Apple dormancy in a changing climate: development of chilling and heat accumulation models to predict bud break

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A thesis submitted for the degree of Doctor of Philosophy

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University of Reading

February 2022

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

The increase in temperature as a consequence of climate change is predicted to impact adversely on UK apple production by affecting the dormancy cycle. Warmer winters, and an increased risk of frost damage due to earlier bud break, are of major concern to UK growers. Although chilling and heat accumulation models are often used to predict time of bud break and anticipate cultivar suitability to new climes, existing models do not consider different cultivar responses or climate change scenarios and so are of limited use.

The aim of this PhD research programme was to improve our understanding of the temperature effect on the dormancy cycle in a range of apple cultivars, and to develop cultivar-specific models to predict cultivar suitability in a climate change context. All experimental work was carried out in the UK between 2017 and 2021, using a combination of methodologies and plant materials including excised shoots, potted trees, floral buds, and xylem sap from orchard trees. Data were collected through experiments in controlled environment conditions, from UK apple orchards, and from analytical work in the laboratory.

The depth of dormancy was highly variable between cultivars, as evidenced by the finding of different sensitivities to warm temperatures during ecodormancy. A lower optimum temperature for chilling accumulation was observed compared to previous models, indicating that future reductions in winter chill accumulation could be more severe than predicted. A new chilling model was created, the *Malus model*, which represents a better approximation of temperature contributions towards chilling accumulation. Results also indicated a partial overlap between chilling and heat accumulation, and a new modelling approach was developed that accounted for the correlation between both temperature-driven processes. Changes in carbohydrate concentrations in apple buds and xylem sap during winter occurred in parallel to chilling and heat accumulation, indicating a link between carbohydrate dynamics and the progression of dormancy.

The outputs from this PhD research programme corroborate the industry's concern that warmer winters will pose significant challenges for UK apple production. Nevertheless, new fundamental knowledge needed to develop more accurate chilling and heat accumulation models was generated, and this will help to inform climate change adaptation strategies so that potential impacts on the UK apple sector can be managed and mitigated more effectively.

Acknowledgements

First of all, I would like to thank my supervisors, Prof. Paul Hadley and Dr. Mark Else; their knowledge and guidance have been integral to this PhD. Thank you for giving me the opportunity and the freedom to explore the areas of research and methodologies I was most interested in. I am grateful for all the interesting conversations we have engaged in during the last four years, they have really motivated me and encouraged me to question my research in different ways. And most of all, thank you for all the moral support and for helping me build up confidence in my own work.

I am deeply grateful to the CTP consortium for funding this research, and to the National Association of Cider Makers (NACM), for technical advice on cider apple production.

I would like to acknowledge past and present technical staff at NIAB EMR and the University of Reading that have helped me in many ways during the past four years; in particular Val, Liam, June, Mike, Teddy, George, Nikki, Emma, Dilly, Pablo, Fernando, Stuart, Alex, James, Josh, Graham, Bianka, Matteo, Lucia and Karen.

I would like to thank Agrii (UK) for giving me permission to collect plant material from their experimental orchard at NIAB EMR throughout this PhD; and for granting me access to climate data obtained with their Adcon system installed at NIAB EMR. I also thank Bardsley Farms (Tonbridge, UK), where I was able to collect apple shoots for the first experiment of this PhD.

To all the friends I have made in East Malling and Reading; thank you for all your support, research chats and advice; and most importantly, thank you for all the Fridays at the bar and fun times that made me temporarily forget about the struggles of the PhD!

Gràcies a tots els meus amics i a la meva familia. En especial als meus pares, Rosa i Victor, i al meu germà Guillem, que tot i tenir-vos lluny, heu aconseguit que us sentís a prop en tot moment. Gràcies per tots els ànims, no ho hauria pogut fer sense el vostre recolzament.

And last but by no means least, to Rob. Thank you for all your support; from helping me to transport trees and prepare samples during weekends, to proof-reading and listening to my presentations. And above all, thank you for all the laughs and welcome distractions, and for keeping my spirits high when it was mostly needed! It would have been a lot harder without you.

