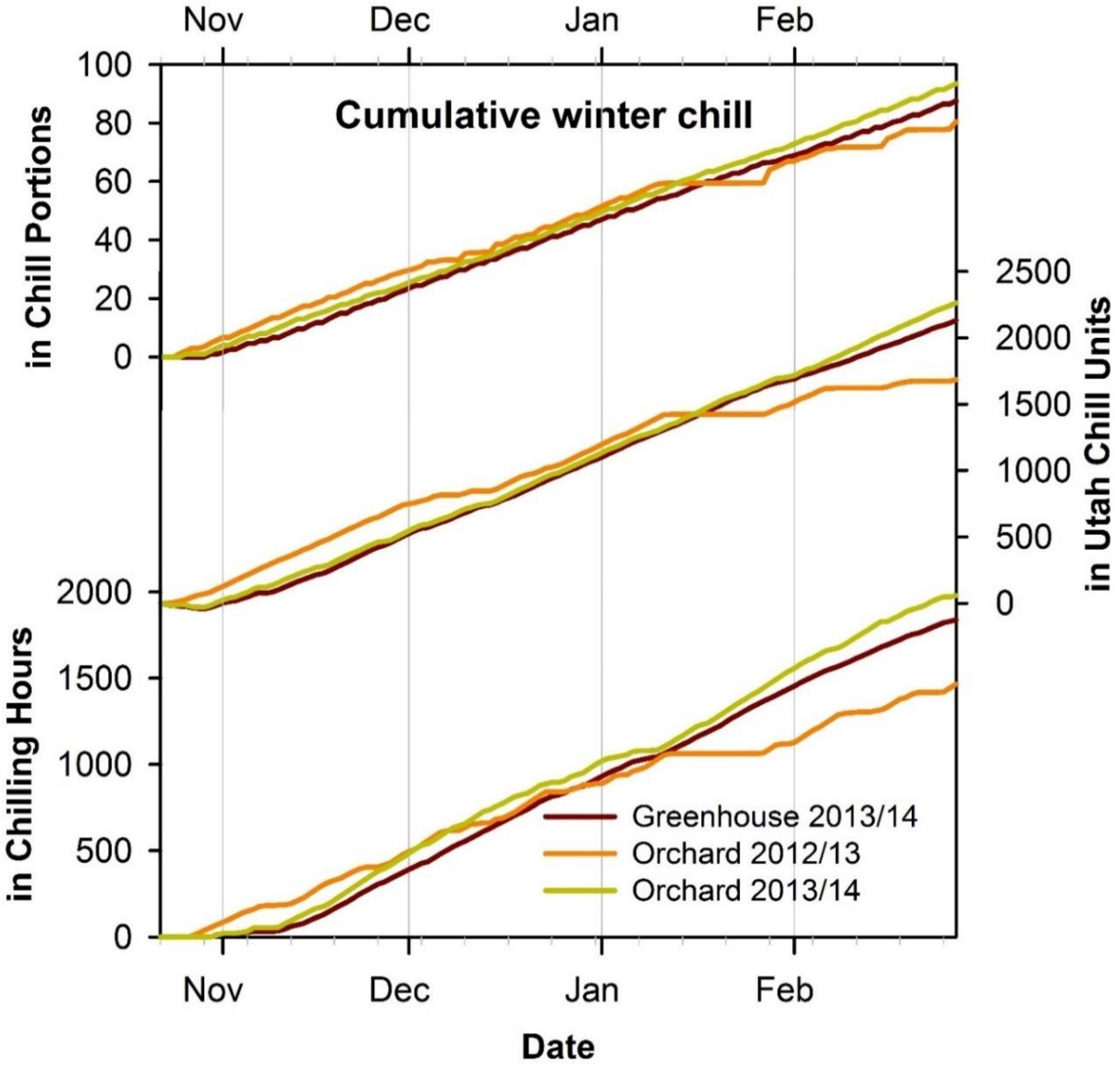

Fig. 2 Chilling development (daily accumulation of the Chilling Hours, Chill Utah and Chill Portion model) in a cold (2012/13) and a warm winter (2013/14) in the orchard and in an unheated greenhouse (2013/14) (simulating global warming) near Bonn, Germany (50.5°N).

3.3 Determination of the physiological starting point for chilling accumulation in the autumn

The second objective of the work was to resolve the uncertainty when trees commence to accumulate chilling and define development stages for low, medium and high chill varieties for the three chilling models. This is based on our regional records obtained of the leaf fall, temperature and chilling and flowering results of three cherry varieties. Leaf fall, as one candidate for the start of chill counting, commenced on 14 October for cv.

‘Brooks’, 20 October for cv. ‘Schneiders späte Knorpelkirsche’ and 30 October 2012 for cv. ’6000CZ’; in 2013, the leaves commenced to fall on 16 October for cv. ‘Brooks’, 23 October for cv. ‘Schneiders’ and 25 October for cv. ’6000CZ’ (Table 1). Interestingly, the *end* of the leaf fall period in our region coincided with the drop in temperature and was inversely related to the chilling requirement of the particular variety. Since the *beginning* of leaf drop preceded the drop in autumn temperature by over a week (7- 10 days; Table 1) in both cold and warm winters, we propose the beginning of leaf drop as start point for counting chill accumulation in all three chilling models and varieties, irrespective of their chilling requirement. This allows ca 10 days leeway and may be transferable to other tree fruit and forestry crops in various regions.

3.4 Development of a concept of chilling ranges

The third objective of the work was to resolve the uncertainty and define chilling ranges for low, medium and high chill varieties in each of the three chilling models instead of a fixed value as commonly reported. In our experiment, cherry trees cv. ‘6000CZ’ receiving <300 CH, cv. ‘Brooks’ <500 CH and cv. ‘Schneiders späte Knorpelkirsche’ <700 CH were unable to flower (result not shown), possibly due to lack of chilling causing acrotony (figure 3b). These values are equivalent of 50% of the assumed chilling optimum of the respective cultivar (table 1) under our regional environmental conditions of double dormancy (sequence of cold - warm - cold winter temperatures).

The experiment with potted sweet cherry trees in the orchard and the unheated greenhouse showed that the low chill sweet cherry cv. ‘6000CZ’ required a range of 402-483 Chilling Hours (CH), 465-684 Chill Units (CU) or 22.3-26.6 Chill Portions (CP) for natural flowering, averaged over four including the two contrasting winters (Table 3). The medium chill cv. ‘Brooks’ required 779-941 CH, 819-1,267 CU or 37.9-54.4 CP for a natural flowering (BBCH 65; F2; 50% open flowers). The wide range (up to 40%) of Chill Units and Chill Portions in cv. ‘Brooks’ is due to a combination of different temperature ranges and their different weighting, in both models and the climate variability, expressed in the two different winters in 2012/13 (cold) and 2013/14 (warm) for the Meckenheim fruit growing region.

Table 3: Range of chilling requirement of the low chill cvs ‘6000CZ’, mid chill ‘Brooks’ and high chill ‘Schneiders späte Knorpelkirsche’ of potted trees under the natural photoperiod in the Meckenheim fruit growing region.

Cherry variety	Literature estimate fixed value in Chilling Hours (CH)	Our observed range in Chilling Hours (CH)	Our observed range in Chill Units (CU)	Our observed range in Chill Portions (CP)
‘6000CZ’	500 CH (estimate)	402-483 CH	465-684 CU	22.3-26.6 CP
‘Brooks’	411 CH*, 720 CH**	779-941 CH	819-1,267 CU	37.9-54.4 CP
‘Schneiders späte Knorpelkirsche’	1,400 CH***	941-1,494 CH	1,267-1,696 CU	54.4-79.3 CP

*Alburquerque et al. 2008, **Gratacós & Cortés 2007, ***Luedeling et al. 2013

Interestingly, the cv. ’Schneiders späte Knorpelkirsche’ required about 941-1,494 CH, 1,267-1,696 CU or 54.4-79.3 CP for natural flowering; this is also a wide relative range (25-37%) of CU, CP and CH values for the variety with the highest chill requirement (Table 3). The responses to environmental changes of cherry varieties with different chilling requirement confirmed our hypothesis that a single fixed value or threshold is insufficient to properly describe the variation in dormancy and flowering; these chilling ranges represent the minimum values for a natural flowering (BBCH 65; F2 Fleckinger stage; Meier et al. 1994; Fleckinger 1955).

4. Discussion

The results identified a) presently maximum chilling availability in the Meckenheim fruit growing region with an expected decrease in the future as shown by the climate change simulation, b) the beginning of leaf fall as a suitable candidate for the initiation point of the chill accumulation for all three chilling models rather than a fixed calendar date (Table 1) and c) ranges (rather than one fixed value) to accurately describe the chilling requirement of the three cherry cultivars under varying environmental conditions in autumn/winter. Our experiments with 160 whole trees accommodated acrotony, as a consequence of lack of chilling, with differential flowering of branches.

Fig. 3 Potted sweet cherry (*Prunus avium* L.) trees at Campus Klein-Altendorf, University of Bonn in the Meckenheim fruit growing region in the unheated greenhouse (simulate global warming)(a, left); Sweet cherry tree with insufficient chill showing flowering disorder (acrotony). Lower branches flowered 2 month before middle/upper part of the tree. (b, right)

Reduced chill induces retarded, uneven flowering of different sections of the tree (acrotony; figure 3b) with a high abortion rate, causing difficulties with insect pollination, subsequent fruit set and yield. Cherry usually requires two different cultivars with different s-alleles and synchronous flowering for successful pollination. Retarded flowering can result in beehives being withdrawn from the orchard and may coincide with adverse weather conditions like frost or heat depending on location (Kaufmann and Blanke 2016).

4.1 Effects of the unheated greenhouse in comparison to global warming

The unheated greenhouse, employed to simulate climate change i.e. global warming, provided the same temperature difference of an averaged of ca. 2.4°C between the cold and the warm winter as in the orchard (Table 2). The average orchard temperatures in the warmer winter (6.0°C) exceeded those in the unheated greenhouse in the cold winter (4.7°C; Table 2), indicating that the natural variation in regional weather patterns exceeded the simulated climate change in the unheated greenhouse. From the results (Table 3) with a simulated warmer winter it can be derived that the maximum chill accumulation was achieved in the winter 2013/14 and warmer winters may reduce the available chill in our region in line with Luedeling et al. (2011a).

4.2 Identification of the initiation point of chilling accumulation under natural orchard conditions (diurnal temperature/ photoperiod)

All calculations of the chilling models and their outcome relate to a theoretical initiation point making it relevant for all chilling issues, especially in low chill crops grown in Mediterranean or Californian climate in warm winters with a slow drop in temperature and a short winter. Hence, the second objective was to determine the initiation point of chill accumulation in the trees, based on our records of the cessation of the shoot growth and leaf fall, or abrupt drop in temperature in the autumn as an alternative to a fixed calendar date in some of the literature.

Our proposal of using the beginning of the leaf fall as an initiation point for modelling chill accumulation (Table 1) is the first concept to define a developmental stage for this topic, irrespective of crop and chilling model, in a situation where the initiation point is not clearly defined (Luedeling et al. 2011a,b,c; Albuquerque et al. 2008; Couvillon and Erez 1985; Cesaraccio et al. 2004) or details of the beginning of the chill accumulation are missing (Alburquerque et al. 2008). Our approach is in contrast to a fixed calendar date (like 11 May in Chile) used in most studies (Guak and Neilsen 2013; Mahmood et al. 2000a,b; Gratacós and Cortés 2007; Couvillion and Erez 1985), which does not reflect the actual temperatures nor the biological development stage of the tree.