Table of Contents

Abbreviations

%	Percentage
<	Less than
>	More than
°C	Degree Celsius
µmol	Micromol
CEL	Crops and Environment Laboratory
CF	Chill Fractions
cm	Centimetre
CH	Chill Hours
CP	Chill Portions
CR	Chilling requirement
CTP	Collaborative Training Partnership
CU	Chill Units
DEFRA	Department for Environment, Food and Rural Affairs Department
DW	Dry weight
g	Gram
GDH	Growing Degree Hours
h	Hour
HR	Heat requirement
K	Potassium
kg	Kilogram
L	Litre
m	Metre
M	Molar concentration (mol/m^3)
MgO	Magensium Oxide
min	Minute
ml	Millilitre
N	Nitrogen
NACM	National Association of Cider Makers
NIAB EMR	National Institute for Agricultural Botany, East Malling Research
P	Phosphorus
Pers. comm.	Personal communication
PLS	Partial Least Squares
s	Second
SD	Standard Deviation
SE	Standard Error
Tb	Base temperature
temp	Temperature
UK	United Kingdom
µL	Microlitre

List of figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of tables

Chapter 1

Introduction

1.1 General introduction

The global climate is changing as a consequence of an increase in greenhouse gas emissions due to anthropogenic activity (IPCC, 2021). Even under a low emissions scenario, by 2070, mean winter and summer temperatures in the United Kingdom (UK) could increase up to 3.3 °C and 2.4 °C, respectively (Murphy et al., 2018). This would impact any apple crops currently being planted and will have devastating consequences for the agriculture and food production industries, which have been identified at high risk by the Intergovernmental Panel on Climate Change (IPCC, 2021).

Apple is the biggest top fruit crop in the UK, with a value of over £260 million for dessert, culinary and cider varieties (DEFRA, 2021). In the UK, potential impacts on apple production as a consequence of higher temperatures have already been identified, including a decrease in winter chilling (Atkinson et al., 2004, 2013; Sunley et al., 2006) but also earlier bud break with the associated risk of frost damage (Harding et al., 2015). The flowering stage in apple is particularly sensitive to changes in climate and significant production losses have occurred in the past due to late spring frosts (DEFRA et al., 2017). Higher temperatures during winter can impact the dormancy cycle (Campoy et al., 2011a; Atkinson et al., 2013) and are negatively correlated with yield (Jackson and Hamer, 1980). Dormancy enables trees to survive winter and less chilling can reduce and/or delay bud break, cause non-uniform flowering and, as a consequence, produce smaller and abnormal fruits (Petri and Leite, 2004).

The dormancy cycle in apple trees is regulated by temperature (Heide and Prestrud, 2005), making apples especially vulnerable to any changes in the climate. A reduction in winter chill (Atkinson et al., 2004, 2013; Sunley et al., 2006), combined with an increased risk of frost damage as a consequence of an early start to the growing season (Harding et al., 2015) create an uncertain and concerning future scenario for apple production. As chilling requirement varies between cultivars (Hauagge and Cummins, 1991a), it is important to anticipate how different varieties are likely to respond to changes in the climate so that informed commercial planting decisions can be made over the next few decades.

1.2 Biology and cultivation of the apple tree

The cultivated apple, *Malus* × *domestica* Borkh. (Korban and Skirvin, 1984), belongs to the *Rosaceae* family and subfamily *Pomoideae*, the pome fruits. Grown worldwide, there are over 2,000 different apple varieties (Brogdale Collections, 2021).

Apple fruit buds can grow terminally on spurs (short shoots) and/or laterally or terminally on long shoots. Whether trees grow flowers only on spurs or also on long shoots, depends on tree age and cultivar (Pratt, 1988; Jackson, 2003); varieties are often informally labelled as "spur-bearers" or "tip-bearers". All fruit buds are born as mixed buds, containing both leaves and flowers; and the final fate of a bud is driven by a range of factors. For buds to start floral initiation, a critical number of nodes (leaf initials) in a shoot must be reached before dormancy, this number varies from 16 to 20, depending on the variety (Jackson, 2003). The first sign of floral commitment is the doming of the apex, which occurs approximately 100-150 days after full bloom if the critical number of nodes has been reached (Foster et al., 2003).

Different floral parts are formed inside the buds during autumn and winter, but final flower development occurs between bud break and anthesis during the following spring; with the full process being completed over two seasons (Pratt, 1988). Flowers are arranged in inflorescences of four to six flowers and colour varies from white to dark pink depending on the cultivar (Jackson, 2003). Most apple cultivars are self-incompatible so successful fruit set requires cross-pollination with other varieties, carried out by insects (Ramírez and Davenport, 2013).

As flower initiation for the following season occurs when current fruits are developing, heavy cropping in one year can reduce the proportion of flowering buds the next year (Jonkers, 1979). This heavy cropping in one year followed by a year with no crop is known as biennial cropping, and whilst some cultivars are more prone to it, orchard management to control excessive fruit setting or the application of plant growth regulators can be used to control it (Jonkers, 1979). The period between bloom and fruit maturity varies between cultivars (Jackson, 2003) and is highly influenced by temperature (Luton and Hamer, 2016).

Apple seedlings have a long juvenile phase where they remain vegetative for more than seven years. Horticultural practices such as grafting have been adopted to shorten this phase and ensure quicker production (Campbell, 1961). Consequently, commercial apple trees are compound trees, formed of a scion and a rootstock. The scion (grafted part) is taken from a mature tree and grafted onto a rootstock with certain characteristics. Dwarfing rootstocks combined with pruning methods are used to control tree size, facilitating fruit harvest, and enabling trees to be planted closer together to achieve higher densities and thus more productive orchards per unit area (Webster, 1995).

1.3 Apple production in the United Kingdom

Apple is cultivated worldwide with China being the biggest producer in the world, followed by the United States (FAOSTAT, 2020). The United Kingdom ranks 27th, and is the 9th most important producer in Europe (FAOSTAT, 2020), producing 447,000 tonnes in 2020, which accounts for over 95% of total orchard fruit production in the country (DEFRA, 2021). After strawberries, it is the most economically important fruit crop in the UK (DEFRA, 2021). Production of dessert varieties has doubled between 2004 and 2020, whilst a slow decrease has been observed in culinary and cider apples during the last decade (DEFRA, 2021). In 2020, approximately 340,000 tonnes of apples were imported to the UK, valued at more than £330 million (DEFRA, 2021), providing a unique opportunity for import substitution by increasing UK grown varieties.

Although over 2,000 different cultivars exist, in the early 90s more than 50% of the world apple production was generated from only seven varieties: “Delicious” (“Red Delicious”), “Golden Delicious”, “Fuji”, “Granny Smith”, “Jonagold”, “Gala” and “Idared” (Jackson, 2003). Whilst this list is likely to have changed, no official current statistics on worldwide production per cultivar are available. In the UK, “Gala” is the most popular cultivar accounting for almost half of the production of dessert varieties; followed by “Braeburn” and “Cox Orange Pippin” (DEFRA, 2021).

1.4 Apple production in a climate change context

Apples are grown worldwide, from warm regions such as Kenya (Griesbach, 2007) and South Africa (Cook, 2010), to cold areas such as Finland (Kaukoranta et al., 2010) or the western Indian Himalaya region (Kumar et al., 2008). However, successful production with significant economic gains is highly variable, particularly if cultivars are grown in areas beyond their climatic requirements. With both winter and summer temperatures predicted to increase, important impacts on apple production have been anticipated (Else and Atkinson, 2010; Luedeling, 2012; Atkinson et al., 2013).

Apple trees require cold temperatures during the winter as insufficient chilling can reduce bud break and flowering (Petri and Leite, 2004). Chilling requirements are highly variable between cultivars (Hauagge and Cummins, 1991a) and fruit production will be compromised if cultivars with high-chill requirements are grown in regions with mild and/or short winters. Future winter chilling is predicted to decline in all major fruit tree growing regions as a consequence of climate change (Luedeling et al., 2011; Darbyshire et al., 2016a). In the UK, significant declines have already been observed over the last few decades, particularly in southern England where most apple orchards are located (Sunley et al., 2006). The application of rest-breaking chemicals such as Hydrogen Cyanamide is used in regions with insufficient chilling to induce bud break (Griesbach, 2007), however, results are

inconsistent and it has been banned in many countries due to phytotoxicity (Siller-Cepeda et al., 2019).