Our proposed initiation point of chill accumulation, viz. beginning of leaf fall, precedes the temperature drop into the chilling effective temperature range in the temperate climate zone. At the time of cessation of shoot growth, which could also be used as a chilling start point, the temperatures are well above the chilling effective range and buds are still developing during the end of fall; especially the stamens and pistils are still developing in September and October (Jackson 2003). In our fruit growing region, cherry harvest in July precedes leaf drop by ca. three month. Biologically, at the time of leaf fall the tree prepares for dormancy, buds are fully developed, and nutrients such as carbon and nitrogen translocated form the leaves to the tree trunk and roots (Tartachnyk and Blanke 2004). Our proposal is in line with that of Cesaraccio et al. (2004), who explained the better performance of their chill day model due to chill accumulation starting with a phenological stage (e.g. harvest or leaf fall), whereas the classical models begin accumulating based on weather data or a fixed calendar date; the authors used harvest in their calculations, which identifies our proposal of the beginning of the leaf fall as a compromise between harvest and the end of leaf fall.

From the results (Table 1; Fig 2) it can derived that, if we start to record the chilling accumulation prematurely, i.e. before trees start to shed their leaves, the tree is not receptive even for cool chilling effective temperatures, and vice versa, if the first chilling temperatures are missed out in the computations, this would lead to a lack of comparability; this physiological chilling interpretation has not been reported before.

The time of leaf fall can vary up to one month in a region depending on variety and mild or cold autumn, thereby excluding a fixed calendar date as initiation date for chilling. Our studies show that longer periods of warm temperatures in late fall/beginning of winter were associated with retarded leaf fall (comparing trees in the orchard to those in the unheated greenhouse) and delayed the start of chilling accumulation. Horticultural practises based on tree physiological knowledge include defoliation as a practical procedure to induce chilling sensitivity, although the nutrients have not been fully translocated from the leaf to the tree trunk in this technique.

Since artificial constant temperature and light conditions in controlled environment chambers may not represent whole tree responses and the winter conditions in an orchard (diurnal temperature and photoperiod), we deliberately exposed whole trees to natural temperatures, light, wind, humidity and rain. Ohashi et al. (2012) found that the effective temperature, viz. air-temperature plus wind chill and radiation is more significant in the prediction of flowering of sweet cherry trees in orchards in Japan than just the air temperature; in beech (*Fagus sylvatica* L.), light viz. the photoperiod can substitute chilling to some extent, but not in other trees (Heide 1993).

4.3 Chilling ranges as concept – regional variation in chilling

This work was based on the hypothesis that Chill Units, i.e. Chilling Hours, Chill Portions, cannot easily be transferred without modifications from region a to region b as consequence of regional environmental change, possibly because of discrepancies in temperature fluctuations or temperature regimes and variations in the beginning of the chilling records in the autumn (Hillmann et al. 2016; Luedeling and Brown 2011a). Our experiments show in a cold and a warm winter how the results, especially in the medium chill cv. Brooks, can vary depending on the chilling model and the winter weather. Our chilling ranges of 800-900 CH, 900-1,200 CU and 40-55 CP (Table 1), respectively, in the two contrasting winters at 50°N differed considerably from two studies in other regions, in Chile by Gratacós and Cortés (2007) and in Murcia, Spain by Alburquerque et al. (2008) with ca. two-fold lower single values of 411 CH, 566 CU and 36 CP. Alburquerque et al. (2008) employed cut branches from trees chilled outside and initiated forcing in an environment chamber at a constant temperature of 24°C without photoperiod. Gratacós and Cortés (2007) reported intermediate values of 720 Chilling Hours (CH) for the same cultivar at constant 6°C in the dark controlled environment chambers. The largest ca. two-fold discrepancy between the single chilling values for 'Brooks' became visible in the Weinberger Chilling Hours (800 CH in Meckenheim vs 411 CH in Spain) and in the Utah Model (900 CU vs 566 CU in Spain). Overall, the data discrepancies of chilling data indicates the inefficiency of available model to compute winter chill for fruit trees and consequently predict flowering. Our results indicate that the largest chilling values originate from the coldest regions, indicating their long term acclimation to regional climate. The results of the present chilling experiment of 450-500 Chilling Hours (CH), 650-700 Chill Units (CU) or 25-27 Chill Portions (CP) (Table 3) for the Californian cv. '6000CZ' provided the first chilling values for this variety.

Similarly, no experimental data exist for cv. 'Schneiders späte Knorpelkirsche', with a high chilling requirement and a worldwide distribution under 20 synonyms in other regions, like '0900 Ziraat' in Turkey and 'Zing' in the USA. A Partial Least Squares (PLS) regression by Luedeling et al. (2013) of historical cherry bloom dates and winter temperatures over 40 years in the Meckenheim fruit growing region at Campus Klein-Altendorf (University of Bonn) resulted in estimates of 1,375 CH, 1,410 CU and 68.6 CP, which compares favourably with those obtained in our experiments with ranges of 941-1,494 CH, 1,267-1,696 CU or 54.4-79.3 CP (Table 2).

5. Conclusion

This work has produced the following insights about the effects of regional environmental change on flowering behaviour:

1. Maximum chill is available in the Meckenheim fruit growing region at the moment; any further increases in winter temperature will decrease the chilling availability to perennial crops
2. Beginning of leaf fall is proposed as initiation point of the chilling sensitivity of the trees for all three chilling models and all varieties, irrespective of their chilling requirement
3. Chilling ranges are preferred over a single fixed chilling value to accommodate differences in winters and environmental changes and transferability to a different growing region

Overall, certain fruit production regions may not remain suitable for traditionally grown high chill cherry varieties in the future due to climate change, with loss of winter chill a likely driver of this process.

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D Changes in carbohydrate levels and relative water content (RWC) to distinguish dormancy phases in sweet cherry[3]

1. Introduction

Climate change including global warming is associated with warmer winters in most parts of the world (IPCC, 2013), particularly in the stone fruit growing regions. Many perennial, deciduous trees undergo a dormancy period during winter, when they shed their leaves after translocation of their nutrients such as carbon and nitrogen skeletons into the woody perennial parts of the tree (Tartachnyk and Blanke, 2004). Chilling, i.e. a cold period during winter, is a prerequisite for flower initiation in spring in perennial plants (Coville, 1920; Lang et al., 1987; Meir et al., 2016, Vegis, 1964) and maybe hampered by climate change (Blanke and Kunz, 2009; Luedeling et al., 2013). Release from dormancy is associated with chilling fulfillment. Carbohydrates play a major role in the control of bud growth and development during dormancy and dormancy release (Bonhomme et al., 2005; Cottignies, 1986; Hillmann et al., 2016; Marquat et al., 1999). To our knowledge, no study combined the physiology of carbohydrate metabolism, relative water content (RWC) in floral buds and chilling accumulation with the dormancy phases introduced earlier by Lang et al. (1987). Cherry as one of the temperate-zone tree species most affected by global warming, e.g. warmer winter, and therefore lack of winter chill in fruit growing regions (Kaufmann and Blanke, 2017a and b) is used as model crop. This research paper is based on the hypothesis that changes in chilling status are associated with changes in the carbohydrate dynamics, relative water content in reproductive buds, thereby relating mechanism to functioning.

Hence, the objective of the present work was to investigate the changes in carbohydrate levels and water relations of cherry buds to identify and distinguish the dormancy phases (para-, endo, eco-dormancy) on a plant physiological base, and to determine transition

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[3] Kaufmann, H., Blanke, M.M., 2017. Changes in carbohydrate levels and relative water content (RWC) to distinguish dormancy phases in sweet cherry. J. Plant. Physiol. 218: 1-5

points between dormancy stages using ca. 10,000 flower buds from nine varieties and bi-weekly sampling over two years. To our knowledge, no study of this kind has been reported in the literature, linking physiological processes of dormancy to concomitant temperature records and chilling status in the orchard. The synthesis of carbohydrate dynamics, changes in relative water content (RWC) in floral buds and chilling status is used to calculate carbohydrate ratios, and to elaborate four transition points as thresholds and representatives of the current dormancy stage and integrated them in a schematic of the changes in relative water content and carbohydrate that drive dormancy and dormancy release.

2. Materials and methods

2.1 Plant material

Ten-year-old sweet cherry (*Prunus avium* L.) trees cv. ‘Titan’, ‘Regina’, ‘Chelan’, ‘Kordia’, ‘Skeena’, ‘Burlat’, ‘Schneiders späte Knorpelkirsche’, ‘Benton’ and ‘Rubin’ on dwarfing Gisela 5 rootstock were grown on the experimental orchard of the University of Bonn, Campus Klein Altendorf, Germany (50°N). Temperatures were recorded using Datahog 2 (Skye Ltd., Pontys, Wales, UK) over the winter 2014/15 and 2015/16 and used to calculate the available chilling (Weinberger, 1950). Overall, ca. 10,000 flower buds were sampled bi-weekly from two-year-old wood in one to two meter tree height from these nine sweet cherry varieties from leaf drop to bud break in spring, weighed for subsequent assessment of RWC and stored at -25°C until further processing.

Three 50 cm long branches of each variety were taken concomitantly to the bud samples for carbohydrate analyses in bi-weekly intervals. The cut branches were kept in a flask of water (recut and water changed twice a week) and were forced in a heated greenhouse (>16°C) as a reference, if the chilling requirement was fulfilled.

2.2 Carbohydrate analysis of cherry flower buds

Carbohydrates were analysed in complete flower buds according to Saied et al. (2004). Samples were freeze-dried (Christ Gamma 1-16 LSC, Osterode, Germany), ground (Retsch MM200, Retsch GmbH, Haan, Germany) and dissolved in distilled water as solvent (0.3-0.5 g DM in 5 mL H_2O 0.1 g/mL). The extract was incubated for 60 minutes in a water bath at 60°C (stirred after 30 min) and the solution centrifuged (Heraeus Multifuge X3 FR, Thermo Fischer Scientific, Darmstadt, Germany) at 4,500 g for 15 min. The supernatant was used for sugar analysis and the pellet was kept for starch analysis. For the sugar determination, 4 mL chloroform was added to the supernatant, stirred and centrifuged for 15 min at 2,000 g. The supernatant was stored at -25°C until analyzing via HPLC. Samples were thawn and centrifuged at 16,100 g in a benchtop centrifuge (Eppendorf 5415R, Eppendorf AG, Hamburg, Germany). This supernatant was analysed for sucrose, glucose, fructose, raffinose, sorbitol and starch using HPLC (ChemStation, Agilent Technologies, California, USA using Software B.02.01.SR2) with a column (type Carbohydrate CA2+ 300 x 8 mm) and a pre-column heated to 75°C with a flow speed of 0.8 mL/min at 30 bar pressure and examined in a RI detector. The starch pellet was dissolved with 2 mL of H_2O, stirred and centrifuged at 4,500 g for 15 min twice and the supernatant was discarded. The pellets were rehydrated with 2 mL of distilled water and heated for two hours in a water bath at 100°C. Centrifuge tubes were cooled down and 2 mL of acetate buffer (0.2 M pH 4.6) and 50 µl amyloglucosidase (250 mg in 10 mL acetate buffer) was added, stirred and kept in a water bath for 20 h at 60°C. This solution was centrifuged at 4,500 rpm for 15 min. The supernatant was treated like the sugar samples with chloroform as described above. Representative changes in carbohydrate levels of the two years are presented in figure 1 and 2 using one cultivar for each group in the same year.