Warmer temperatures in spring are also of concern as they might induce earlier bud break; advances in blooming dates in apple have already been observed in many European countries (Legave et al., 2013; Drepper et al., 2020). If buds open too soon there is an increased risk of frost damage, which has already caused significant yield losses in the UK in recent years (DEFRA et al., 2017). An increase in spring temperatures might also cause a loss of synchrony between flower and pollinator appearance, having a negative impact on final fruit production (Korösi et al., 2018).

Excessive heat and solar radiation during fruit development can cause sunburn, a physiological disorder causing fruit necrosis and browning, responsible for important fruit losses in warmer regions (Racsko and Schrader, 2012). Whilst some management practices exist to reduce sunburn, the predicted increase in temperatures is expected to expand its incidence to growing regions not currently being highly affected (Racsko and Schrader, 2012).

The impact of warmer temperatures on the dormancy cycle (Campoy et al., 2011a), in combination with other factors such as water scarcity and an increased risk of pests and diseases (IPPC Secretariat, 2021) create a concerning scenario for future apple production. Overall, understanding how different apple cultivars will respond to the predicted changes in the climate is key to ensure future production and provides an opportunity to identify better suited varieties.

Chapter 2

Literature review and research objectives

2.1 Dormancy: definition and phases

Dormancy is part of the annual cycle of perennial woody plants growing in temperate regions, and is defined as the temporary suspension of visible growth of any plant structure containing meristems (Lang et al., 1987). Meristematic regions are formed of undifferentiated cells with the capacity of continuous cell division, such as the apical meristem found at the tip of buds and roots. Bud dormancy is the focus of this study as it determines time and quality of flowering, it is linked to tree survival during winter months and ultimately to fruit production (Saure, 1985; Faust et al., 1997; Rohde and Bhalerao, 2007).

Dormancy has been artificially divided into three phases which often receive different names (Doorenbos, 1953; Saure, 1985; Lang et al., 1987) (Figure 2.1). Although these phases were described earlier by other authors (Doorenbos, 1953; Saure, 1985), Lang's terminology (Lang et al., 1987) is the most widely adopted and will be used throughout this thesis. Lang (1987) named the three dormancy phases as (i) paradormancy, (ii) endodormancy and (iii) ecodormancy; and proposed that they are differentiated mainly by two parameters, (i) the event responsible for changing the state of dormancy (i.e. temperature) and (ii) the tissue where this change is perceived (i.e. the bud).

During paradormancy, inhibition of growth is regulated by intrinsic physiological factors located outside the organ undergoing dormancy. Champagnat (1989) called these inhibitory factors "long distance correlative inhibitions (LDI's)". Apical dominance is an example of LDI by which the terminal bud inhibits growth of axillary buds on growing shoots. As discussed in more detail in section *2.6.1 Hormones*, apical dominance is strongly regulated by hormones.

In apple, colder temperatures induce the transition towards endodormancy (Garner and Allard, 1923; Heide and Prestrud, 2005), when growth is prevented by internal bud signals (Lang et al., 1987). Endodormancy is overcome by extended periods of chilling (Lang et al., 1987), which is thought to remove the physiological blocks that prevent growth. The amount of chilling needed for endodormancy break is known as chilling requirement (CR); CR in apple is cultivar-specific and highly variable between varieties (Hauagge and Cummins, 1991a).

After CR satisfaction, trees remain ecodormant until environmental conditions are favourable for growth, this is key not only for plant survival during winter months, but also as a mechanism to avoid

the resumption of growth when environmental conditions fluctuate between favourable and unfavourable (Rohde and Bhalerao, 2007). Warmer temperatures are needed to exit ecodormancy and promote bud development and blooming. The minimum amount of heat needed for bud break is often referred to as the Heat Requirement (HR).

Other names used to identify these three phases are (i) summer-dormancy, (ii) winter-dormancy and (iii) imposed dormancy (Doorenbos, 1953); or Saure (1985) called them (i) predormancy, (ii) true dormancy and (iii) imposed dormancy. More recently, other definitions of dormancy have been proposed, Rinne et al., (2001) used changes in cell-to-cell communication at the apical meristem to also differentiate three phases (i) the online state, when active growth and cell division occurs; (ii) the offline state, symplasmic cell to cell communication is blocked and buds are dormant (edodormancy); and (iii) the standby state, symplasmic communications have been restored but the apical meristem cells remain inactive due to unfavourable environmental conditions (ecodormancy).

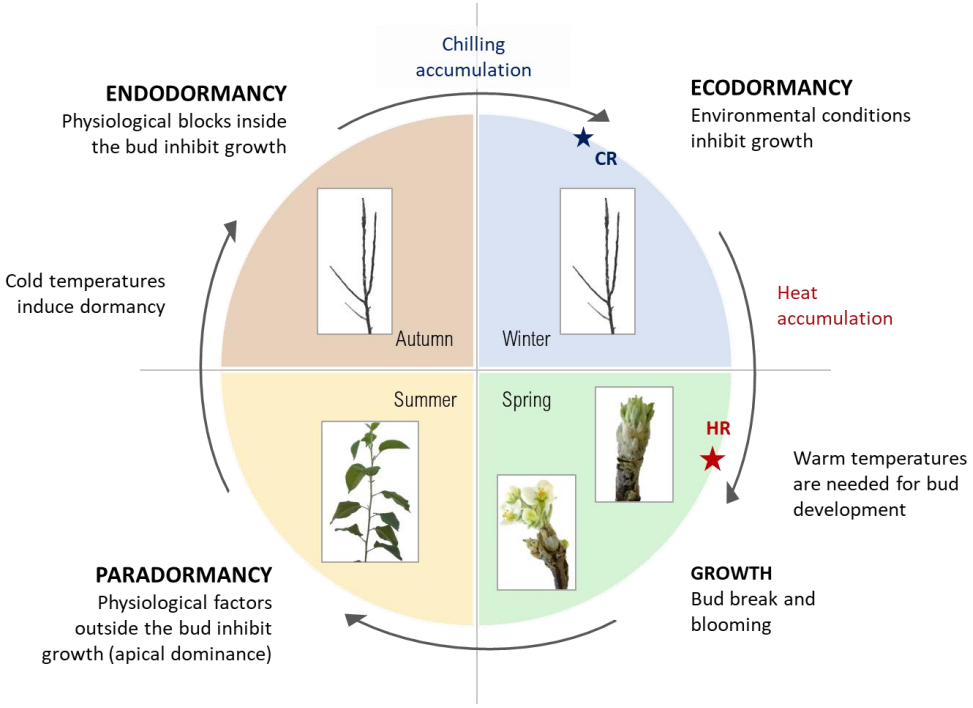


Figure 2.1 – Diagram representing the phases of the dormancy cycle (Lang et al., 1987). CR: Chilling requirements fulfilled. HR: Heat requirements fulfilled.

Dormancy is a continuous annual process with huge implications for fruit production; whilst paradormancy influences trees structure (Champagnat, 1989), endodormancy and ecodormancy release will determine time of bud break and flowering and are the focus of this review. A range of inter-linked factors are involved in regulating the dormancy process, including environmental conditions and changes in the balance of hormones, but important gaps in our understanding of the cycle still exist. Being an annual process, all phases are tightly associated; for instance, environmental conditions during dormancy induction have been shown to affect depth of dormancy (Heide, 2003).

2.2 Environmental regulation of apple dormancy

At the end of summer, beginning of autumn, shorter and colder days trigger growth cessation, bud set and induce dormancy. Photoperiod and temperature are the two environmental cues regulating this change, but their relative importance varies between species (Garner and Allard, 1923).