3. Results

The objective of the present work was to investigate the changes in relative water content (RWC) and carbohydrate dynamics of cherry buds to identify and distinguish the dormancy phases (para-, endo, eco-dormancy) on a plant physiology base and to determine transition points between dormancy stages for orchard management practices.

3.1 Carbohydrate dynamics of cherry flower buds during dormancy

Based on these 10,000 flower buds and their carbohydrate dynamics, two groups of cherry varieties could be distinguished analogue to the distinction between (mostly containing larger amounts of starch) climacteric and non-climacteric fruit (with lower starch levels). Group 1 includes ‘Schneiders’, ‘Burlat’, ‘Kordia’ and ‘Skeena’ with an initial lower and constant starch and sorbitol (10 to 15 mg/g DW) content, whereas group 2 includes cherry varieties ‘Regina’, ‘Chelan’ and ‘Tieton’ with an initial larger starch and sorbitol content (>20 mg/g DW), both decreasing, to a different extent, over the time. Carbohydrate dynamics of cv. ‘Schneiders’ in the winter 2014/15 was chosen as a representative example of group 1 and of cv. ‘Regina’ as representative of group 2. For each of the two groups, we propose five developmental stages with four transition points during dormancy.

3.2 Carbohydrate dynamics of cherry flower buds during para-dormancy

A Para-dormancy is the first dormancy phase in fruit trees in autumn with a continuous inhibition of lateral growth. In this para-dormancy, the contents of glucose, fructose and sorbitol diminished to a low level of about 10-15 mg/g DM (figure 1); starch contents either remained constant at levels of 10-15 mg/g DM (group 1), or decreased to about half the value (e.g. from 20-25 mg/g DM to 10-15 mg/g DM; group 2). The most pronounced difference between group one and two was detected in this early para-dormancy phase and the initial content of starch and sorbitol. Water content remained steady in this para-dormancy between 51 % and 54 %, irrespective of group (figure 1 and 2). The first transition point (table 1) between para- and subsequent endo-dormancy is characterized as a change from a constant level (group 2) or a decrease (group 1) to a sharp increase in hexoses and sorbitol and a drop of starch content.

Table 1. Characterization of transition points during dormancy stages of trees

Transition Point	Content changes	Chilling
1. Para- to d-endo-dormancy	Trough in hexoses	Ca. 50 CH
2. D-endo to f-endo-dormancy	Trough in starch	Ca. 650 CH
3. F-endo to eco-dormancy	Trough in water content	Ca. 1,000 CH
4. Eco-dormancy to forcing	Water content increase continues	Ca. 1,550 CH

3.3 Carbohydrate dynamics of cherry flower buds during d-endo-dormancy

A Deep-endo-dormancy is the second phase starting at the beginning of winter, when the tree accumulates cold temperatures and growth is suppressed until this phase has been completed. In this d-endo-dormancy, the contents of glucose, fructose and sorbitol increased from the low level in the para-dormancy phase. Both hexoses *increased* about 1.5 fold in concentration (up to 25 mg/g DM), whereas sorbitol *increased* over proportionally by ca. 2 fold to about 30-35 mg/g DM (figure 1 and 2). In this phase, starch contents *decreased* to below 5 mg/g DM in both groups. Water content decreased in this endo-dormancy from 52% to 49 % and from 53% to 50%. The second transition point between the deep d-endo-dormancy and facultative f-endo-dormancy is characterized as the changes in both hexoses (increase) and starch (decrease) in both groups (figure 1 and 2). From the chilling computation in our concurrent forcing experiment, our cherry trees had reached ca. 650 CH, at this stage, i.e. insufficient chilling for flowering of temperate forest and fruit trees and absence of flowers on the forced cut branches.

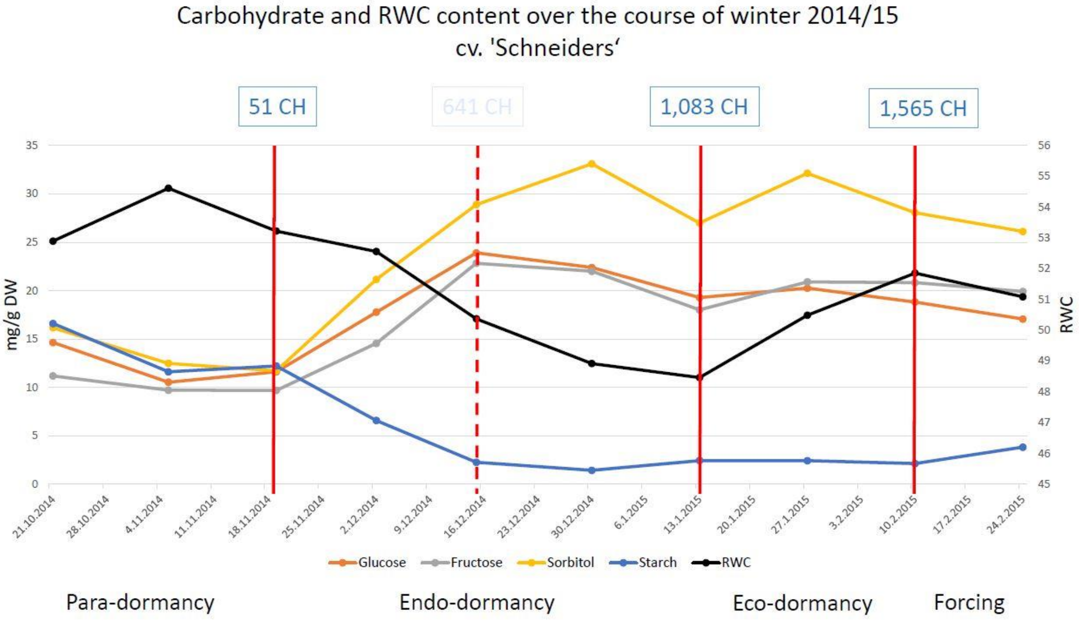

Figure 1. Carbohydrate, relative water content chilling availability and dormancy phases during the dormant period (2014/15) of floral buds of cherry cv. 'Schneiders' representing group 1 (n= 100 floral buds per sampling date and SDs)

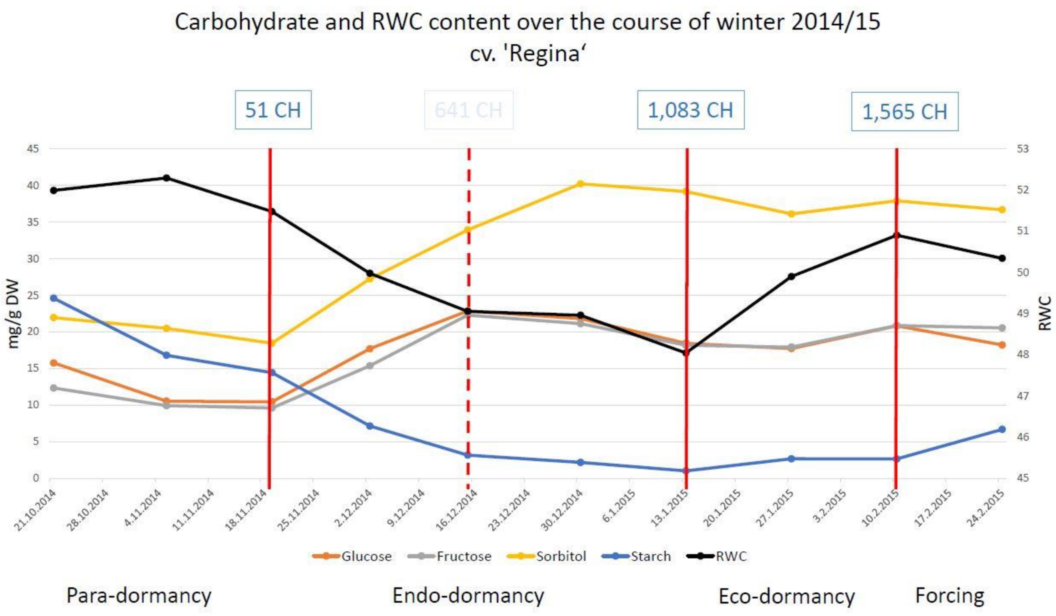

Figure 2. Carbohydrate, relative water content chilling availability and dormancy phases during the dormant period (2014/15) of floral buds of cherry cv. 'Regina' representing group 2 (n= 100)

3.4 Carbohydrate dynamics of cherry flower buds during f-endo-dormancy

Facultative endo-dormancy is the second part of endo-dormancy and is characterized by the transition to eco-dormancy. In this f-endo-dormancy, the contents of glucose and fructose *decreased* slightly from the d-endo-dormancy phase. Sorbitol, however *increased* further and peaked at 35 mg to 45 mg/g DM (figure 1 and 2). In this phase, starch contents remained below 5 mg/g DM in both groups. Water content decreased to a trough of about 48% at the end of this f-endo-dormancy (figure 1 and 2). After the decrease in relative water content (RWC) during f-endo-dormancy, this trend reversed with an increase in water content. The third transition point at the end of the f-endo-dormancy phase identifies the start of the rehydration of the flower bud. This third transition point between f-endo-dormancy and eco-dormancy is characterized by ca. 1,000 Chilling Hours, which coincides with the minimum chilling requirement of these cherry varieties. Our concomitant experiment with branches cut at this third transition point, forced in a heated greenhouse, reached sufficient bloom flowering (colour supplement).

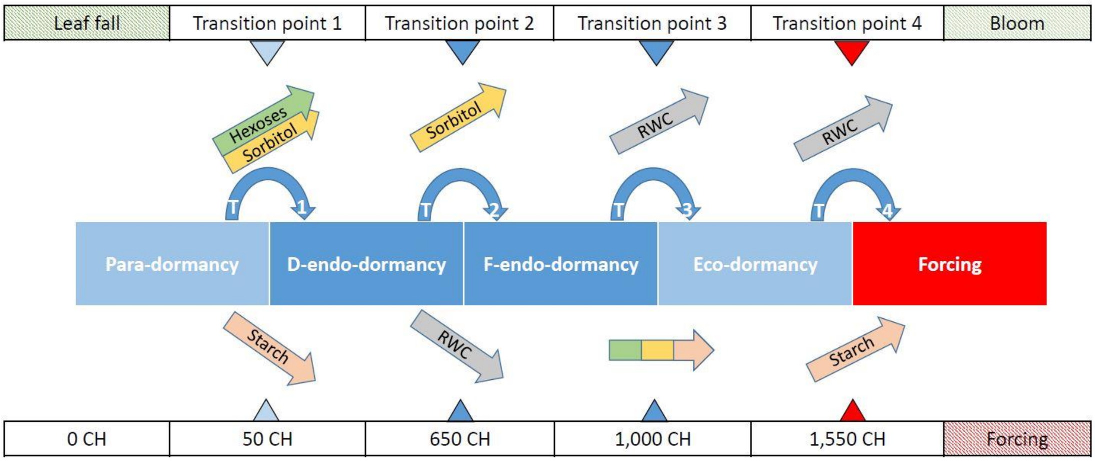

Figure 3. Visualization of RWC, carbohydrate dynamics, chill accumulation and transition points

3.5 Carbohydrate dynamics of cherry flower buds during eco-dormancy

After completing their chilling in the endo-dormancy phases, trees enter into eco-dormancy, in which environmental factors like warm temperatures and longer photoperiod can initiate flowering. In this eco-dormancy, the contents of glucose, fructose, sorbitol and starch remained constant (figure 1 and 2), whereas the relative water content (RWC) increases to about 50% (figure 1 and 2). This fourth and last transition point between after endo-dormancy is characterized by a further increase in water content and coincided with ca. 1,550 Chilling Hours (CH), which provides optimum chilling for cherry varieties. The concomitant forcing experiment with branches cut at this fourth transition point, forced in a heated greenhouse (>16°C), reached natural bloom initiation after 8,000 - 10,000 growing degree hours (GDH).