Day length is perceived by phytochromes (Smith, 1995), dimers located in the leaves of the plant. This signal is transmitted to the shoot apex which responds to shorter photoperiods by becoming dormant (Garner and Allard, 1923); it also leads to changes in the expression of genes responsible for regulating physiological light responses (see section *2.7 The genetics of bud dormancy*). Short photoperiods have a clear dormancy-inducing role for many species (Garner and Allard, 1923; Olsen et al., 1995a; Rohde et al., 2011); but apple does not react to this environmental cue and rather than light, it is low temperature that induces growth cessation and dormancy, regardless of short- or long-day conditions (Garner and Allard, 1923; Heide and Prestrud, 2005). Other species from the *Rosaceae* family such as pear (*Pyrus communis*) also do not react to photoperiods (Heide and Prestrud, 2005). An interaction between photoperiod and temperature has been observed in various *Prunus* species as trees appeared to be insensitive to photoperiod at temperatures above 21 ˚C whereas both factors had a growth cessation effect at lower temperatures (Heide, 2008).

Cessation of growth is a prerequisite for dormancy induction (Olsen *et al.*, 1997a). Morphologically, it is associated with two processes taking place at the apical meristem: (1) the suspension of cell division and elongation and (2) the formation of bud scales (Cooke et al., 2012). The timing of bud set, and the degree of bud development when dormancy starts, vary between species; in apple, floral buds have bractlets and sepals before dormancy starts (Foster et al., 2003). As growth stops, dormancy is progressively induced so it is difficult to establish a precise moment for the start of endodormancy (see section *2.4.4 Selecting starting dates for chilling and heat accumulation*).

As winter progresses, extended periods of cold temperatures (accumulation of chilling) are needed for endodormancy release. In apple, it is generally assumed that the same temperature range is needed for both dormancy induction and release (Heide and Prestrud, 2005). Whilst 7 °C was formerly adopted as the ideal temperature for chilling accumulation in temperate fruit trees (Samish, 1954; Vegis, 1964); it was later observed that not all temperatures contributed equally to chilling (Richardson et al., 1974; Thompson et al., 1975; Shaltout and Unrath, 1983). The amount of chilling required to release endodormancy (Chilling requirement, CR) can be quantified with statistical models, and the most recently developed models consider different temperature effectiveness's at releasing endodormancy (see section *2.4.2.1 Sequential models*). Chilling is needed for breaking dormancy of both vegetative and floral buds, CR is lower for floral buds (Thompson, Jones and Nichols, 1975; Hauagge and Cummins, 1991; Naor *et al.*, 2003; Campoy *et al.*, 2011) and higher for axillary buds compared to apical buds (Naor et al., 2003).

A great variability in chilling requirements exists in apple cultivars (Hauagge and Cummins, 1991a); and different responses to temperature have also been observed, with different temperature ranges and optimum chilling temperature being reported in the literature (see section *2.4.2.1 Sequential models*). High temperatures during dormancy induction appear to increase chilling requirements and delay bud break in apple (Cook and Jacobs, 2000) and forest boreal trees (Heide, 2003). Other studies have highlighted a negative impact of high temperature interruptions during dormancy as they can reduce bud burst (Richardson et al., 1974; Thompson et al., 1975; Anzanello et al., 2014). However, a promoting bud break effect of high-low temperature cycles compared to moderate and low temperatures has also been observed (Erez and Couvillon, 1987; Campoy et al., 2011b), and this is included in the Dynamic chilling model (see section *2.4.2.1 Sequential models*). Overall, the effect of high temperature varies depending on the dormancy stage when they occur, the length of these warm periods and whether CR has been previously satisfied (Erez and Couvillon, 1987; Fishman et al., 1987).

When chilling requirements are met, trees enter ecodormancy. At this stage, warm temperatures (accumulation of heat) are required for bud break, further bud development and blooming. Therefore, two temperature driven processes regulate the dormancy cycle, chilling accumulation for endodormancy break, and heat accumulation for ecodormancy release and blooming. There is clear evidence that chilling requirements are highly variable between apple cultivars (Hauagge and Cummins, 1991a), but less information exists in regard to heat requirements. Whilst it was initially thought that the effect of chilling and warm temperatures was sequential, a correlation exists between both processes (Ruiz et al., 2007; Darbyshire et al., 2013; Guo et al., 2014) and a growing number of studies are providing evidence for a certain degree of overlap (see section *2.4.2.3 Overlap models*).