4. Discussion

4.1 Relationship between carbohydrates and autumn weather

The cooler autumn temperatures from 23rd September onwards (Figure 4) indicate the induction of starch breakdown from 29th October. The resulting increase in hexoses in the first year also contribute to cold hardiness of flower buds, in our cherry growing climate (50°N), where the first frost occurred on 25th November, i.e. one month later (Figure 4)

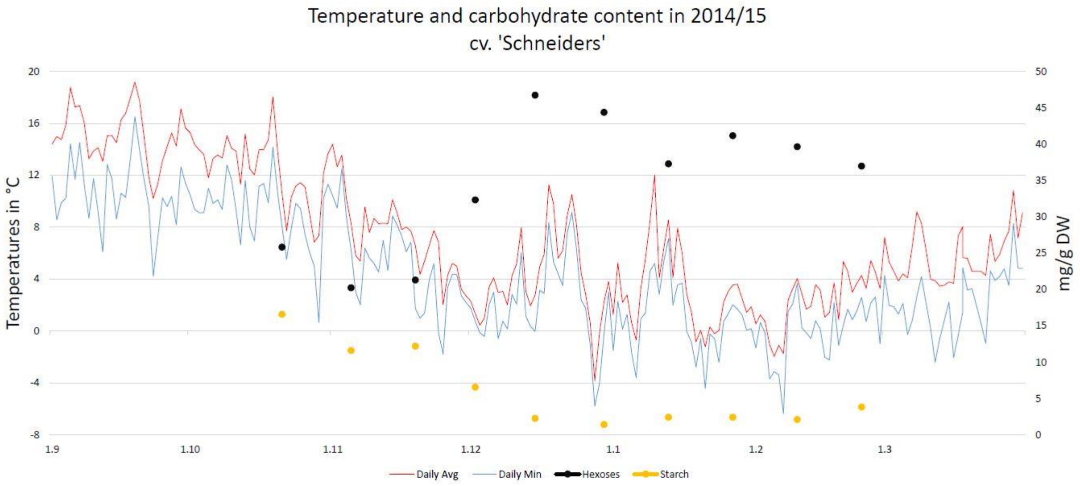

Figure 4. Daily average and minimum temperature (continuous lines) in relation to starch breakdown and hexose biosynthesis in floral cherry buds (dots) from 1 Sept 2014 until 1 April 2015

4.2 Visualization of the dormancy stages

Based on previous definitions of dormancy (Faust et al., 1997; Lang et al., 1987), we aimed to visualize the four stages of winter dormancy and relate them to the degree of dormancy degradation (Figure 5). In the first proposed phase para-dormancy, growth can resume depending on environmental factors, whereas growth comes to a standstill in both endo-dormancy phases, when the tree accumulates winter chilling. In the eco-dormancy phase, favourable environmental conditions such as warm temperatures and longer photoperiod lead to flower budburst.

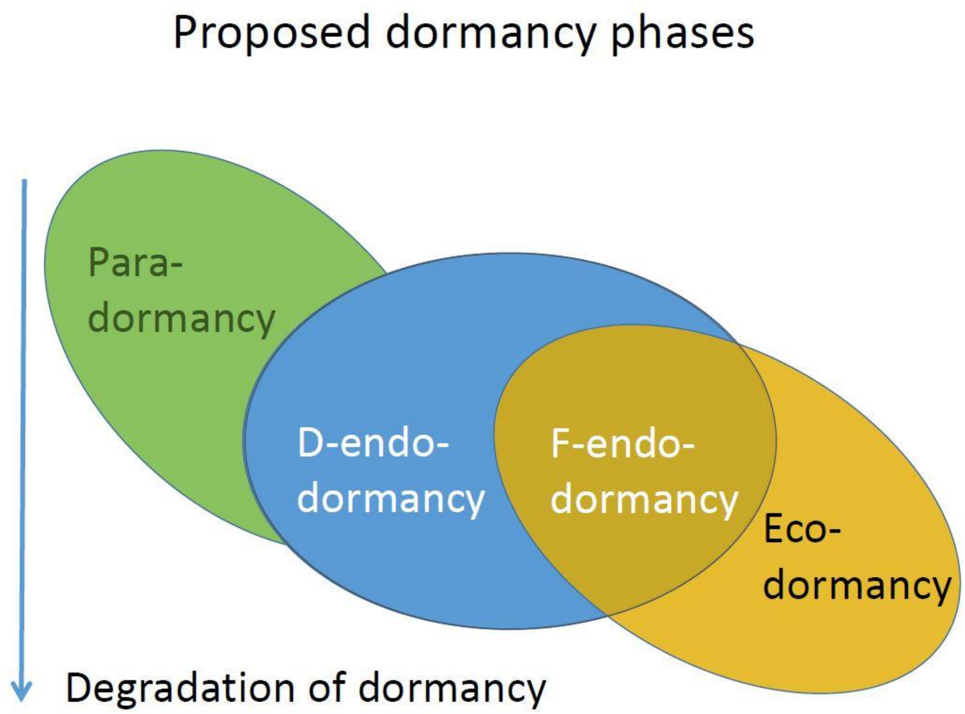

Figure 5. Proposed scheme to visualize the four dormancy stages in relation to overcoming dormancy during the winter

4.3 Carbohydrate dynamics of cherry flower buds during eco-dormancy

Based on the carbohydrate dynamics in the flower buds during dormancy, we calculated carbohydrate ratios in order to more clearly identify start and end points of dormancy stages. The most prominent change was found between para-dormancy to d-endo-dormancy. This first transition point (table 1) is characterized by a several fold increase in different ratios (glucose: starch; fructose : starch) from <1 to ratios >1 up to 10. Concentrations of hexoses (pooled glucose and fructose) to starch increase from <2 up to 30 after transition point 1 (table 2). The advantage of the proposed ratios is that they maybe used in upscaling, since ratios are independent of absolute values.

Table 2. Ratios of hexoses (glucose and fructose) : starch for the two variety groups of cherry at the four transition points and subsequent forcing phase to identify the dormancy stages of trees

	Transition point 1	Transition P 2	Transition P 3	Transition P 4	Forcing
Group 1	1.7	20.6	15.3	18.6	9.6
Group 2	1.4	14.3	15.0	19.5	10.1

Our results (Figure 1-4) may be a valuable addition to a previous study (Keller and Loescher, 1989) on carbohydrate dynamics in cherry trees, which concentrated on trunk bark and also found decreasing starch and increasing sorbitol concentration during dormancy in spurs of two-year-old shoots without relating them to the individual dormancy phases or chilling status.

4.4 Carbohydrates in cherry buds during dormancy and climate change

Information on carbohydrate dynamics in trees is scarce, although cherry may be one of the most affected fruit crop by climate change (e.g. cv. 'Schneiders späte Knorpelkirsche' with a chilling requirement of up to 1,500 CH; Kaufmann and Blanke, 2017a and b). Global warming and hence warmer winters increase the risk of insufficient chilling in the majority of cherry growing regions (40-60°N), whereas more northern regions may see an increase in winter chilling (Luedeling et al., 2013). Our results identify four transition points and their plant physiological bases may be up-scaled to other woody species for future studies on climate change, chilling and possible bio-indicators to identify, when chilling is fulfilled for a tree.

5. Conclusion

The objective of this research paper was to relate mechanisms, i.e. sugar metabolism, to functioning. To our knowledge this is the first time that dormancy phases are clearly linked to all four parameters, carbohydrates, water status, temperatures and chilling hour accumulation; in addition, four transition points are suggested and clearly described as to their plant physiological meaning. A ratio of hexoses (glucose plus fructose) to starch content (< 2:1) appeared to be a potential indicator of the beginning of chilling (para-dormancy); a ratio of 14-20:1 typical for endo-dormancy, whereas the release from dormancy was associated with a decline to less than 10:1 at the end of winter (eco-dormancy). Future research will show how these proposals can be confirmed or modified for other locations, species and other climates.

6. Acknowledgement

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E Substitution of winter chilling by spring forcing for flowering using sweet cherry as model crop[4]

1. Introduction

Many horticultural crops such as apple, pear, plum and cherry, as well as strawberries and perennial vegetables such as *Asparagus* require chill, i.e. a period of cool temperature during the winter season to induce buds to flower in spring (Lang et al., 1987). Winter temperatures differ from the colder Scandinavian climate to the Mediterranean climate, the latter associated with possibly insufficient winter chilling (cold period) followed by a longer period of forcing. Climatic conditions in temperate zones, where cherry is grown, as an intermediate situation are characterized by variable winter chilling followed by a forcing period (Couvillon and Erez, 1985); recent climate change may reduce available chilling in temperate zones (IPCC, 2013). In the past, the emphasis of studies was on chilling, whereas the subsequent forcing received relative little attention. Hence, it remains unclear, whether shorter or longer chilling periods in the winter can be compensated or substituted, and to which extent, by longer or shorter forcing periods, to ensure flowering and hence yields in the orchard.

Among fruit crops, sweet cherry (*Prunus avium* L.) requires the greatest chilling with up to 1,500 Chilling Hours (CH) (Kaufmann and Blanke, 2017a), which is hence categorised as one of the most affected tree crop by environmental change and consequent temperature rises, particularly in warmer winters (Luedeling et al., 2011a) and was chosen here as model crop. In Southern France, mild winters with insufficient chilling led to an average of 32 % yield loss in sweet cherry (Millan et al., 2009). The relative portions of forcing and chilling requirements are difficult to quantify due to uncertainty of a number of issues. Past chilling experiments with cut branches in moist paper in the dark at a constant temperature in a cold chamber (Mahmood et al., 2000a; Albuquerque et al., 2008; Ramos et al., 2018) allow relatively easy quantification of chill effects, but

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[4] Kaufmann, H., Blanke, M., (2018) Substitution of winter chilling by spring forcing for flowering unsing sweet cherry as model crop. Scientia Horticulturae 231;00:00 (in print)

conclusions drawn from such artificial environments are difficult to apply to whole trees under natural weather and light viz. environmental conditions (photoperiod) (Mahmood et al., 2000b). To our knowledge, no study has been carried out with in-situ observations (over several years) on (potted viz. transportable) entire intact cherry trees of different varieties with a broad range of chilling requirement and exposed to 24 scenarios (different chilling and forcing conditions) each winter including natural winter temperature regimes and modified environmental conditions in an unheated greenhouse to simulate climate change.

The hypothesis of this work was that chilling and forcing can be substituted by each other to a certain extent and one can compensate for the other, irrespective of variety, a result, which can be used in upscaling and that negative temperatures contribute to chilling accumulation.

The objective of this project was to investigate the effect of diminishing available chill as a result of climate change on forcing accumulation. This study further aims to elaborate thresholds for minimum chilling fulfilment and its interaction with forcing accumulation. The work includes possible effects of slightly negative temperatures (0 °C to -5 °C) and the effects of simulated climate change with a predicted 2 °C global temperature increase (IPCC, 2013) on chilling availability in the temperate climate zone (50 °N), the major pome and stone fruit growing belt in Europe using climate sensitive sweet cherry as model crop. To achieve these goals, 160 potted sweet cherry trees of three varieties with a wide range of chilling requirements were raised over two years to initiate uniform flower buds before applying 24 chilling scenarios (8 scenarios per variety) per year followed by forcing to determine the effect on flowering.

2. Materials and methods

2.1 Location and environmental conditions

Klein-Altendorf Research Centre is located near Bonn, Germany (50 °N) with an averaged 9.8 °C annual temperature and a mild Westerly wind climate buffered by the Rhine valley during the winter (Blanke and Kunz, 2009). Chilling accumulated in winters 2012/13, 2013/14, 2014/15 and 2015/16 either outside in the orchard or in an unheated greenhouse to simulate recent climate change at Campus Klein-Altendorf of the University of Bonn (Table 1).

Table 1

Average (based on hourly records) temperatures in the orchard and unheated greenhouse during the chilling period in the winter

Date	Location	Winter Temperature in °C*
Winter 2012/13	Orchard	3.4
	Unheated Greenhouse	4.7
Winter 2013/14	Orchard	6.0
	Unheated Greenhouse	7.0
Winter 2014/15	Orchard	4.7
	Unheated Greenhouse	6.8
Winter 2015/16	Orchard	6.7
	Unheated Greenhouse	8.2

*22 October till 28 February

2.2 Materials - sweet cherry trees

A The 160 sweet cherry (*Prunus avium* L.) trees (colour supplement 1) were grafted on dwarfing GiSelA 5 rootstock and planted in 35 litre pots on 24 March 2011 in order to initiate uniform flower buds over the 1.5 years before chilling treatments commenced in October 2012. The sweet cherry varieties were chosen to cover their widest possible range in chilling needs, a high chill variety '*Schneiders späte Knorpelkirsche'*, '*Brooks*' as a medium chill and '*6000CZ'* as a low chill variety (Gratacós and Cortés, 2007; Luedeling et al., 2013; Kaufmann and Blanke, 2017b). The cv. '*Schneiders späte Knorpelkirsche*' is an old widespread variety and first archived in 1850 in Europe, while both cvs '*Brooks*' and '*6000CZ*' are from California, the latter especially bred for its low chilling environment.

2.3 Methods – Experimental layout and scenario description

Eight groups of four trees were formed for each variety. To acquire chilling, trees of the first three groups of each variety were placed in an unheated greenhouse in the autumn, while those of the second three groups were left outside in a cherry orchard. For these two groups, viz. unheated greenhouse and orchard, a control group with four trees of each variety was set up under the two environments. Each group of four trees equals one climate scenario; a certain amount of natural accumulated chilling and forcing in a heated greenhouse.

Chilling was assessed using beginning of leaf fall as physiological plant parameter (Kaufmann and Blanke, 2017b) with intact potted trees subjected to one of eight climate scenarios (Table 2) under natural conditions in terms of diurnal temperature and photoperiod fluctuations; this is in contrast to previous reports with cut branches stored in a refrigerator at a constant temperature without light. Our scenarios included exposure to either about 50 % less chilling of the estimated chilling optimum, or up to 50 % additional chilling on top of the chilling optimum to cater for all possible weather extremes, possibly associated with environmental change and areas of increased chill projection.

After the targeted chill accumulation was reached, the potted trees were transported from the orchard or the unheated greenhouse to a heated greenhouse with the natural photoperiod and diurnal temperature fluctuation (heated to >12 °C) to prevent any further chilling and subject the cherry trees to environmental conditions to start forcing and induce flowering. In the heated greenhouse, flower buds were counted on each tree and full bloom assessed, when 50 % of flowers for a tree opened (BBCH 65; Meier et al., 1994 equivalent to F2; Fleckinger, 1955). Groups viz scenarios are labelled as follows. The first letter of the scenario abbreviation denotes the cherry variety (C= ‘*6000CZ*’; B=’*Brooks*’; S=’*Schneiders*’), the first number the year (2012/13=1…2015/16=4), the second the letter location (orchard=O, unheated greenhouse=G) and last number the group in each year (Table 2).

Table 2

Sweet cherry varieties employed in the present experiment, their origin, chilling estimates, treatment (chilling exposure), treatment designation and time of leaf drop.

Variety	Origin (country)	Environment (winter climate) at origin	Alleged chilling hours (CH)	Chilling applied in experiment **(scenarios)**	Begin leaf fall in 2012 till 2015
‘6000 CZ’	California, USA	Mild maritime med	ca. 500 CH	250 CH up to 800 CH **(C2O2; C2G2)**	30 October 2012 25 October 2013 03. October 2014 22 October 2015
‘Brooks’	California, USA	Mild maritime med	411.5 CH*, 720 CH**	400 CH up to 1,400 CH **(B2O2; B2G2)**	14 October 2012 16 October 2013 01 October 2014 20 October 2015
‘Schneiders späte Knorpelkirsche’	Germany	Cold Continental	ca. 1,400 CH***	800 CH, up to 2,000 CH **(S2O2; S2G2)**	20 October 2012 23 October 2013 08 September 2014 16 October 2015

*Alburquerque et al., 2008; **Gratacós and Cortés, 2007; ***Luedeling et al., 2013

2.4 Computation of the three chilling models based on our own hourly temperature records

Chilling Hours, Units and Portions were computed from our own on-site meteorological data obtained at 10 minutes intervals from temperature loggers (Datahog, Skye Ltd., Pontys, Wales, UK) placed at 2 m height between the trees. Chilling computation started at the beginning of leaf fall, the period identified when the tree prepares for dormancy (Hillmann et al., 2016; Kaufmann and Blanke, 2017b) (Table 2). Chilling was computed using the Utah model, the Dynamic model and the Growing Degree Hours with the program “R” (“R” version 2.15.3, Lucent Technologies, USA) and R-chill package (Luedeling et al., 2011b). In the computation, we used the oldest and most widespread Chilling Hours model, which adds the number of hours with temperatures of 0 to 7.2 °C (Weinberger, 1950). It was originally developed for peaches in Georgia (USA), but is now applied to many other types of fruit crops in other climates without adaption. Since the Chilling Hour model originated from warm winter regions without frost, we computed two versions of the Chilling Hour model, the original approach (Weinberger,

1950) and our own modified version for an environment with slightly negative temperatures (-5 °C to 7.2 °C), typical for the temperate zone fruit growing belt along 50 °N. The third chilling model applied was the Utah model, also developed for peaches in Utah (Richardson et al., 1974), in which temperatures are weighted and especially warm temperatures can nullify already accumulated chilling. In the Utah model, effective chilling temperatures range from 1.4 °C to 12.4 °C and temperatures above 15.9 °C can overcome previous chilling effects. The Dynamic Model (Fishman et al., 1987; Erez et al., 1990) was also developed for peaches in Israel. It divides the chilling in a two-phase process. In the first phase, cold winter temperatures lead to a precursor of the chilling accumulation. This precursor is “vulnerable” and can either be negated by warm temperatures or taken into account (realized) as one Chill Portion (CP) by cool temperatures. These Chill Portions will then remain until the end of the winter. Physiologically, this model (CP) is based more on speculation rather than biological facts, but in comparison to the other models it seems to be plausible.

2.5 Forcing – using GDH

Once trees reached their chilling target, we transported the potted cherry trees to a greenhouse heated to >12 °C to start forcing; forcing progress was computed based on the Growing Degree Hours (GDH) model by Anderson et al. (1986), with a base temperature of 4 °C and maximum temperature of 36 °C with a maximum effective point of 25 °C until 50 % flowers of a tree opened. Trees, which had not flowered or abandoned their flower buds during 24,000 GDH of forcing, were categorised as not fulfilled their chilling requirement.

3. Results

The first objective of this present work was to investigate, to which extent forcing can compensate for chilling and vice versa and elaborate thresholds for minimum chilling fulfilment for natural flowering.

3.1 Interchangeability of chilling and forcing

Figure 1 shows 19 scenarios of winter chill, expressed as Chilling Hours (CH), versus forcing expressed as Growing Degree Hours (GDH), based on trials over four consecutive winters and three (low, medium and high chill) varieties. In the experiment, potted cherry trees had been kept either under natural environmental viz. winter conditions at 50 °N or in an unheated greenhouse (to simulate climate change) with natural day night light cycle. After the intended chilling accumulation, the trees were transported to a heated greenhouse to cease further chilling and start the forcing phase.

Figure 1 includes up 19 scenarios of different chilling to forcing relationships starting from 50 % less chilling up to 50 % more chilling than required by each variety. The blue curves for chilling accumulation in figure 1 start with excess chilling (+50 %) of the respective chilling needs and the lowest forcing at a minimum of ca. 4,000 GDH, irrespective of variety, to reach full bloom (Fig. 1). As trees had been exposed to less chilling, e.g. by earlier removal from the orchard or from the unheated greenhouse, their forcing requirement increased to reach full bloom. Minimum chill fulfilment ranged from 400 CH in the low chill, 550 CH in the medium chill and 750 CH in the high chill variety to reach full bloom. As a result, forcing increased from ca. 4,000 GDH to 11,000 GDH for low chill, 12,000 GDH for medium chill and to 13,000 GDH for high chill varieties to reach full bloom. In all these cases, the intersection of the chilling and the forcing curves in figure 1 was between 7,000 - 8,000 GDH, irrespective of the variety.

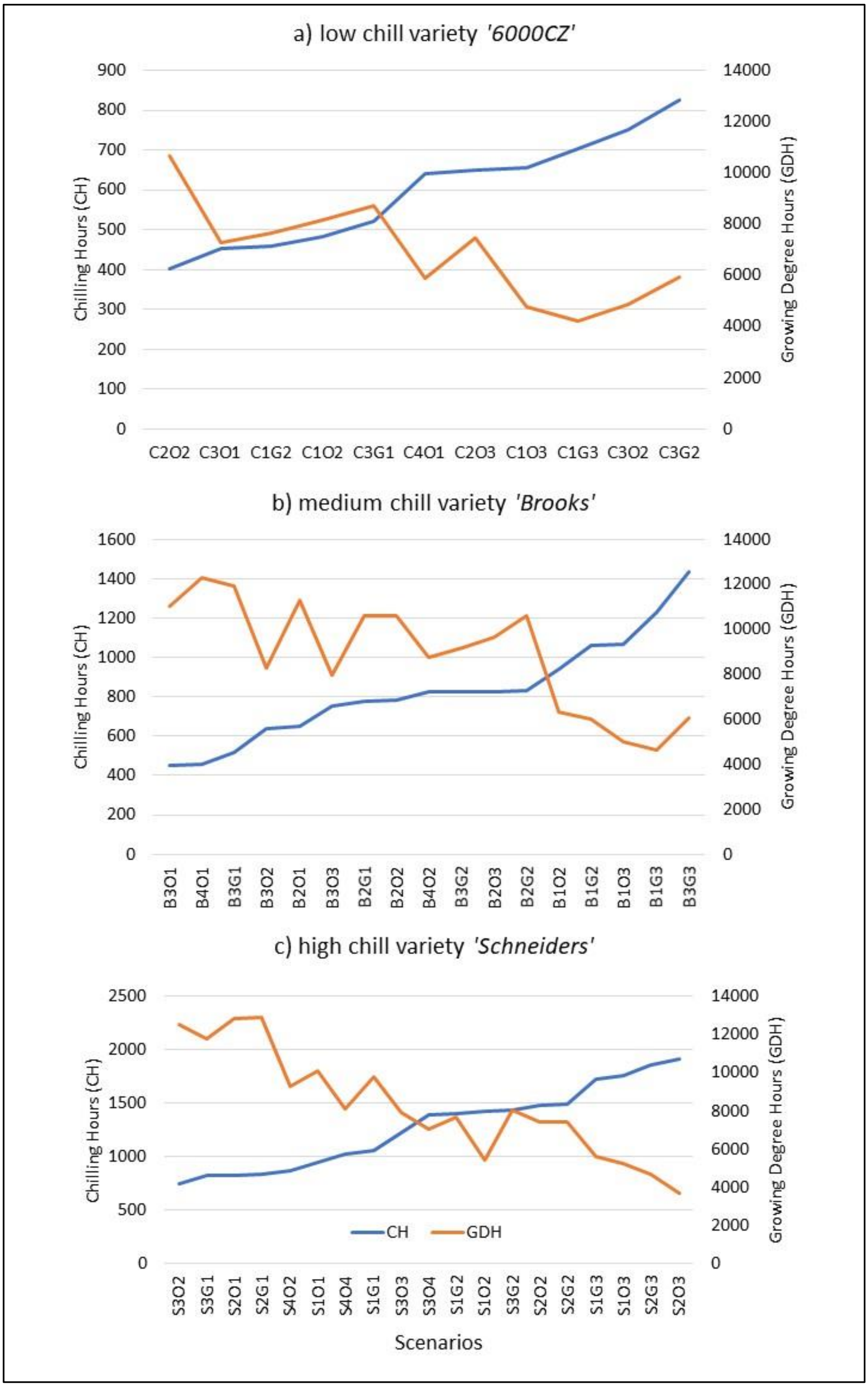

Fig. 1. Chilling and forcing scenarios (from winter 2012/13 till 2015/16) of a) low, b) medium and c) high chill cherry varieties in ascending chilling accumulation (scenarios with insufficient chilling did not reach flowering and are hence not shown).