Therefore, the importance of temperature as a dormancy regulating factor is evident; however, the relationship between temperature and bud break has not been clearly defined due to a complex interaction between chilling and heat accumulation and a lack of understanding of the physiological mechanisms regulating dormancy. Furthermore, this relationship is cultivar-specific (Thompson et al., 1975; Naor et al., 2003), creating a greater challenge to understand responses in apple varieties that have not been previously studied.

2.3 Experimental approaches to study dormancy

One of the main difficulties facing researchers studying dormancy, is that there are no visible changes in the buds during the transition from endo to ecodormancy, making it challenging to establish when it occurs. A range of methodologies have been used over the years to explore dormancy progression (Fadón and Rodrigo, 2018), but a lack of standardisation has hindered the development of clear protocols and comparisons between studies (Dennis, 2003).

The most common approach to determine chilling requirements is to investigate the dormancy status of the plant. This is done by exposing plant material (usually excised shoots, potted trees or single-node cuttings) to chilling conditions for different durations of time before transferring them to warmer temperatures to induce bud break (Ruiz et al., 2007; Campoy et al., 2012; Jones et al., 2013; Parkes et al., 2020). Chilling requirements are considered fulfilled when it takes less than a certain number of days (10-15 days) to reach a predefined percentage of bud break (20-50%). This protocol seems to have been established arbitrarily and different values can be found in the literature (Hauagge and Cummins, 1991b; Naor et al., 2003; Ruiz et al., 2007; Campoy et al., 2012; Jones et al., 2013; Parkes et al., 2020). Chilling and heat accumulation models are then used to quantify chilling and heat accumulated depending on the temperature and duration of the treatments (see section *2.4 Phenological models*).

Another approach to determine the date of endodormancy release is the Tabuenca test (Tabuenca, 1964), which proposes changes in the weight of floral buds to distinguish dormancy phases. The test is based on comparing the weight of buds placed under forcing conditions for a week with the weight of buds collected directly from the field. Whilst the original protocol used dry weight (Tabuenca, 1964), other studies have considered fresh weight (Malagi et al., 2015; Fadón et al., 2018). Endodormancy is considered to be broken when a significant weight increase is observed in buds that have been forced compared to buds collected from the field, but the original method does not define a weight increase threshold (Tabuenca, 1964). Some important aspects should be considered when using this method, as external factors, such as water availability, can impact bud weight (Bartolini et al., 2020) and mask the effect dormancy progression. The lack of a defined weight increase threshold, based on scientifically robust observations, has probably hindered its broader application, and some studies have suggested that it might not be adequate in areas with mild winters (Malagi et al., 2015).

To get a better understanding of the physiological mechanisms regulating dormancy, analytical or histochemical techniques are usually combined with one of the two methods described above to establish chilling requirements (Fadón and Rodrigo, 2018). This is important as it links any physiological observations to the dormancy status of the plant. Analytical techniques aim to investigate compounds linked to dormancy progression, such as hormones (see section *2.6.1 Hormones*) and carbohydrates (section *2.6.4 Carbohydrates*). These studies are usually based on quantifying the concentration of these substances in floral buds (Wen et al., 2016; Fernandez et al., 2019), or on exogenous application of these compounds to determine their role (Junttila and Jensen, 1988; Guak and Fuchigami, 2001). They are destructive and time-consuming methods, which have the intrinsic limitation that a singular bud or plant cannot be followed throughout the dormancy cycle, as samples are destroyed after analyses. These limitations are also encountered when using histochemical techniques, which allow visualisation of internal parts of the plant at different

dormancy stages, such as bud cells or other structures. Microscopic observations, for example, have allowed identification of different changes at the cellular level during dormancy (Rinne et al., 2001; Fadón et al., 2018) (see section *2.6.2 Cytological changes*).