3.2 Partial substitution of insufficient chilling by forcing between orchard and greenhouse

Trees in their natural environment in the orchard and in the unheated greenhouse (simulated climate change) showed commensurate courses in their chilling and forcing accumulation. Sweet cherry cv. ‘*Schneiders späte Knorpelkirsche*’ was chosen as typical representative starting at the scenario of ca. +50 % chill (1,900 CH) to -50 % chilling (750-800 CH) (Fig. 2). Optimum chilling was fulfilled at about 1,400 CH and about 8,000 GDH until ‘*Schneiders*’ reached full bloom, irrespective of environment (orchard or unheated greenhouse). The response curve of trees placed in an unheated greenhouse followed a more linear path of chilling and forcing due to fewer temperature fluctuations; both environments provided a minimum of ca. 800 CH chilling; any missing chill above 800 CH was compensated by forcing of 13,000 GDH or less to reach full bloom (Fig. 2). By contrast, excessive chilling of ca. 1,900 CH combined with lesser forcing of between 4,500 GDH in the unheated greenhouse (Fig. 2a) and about 3,500 GDH in the orchard (Fig. 2b) provided natural flowering. This discrepancy in forcing velocity could result from a greater portion of chilling below 0 °C accumulated in the orchard, which is not accounted for in the chilling model.

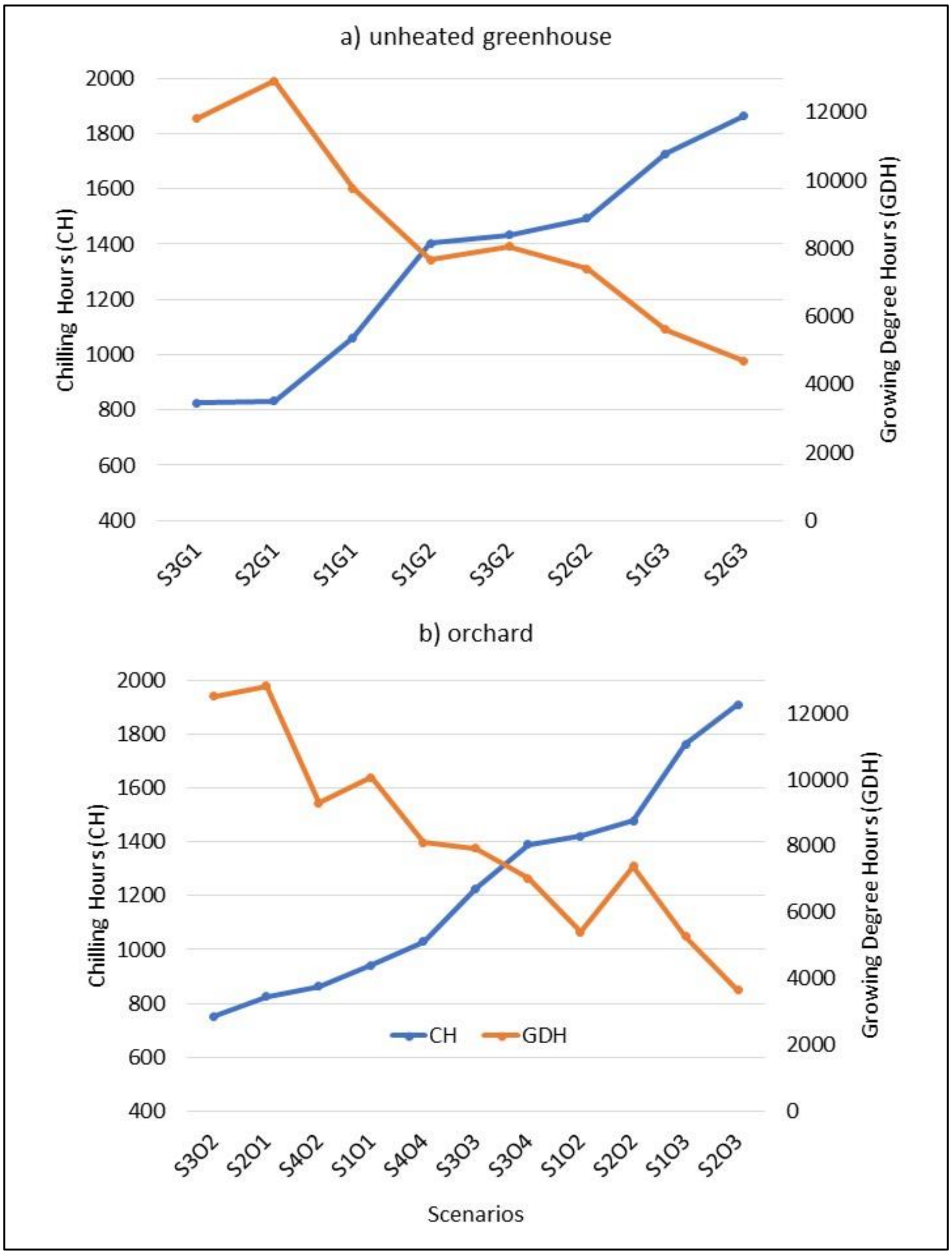

Fig. 2. Chilling and forcing of sweet cherry cv. '*Schneiders späte Knorpelkirsche*' in a) an unheated greenhouse (to simulate climate change) and the b) orchard over four consecutive winters from 2012 until 2016 in ascending chilling accumulation.

3.3 Ratios of forcing to chilling for natural flowering

In order to determine the environmental conditions required for natural flowering, the forcing to chilling ratios were computed from the chilling data in figure 1; synchronous flowering of a tree and trees in the orchard is required for successful pollination. Ratios of forcing (Growing Degree Hours; GDH) to Chilling Hours (CH) or Chill Units (CU) of greater than 15:1 for high chill varieties, 20:1 for medium chill varieties and 25:1 for low chill varieties were an indicator of retarded or sporadic flowering. Overall, 25 times more forcing hours (e.g. 10,645 GDH) than Chilling Hours (e.g. 402 CH) are required for natural flowering in scenario C2O2 (Fig. 1). With Chilling Portions this is equivalent to GDH to Chill Portions (CP) ratios greater than 200:1 for high chill varieties, greater than 300:1 for medium chill varieties and greater than 400:1 GDH to CP for low chill varieties (Table 3). Optimum chilling with natural flowering was found, when forcing (GDH) to chilling (CH or CU) ratios are lower than 5:1 or GDH to CP lower than 100:1 for *high* chill varieties. For *medium* chill varieties, GDH to CH or CU ratios were lower than 10:1 or GDH to CP was lower than 200:1; for *low* chill varieties ratios of GDH to CH or CU were lower than 15:1 or GDH to CP was lower than 300:1 to obtain natural flowering in the cherry trees (Table 3).

Table 3

Forcing to chilling ratios for optimum and minimum flowering

Ratio of Growing Degree Hours to	High chill variety	Medium chill variety	Low chill variety
Optimum forcing to chilling ratios for optimum flowering			
Chilling Hours & Chill Units	5	10	15
Chill Portions	100	200	300
Minimum forcing to chilling ratios for flowering			
Chilling Hours & Chill Units	15	20	25
Chill Portions	200	300	400

(Example for high chill varieties: with 7,500 GDH forcing and 1,500 CH chilling results in the optimum flowering ratio of 5 whereas 12,000 GDH forcing and 800 CH chilling result in minimum flowering ratio of 15; ratios in excess of 15 prohibit flowering)

3.4 Influence of slightly negative temperatures (0 °C to -5 °C)

The conspicuous trough in figure 3a can be explained by the different computing approaches: The original Weinberger Chilling Hour model produced a conspicuous trough in forcing marked red associated with the respective chilling value marked in green. In the modified Weinberger version when we included slightly negative temperatures, trees in this scenario S1O2 consequently accumulated over 30% more chilling (2,113 CH- vs 1,421 CH) (Fig. 3). However, both approaches interestingly resulted in the same amount of forcing (5,405 GDH for flowering). This result is interpreted in a way that slightly negative temperatures may contribute to chilling of a tree, however, to a lesser extent than slightly positive temperatures. In winters without negative temperatures, cherry trees with 1,400 CH needed about ca. 7,500 GDH of forcing.

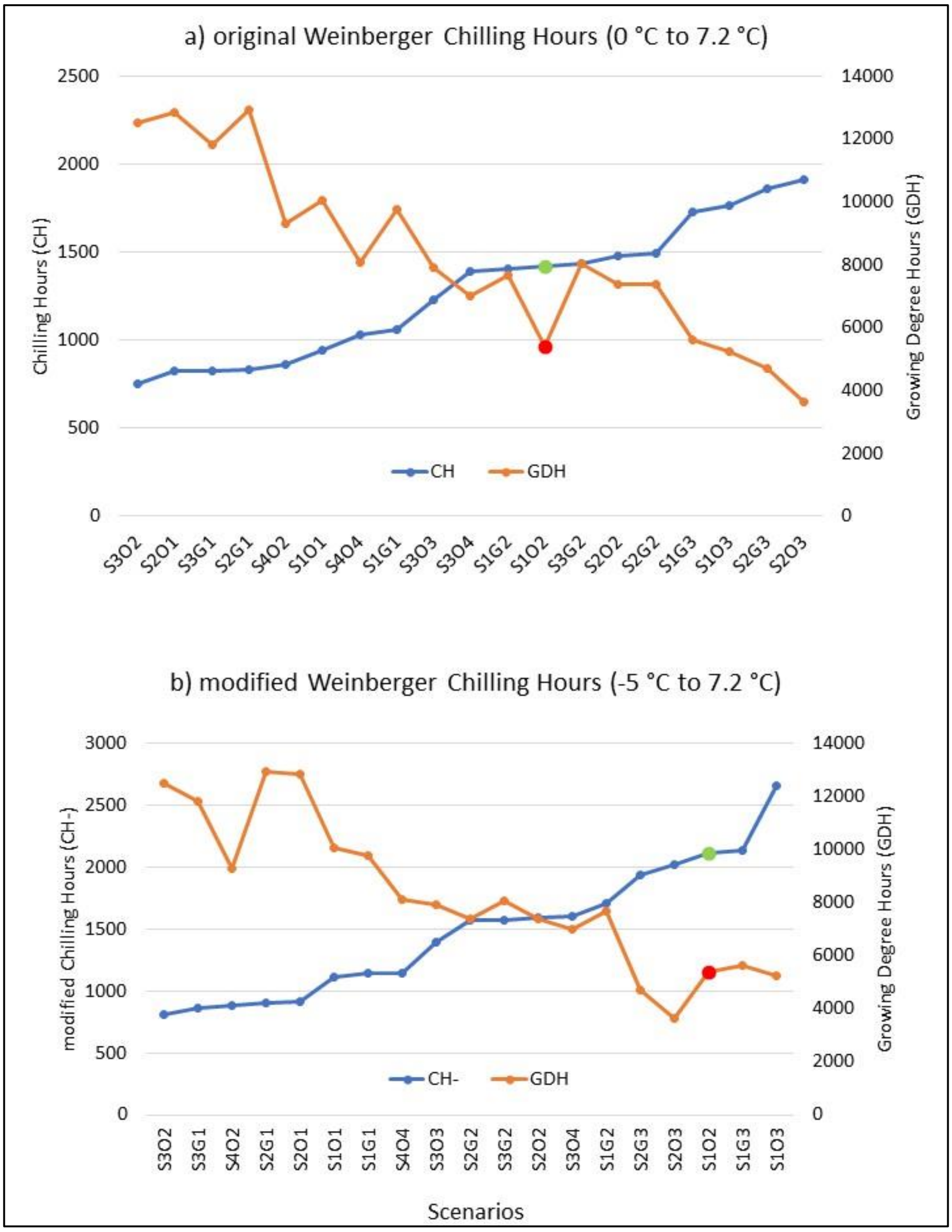

Fig. 3. Comparison of a) the original Chilling Hour model (Weinberger, 1950) and b) our modified version including slightly negative temperatures (0 to -5 °C). Green (chilling) and red (forcing) dots denote values in scenario S1O2, in which cherry trees received 1,421 Chilling Hours (above 0°C) and 2,113 modified Chilling Hours (including negative temperatures down to -5°C), respectively and 5,405 Growing Degree Hours forcing.

4. Discussion

The overall objectives of the present study were to elaborate, whether forcing can partially substitute for lack of chilling (and to which extent) and whether slightly negative temperatures down to -5 °C can contribute to chilling accumulation. The results have shown forcing to chilling ratios of 5:1 to 15:1 for flowering in high chill varieties and in the light of recent climate change that up to 50% of chilling can be substituted by additional forcing viz a longer forcing period and slightly negative temperatures (-5 °C to 0 °C) contribute to chilling fulfilment and have to be included in the chilling period.

4.1 Interchangeability of chilling and forcing

Our results (Fig. 1) showed that a minimum chilling is required before forcing can become effective, a finding, which is in contrast to the ‘Parallel model’ (Landsberg, 1974), who assumed that both chilling and forcing accumulation start parallel at leaf fall. Changes in carbohydrates in the buds during dormancy were assessed (Chmielewski et al., 2017; Kaufmann and Blanke, 2017c) indicating a minimum chilling requirement has to be fulfilled before forcing accumulation enable the bud to flower (Fig. 4). Genetic studies on dormancy indicate that a minimum chill accumulation must be met first before heat accumulation can enable the expression of DAM genes that result in the promotion of flowering (Horvath, 2009; Leida et al., 2012).

Our approach also differs from that of Pope et al. (2014) and the more theoretical overlap model developed in the warm winter climate of California, USA. Our approach is based on the finding of the optimal forcing of 8,000 GDH, irrespective of cultivar (low, medium, high chill), irrespective of scenario (-50% to +50% chilling) and chilling model. These results from 24 scenarios with portable intact cherry trees clearly of very different chilling requirement enable to distinguish between actual chilling due to temperate climate zone winters and forcing in a heated greenhouse. The basis of this threshold of 8,000 GDH of forcing maybe a useful requisite for horticulturists to calculate and predict effects of insufficient chilling e.g. in the Mediterranean and excessive chilling in e.g. Scandinavia. For cherry, up to 50% of chilling may be compensated by forcing (in the scenario with the lowest chill accumulation with sufficient flowering) and vice versa. This may enable a comparison of cherry and other fruit crops as their suitability of cultivation in a particular location and possibly predict flowering. If more than 8,000

GDH are needed for full bloom, chilling availability at this growing location declines for those varieties and vice versa.

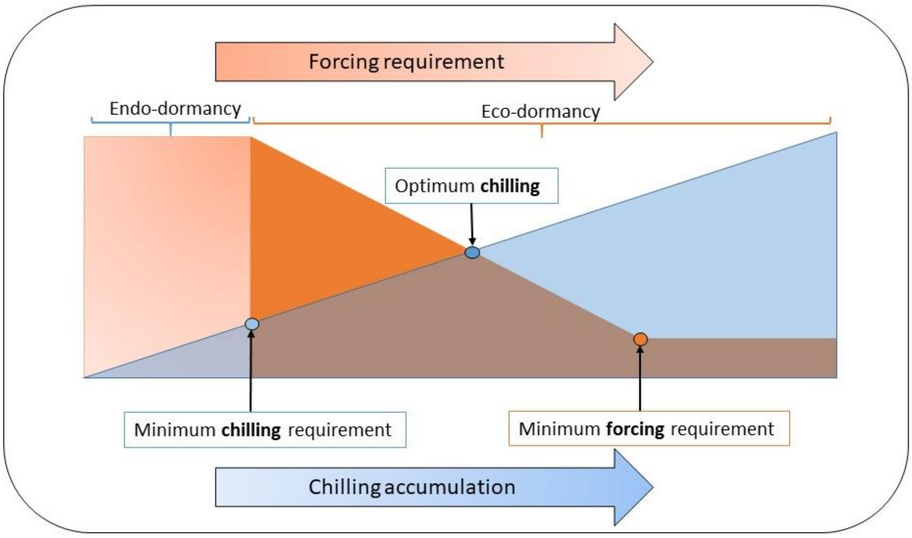

Fig. 4. General scheme for substitution of lack of chilling by more forcing. Minimum chilling and forcing requirements are included as well as the optimum chilling, irrespective of species and cultivar.

Our results have shown that up to 50 % of chilling can be substituted by additional forcing (Fig. 3/Table 3), i.e. exposure of the trees to longer spells of warm temperatures. This finding is in line with Couvillon and Erez (1985), who were probably the first to suggest such an idea for fruit species grown in Georgia, Athens, USA, but without designating ratios to the different species in relation to their chilling requirement (based on cut branches and concluding that bud break is determined by chilling requirement and not forcing accumulation). This finding of chilling substitution is relevant in the context of recent climate changes like warmer winter temperatures (IPCC, 2013) with a projected decline in available chilling in Europe (Luedeling et al., 2011a). As a result of climate change, bloom time of deciduous fruit trees is advanced by 6 to 10 days (Legave et al., 2012; Chmielewski and Rötzer, 2001; Cleland *et al.*, 2007). In the past, most studies focused on the chilling period (Cesaraccio et al., 2004; Erez et al., 1990; Luedeling et al., 2011b) or rarely included forcing and then ended their observations as early as the green tip without waiting for the outmost chilling success, the flower (Alburquerque et al., 2008; Campoy et al., 2011; Marra et al., 2002). In Norway, a cold winter environment with sufficient chilling, only Growing Degree Hours (ca 4.000 GDH) are used for the prediction of flowering of sweet cherry (Meland et al., 2017) which compares favourably with our forcing values (3,500-4,000 GDH) with excessive chilling accumulation under our temperate zone climate (Fig. 1).

4.2 Effect of simulated climate change on chilling in the orchard and unheated greenhouse

In our experiment, the temperature in the unheated greenhouse exceeded that in the natural environment (orchard) by ca. 2 °C (Table 1) to simulate climate change. Although chilling and forcing values were similar in both locations, trees in the unheated greenhouse (climate change scenario) reached their chilling earlier than those outside in the field due to the rise in winter greenhouse temperature. Chilling built up faster in the beginning of dormancy in the orchard due to buffered temperature in the unheated greenhouse. During winter, the situation reverses and chilling accumulated faster in the unheated greenhouse due to fewer temperature drops below 0 °C, without chilling effect in the chilling models (Weinberger, 1950; Richardson et al., 1974; Erez et al., 1990). This finding enables the proposed forcing and chilling interaction possibly applicable to other locations within the temperate zone winters. Due to warmer spring temperatures in the unheated greenhouse, the full bloom of control trees was enhanced by about 7-12 days depending on year and variety. To our knowledge, this has not been investigated before so further discussion does not apply.

4.3 Ratios of chilling to forcing for sufficient flowering

As a result of the chilling and forcing values obtained from the different scenarios in our experiments (Fig. 1), the minimum value for flowering for the ratio of forcing (GDH) to chilling (e.g. CH) is visualized in a graphical abstract (Fig. 5). To our knowledge, this is the first time that ratios of GDH to CH/CU/CP for (in-) sufficient flowering and forcing values were established for the fruit crop most affected by climate change, sweet cherry (Kaufmann and Blanke, 2017a), irrespective of their chilling requirement. Since a comparable visualization has not been published to our knowledge, discussion does not apply.

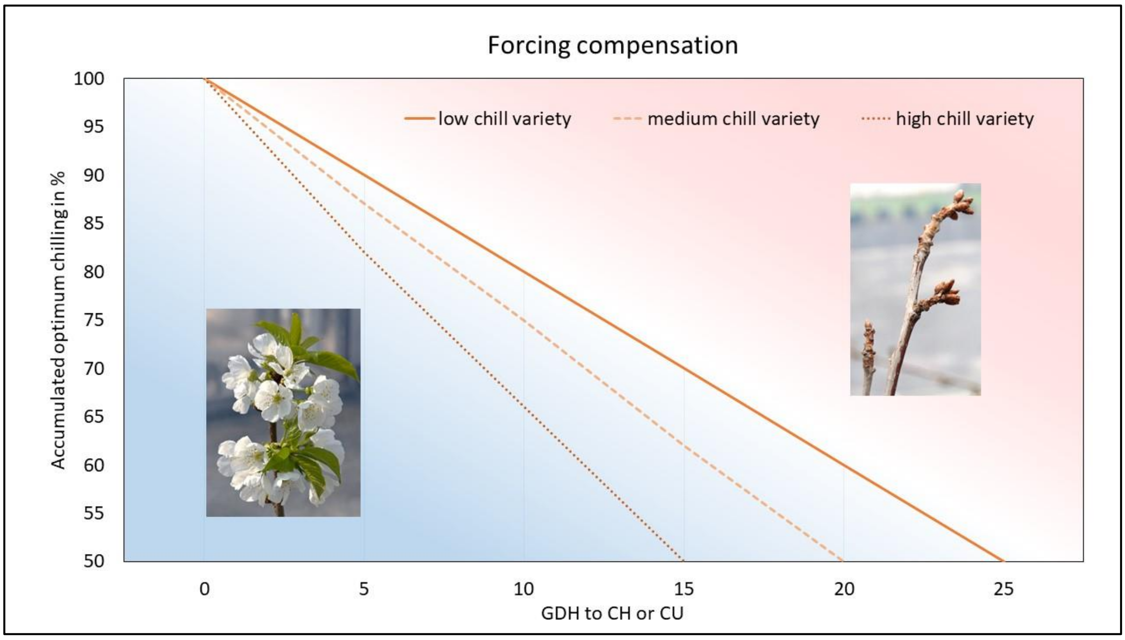

Fig. 5. Forcing can successfully compensate inadequate chilling levels at 50-100% of optimal chilling at ratios GDH to CH or CU of <15:1 for high chill varieties, <20:1 for medium chill varieties and <25:1 with low chill varieties; the top right hand corner symbolises inadequate chilling and no flowering (the trees did not reach full bloom >50% flowering), whereas the left bottom corner represents sufficient chilling and forcing, which lead to optimum flowering.

4.4 Influence of slightly negative temperatures (0 °C to -5 °C) on winter chill

The three most common chilling models ignore temperatures below 0°C and may result from their warm winter origins. In the temperate climate zone, where many fruit trees are grown, winters may exhibit temperatures below zero. Trees therefore adapted to these temperatures like “*Schneiders späte Knorpelkirsche*”, which is grown from warm winter climates in Turkey (under its synonym ‘Ziraat’), where chilling requirements are hard to fulfil, up to Norway with constant freezing temperatures during winter. Although Mahmood *et al*. (2000a) had already suggested that slightly negative temperatures of -1.2°C down to -5.6 °C might have an effect of overcoming dormancy, his experiments with cut one-year-old sweet cherry shoots from two-year-old trees held in a fridge at constant -1.2°C in the dark to overcome dormancy ended before flowering and without calculating the required forcing.

Our results with fully-grown, intact trees show that sub-zero temperatures in the winter may have an effect on chilling and dormancy breaking in line with Mahmood et al. (2000a and b) expectation. The successful result is seen in the example of our modified Weinberger Chilling Hours version including slightly negative temperatures (Fig. 3b) vs. the original Weinberger Chilling Hours model (Fig 3a), trees in this scenario S1O2 consequently accumulated over 30% more chilling (2,113 CH- vs 1,421 CH). However both approaches interestingly resulted in the same amount of forcing (5,405 GDH). This result is interpreted in a way that slightly negative temperatures contribute to chilling of a tree, however to a lesser extent than slightly positive temperatures. In winters without negative temperatures, trees with 1,400 CH needed about ca. 7,500 GDH of forcing.

5. Conclusion

Warmer winters as a result of climate change (IPCC, 2013) require adaption strategies in horticulture (Bindi and Olesen, 2011), including those to overcome the lack of chilling. Since cherry is one of the most affected fruit crop by climate change (Luedeling et al., 2011a; Kaufmann and Blanke, 2017a) the thresholds, ratios and optimum forcing values presented in this study may also help growers and breeders to combat upcoming environmental changes. The interchangeability of chilling and forcing may also help to compare and explain varying chilling values determined in different regions (warmer or colder winters) for the same cultivar.

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8. Colour supplement

Colour supplement -CS1: Potted sweet cherry trees in the orchard at Campus Klein Altendorf, near Bonn, Germany.

F Summary and conclusions

The main objective of this work was to contribute to the understanding of dormancy by investigating the chilling and forcing requirements of sweet cherry trees in one of the major fruit growing regions in Germany. In order to quantify these requirements at an entire tree level rather than single buds or cut branches, 160 potted sweet cherry trees of three varieties with a very wide range (3-fold) of chilling requirements were raised over two years to initiate uniform flower buds before applying 24 chilling regimes (8 regimes per variety) per year followed by forcing to determine the effect on flowering. Also, a natural photoperiod and day and night temperature cycles, rather than artificial conditions, were part of the study. This expense was needed to see the real flower behaviour of the entire fruit tree. To assess future scenarios for fruit growing (e.g. global warming), potted trees were placed in an unheated greenhouse with an average of ca. 2°C temperature increase.

A second objective was to investigate the changes in carbohydrate levels and water relations of cherry buds to identify and distinguish the dormancy phases para-, endo, eco-dormancy on a plant physiological base, and to determine transition points between dormancy stages using ca. 10,000 flower buds from nine varieties and bi-weekly sampling over two years. The understanding of these changes in relative water content and carbohydrate levels may contribute to manage insufficient chilling in the orchard and support climate change studies with trees. The insights gained ultimately highlight the need for cross-sectoral, adaptive management practices that jointly target a sustainable regional development. Overall, the work has shown that environmental change has an effect on chilling availability in Meckenheim, Germany and possibly other fruit growing regions along 50°N latitude.

The major results presented in single chapters can be summarized as follows:

1. When applying the three commonly used chilling models to calculate winter chilling in a cherry orchard at Klein-Altendorf, Meckenheim, Germany, to an unheated greenhouse (average temperature increase by ca. 2°C) to simulate global warming, the available chilling increased by 12 % (Chill Units - CU), 15% (Chill Portions - CP) and 20% (Chilling Hours - CH), respectively (in the unheated greenhouse). In climate change predictions for other locations,

especially in the South of Europe or in the North of Africa, the warmer winter temperatures often induce lack of chilling. Options and limitations of countermeasures in terms of cultivation methods such as microclimate manipulation, rest breaking agents, change of orchard location to a higher altitude and breeding are options to counter a possible lack of chilling.

2. The Meckenheim fruit growing region in Western Germany may be affected as a consequence of climate change by lack of chilling. The orchard temperatures in the warmer winter (6.0°C - October until the end of February) exceeded those in the unheated greenhouse (4.7°C) in the cold winter. Maximum chill accumulation at this location is currently achieved in very warm winters, so that even warmer winters may reduce the available chill, but there will be still enough to grow high chilling varieties. Chilling computations for this region with all three major chilling models, showed that cherry trees of the low chill cultivar '6000CZ' required 22.3-26.6 (CP), 465-684 (CU) or 402-483 (CH), medium chill cv. 'Brooks' about 37.9-54.4 CP, 819-1,267 CU, or 779-941 CH and the high chill cv. 'Schneiders späte Knorpelkirsche' about 54.4-79.3 CP, 1,267-1,696 CU, 941-1,494 CH, respectively, for natural flowering. Cherry trees of cv. '6000CZ' receiving <300 CH, cv. 'Brooks' <500 CH and cv. 'Schneiders späte Knorpelkirsche' <700 CH were unable to flower, equivalent of 50% of the assumed chilling optimum of the respective cultivar. The beginning of leaf drop was identified as a suitable initiation point for computing chill accumulation in sweet cherry.
3. Four transition points were proposed to clearly distinguish dormancy phases by relating carbohydrate and relative water content (RWC) in reproductive buds to concomitant chilling fulfilment. Furthermore, two groups of cherry varieties were defined based on their different initial sorbitol and starch level in the autumn. The first separation between para- and (deep) d-endo-dormancy is characterized as a transition from a decrease (variety group 1 with cv. 'Schneiders späte Knorpelkirsche' as model variety) or a constant level (variety group 2 with cv. 'Regina' as model variety) to a sharp increase in hexoses, sorbitol and a drop of starch content. The second transition point (d-endo- to f-endo-dormancy) is characterized as the changes in both hexoses (glucose and fructose) (increase) and starch (decrease) terminate, with ca. 650 Chilling Hours (CH), i.e. insufficient chilling as measured in the concomitant forcing experiment. This

third transition point (f-endo- to eco-dormancy) was characterized by ca. 1,000 CH, the minimum chilling requirement and restrained flowering (cut branches). The fourth transition point (forcing initiation) marked an increase in water content at ca. 1,550 CH, optimum chilling for cherry and coincided with natural flowering. The ratio of hexoses to starch content (< 2:1) appeared to be a potential indicator of the beginning of chilling (para-dormancy); a ratio of 14-20:1 typical for endo-dormancy, whereas the release from dormancy was associated with a decline to less than 10:1 at the end of winter (eco-dormancy).

4. The effects of more forcing due to diminishing available chill as a result of climate change and the possibility of substitution of chilling by forcing were investigated. In the scenarios applied, *minimum* chill fulfilment ranged from 400 CH (Chilling Hours) in low chill, 550 CH in medium chill and 750 CH in the high chill variety with maximum forcing of ca. 11,000 Growing Degree Hours (GDH) for low, ca. 12,000 GDH for medium and ca. 13,000 GDH for high chill varieties for sufficient flowering. With *optimum* chill, the optimum forcing was ca. 8,000 GDH (>12°C), irrespective of variety, allowing upscaling of the results to other varieties. Trees exposed to excess chilling (150%) required less forcing (ca. 4,000 GDH) to reach full bloom. Hence, chilling can compensate for up to half of the required forcing, i.e. ca 4,000 GDH. Ratios of forcing to chilling were computed for future comparisons, which ensure flowering in the orchard. Slightly negative temperatures (-5°C to 0°C), which are presently exempt in the common chilling models, contributed to chilling accumulation of the fruit trees. Overall, the results have shown that diminishing chilling as a result of climate change can be compensated for, in part (up to 50%), by a larger amount of forcing to obtain natural flowering in the orchard.

Climate change and therefore resulting warmer winter can cause very different effects depending on the location. Regions with relatively cold winters (mostly <0°C) gain available chilling. In combination with earlier war temperatures in spring trees flower earlier, hence are more vulnerable to frost events. Regions with very warm winters (mostly >10°C) may lack available chilling for some cultivar chill requirements. In combination with even warmer spring temperatures the bloom will be delayed, or the tree will flower uneven (acrotony). One of the major challenges in the determination of chilling and forcing requirements are the heterogeneous attempts to quantify and the limitations of the given chilling models. Furthermore, the actual beginning of the chilling period is not clearly defined throughout species and regions. Certain calendar dates are uniform but do not reflect seasons in different parts of the world. Physiological stages like termination of shoot growth or beginning/end of leaf fall are easy to monitor and are suitable for some deciduous fruit trees but can also greatly vary between species. Due to no physiological appearance of the end of chilling and start of forcing period definite chilling and forcing requirements are difficult to distinguish. In addition, as chilling commences or at the end of forcing, some studies assess the weight gain of single buds, others use the green tip stage or full bloom stage, which lead to great disharmony when comparing data sets. This also depends on the plant material used in the study. Buds of single cut branches, which were stored for weeks in a cold chamber behave differently than those of intact trees. New approaches and technologies with the evaluation of carbohydrate, water content, plant hormone fluxes as well as monitoring gene expression will give valuable information about dormancy stages. These new insights have to be incorporated into a model, since the available chilling models just rely on temperature and do neither reflect biological processes nor abiotic factors like photoperiod or light intensity. Temperature ranges seem to be fitted to the original cultivar and location. Chilling values measured for one cultivar at one location cannot easily be transferred to another location. This makes comparability among studies nearly impossible. Also, looking at the given models, the chilling period is consequently isolated from the forcing period, although interactions are very likely and often indicated. Hence, published chilling data or future publications should be complemented by forcing values to give indications if rather low chilling values were compensated by higher forcing values.

It is indispensable for future research on chilling to have defined parameters that reflect actual plant processes and integrate these into a model that include newest insights. Therefore, experiments have to be carried out in many similar locations with identical management practices to rule out influences of too many factors. For the assessment of future climate scenarios covered orchard fruit trees (e.g. polytunnels) or portable potted trees in unheated greenhouses should be next to orchard fruit trees. Thereby the actual temperature ranges (air temperature) for effective chilling can be assessed as they are all originally established for peaches in warm winter climates. It is also yet unknown to what extend soil or root temperature as well as the actual bud temperature play a role in chilling effectiveness. For future research, a general protocol how to assess chilling stages and forcing has to be followed by all researchers to guarantee comparability. As desirable outcome general pattern for most species should be detected and worked into a general model, where more cultivar specific brackets can be added. The resulting value of this new model should rely on actual physiological data and can be transferred to other locations. Then, scientists, growers and breeders would benefit for their upcoming research, orchard planning or specific breeding.

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