Molecular approaches have become more popular in the last decade to study dormancy, including the use of transgenic individuals to determine the role of specific genes (Wisniewski et al., 2015; Wu et al., 2017), or transcriptomic analyses to understand changes in gene expression during dormancy progression (Porto et al., 2015, 2016). These methodologies have provided key information on the molecular mechanisms regulating dormancy (section *2.7 The genetics of bud dormancy*), and as they become more easily accessible, a greater contribution to the global understanding of the dormancy cycle is anticipated.

2.4 Phenological models: predicting time of bud break and flowering

Statistical phenological models are used to predict time of flowering or bud break (Darbyshire et al., 2013; Chuine et al., 2016). Bud break and flowering are phenological stages which are highly sensitive to changes in the climate and are of key importance for successful fruit production. These predictions allow anticipation of how apple cultivars will be expected to adapt to climate change and their suitability to new growing regions. Existing models use air temperature as a predictor variable, as it is the most important environmental cue regulating dormancy and blooming (Richardson et al., 1974; Chuine, 2000; Linkosalo et al., 2008). Some studies have incorporated other variables such as rainfall or solar radiation (Pope et al., 2014) but predictions were not improved.

As described in section 2.2 (*Environmental regulation of apple dormancy*), two temperature-driven processes regulate bud break and blooming, chilling accumulation to release endodormancy, and the accumulation of warm temperature, required for further bud development. Overall, two types of phenological models are commonly used in fruit trees, those that do not consider if chilling requirements have been fulfilled (Thermal time models) and those that separately model chilling and heating accumulation (Two-phase models).

2.4.1 One-phase models

The simplest phenology models are one-phase models, also called Thermal time models, which quantify the amount of heat required to reach a specific stage of bud development, such as bud break or full bloom (Ritchie and Nesmith, 1991; Trudgill et al., 2005). Thermal time models are commonly used in crop sciences to predict the rate of development of many plant processes driven by temperature, such as seed germination (Steinmaus et al., 2000) and leaf development (Ellis et al., 1986; Granier and Tardieu, 1998).

Thermal time models assume that the rate of development increases linearly with temperature (Ritchie and Nesmith, 1991; Trudgill et al., 2005). Heat accumulation is calculated as the degree-day or degree-hour summation until the development stage is reached (Anderson et al., 1986; Trudgill et al., 2005). Degree-days or degree-hours are the daily/hourly temperature accumulated above a specific base temperature, which varies depending on the species/cultivar and the stage of development (Hadley et al., 1983).

Thermal time models have the implicit assumption that chilling requirements are always satisfied, as they only describe heat accumulation during ecodormancy, omitting the endodormancy phase. Whilst they are often used to predict flowering time in annual plants (Hadley et al., 1983, 1984), one-phase models alone are not generally used in fruit trees as bud burst in these species also depends on chilling accumulation (Vegis, 1964; Lang et al., 1987; Heide and Prestrud, 2005). Blooming predictions with Thermal time models in fruit trees are often inaccurate, particularly in regions with mild winters where chilling requirements are rarely met, or under climate change scenarios with higher temperatures affecting chilling accumulation (Legave et al., 2008, 2013).

2.4.2 Two-phase models: chilling and heat accumulation models

Current phenology models combine a chilling model to quantify chilling accumulation and predict endodormancy release (i.e. Fishman et al., 1987; Richardson et al., 1974; Weinberger, 1950) (Table 2.1), with a Thermal time model used to calculate heat accumulation during ecodormancy and until bud break or flowering (Anderson et al., 1986). Two-phase models can be classified according to the interaction between the chilling and heat accumulation sub-models, including the Parallel (Landsberg, 1974), Sequential (Cannell and Smith, 1983) and Overlap models (Pope et al., 2014). Whilst many other models exist (see Chuine et al., (2013) for an extended review), only those relevant for apple phenology are reviewed below.

2.4.2.1 Sequential models

Sequential models are the most commonly used models in fruit trees and assume heating starts accumulating after satisfaction of chilling requirements (i.e. Fishman et al., 1987; Richardson et al., 1974; Weinberger, 1950) (Table 2.1).

Regarding the chilling sub-model, the first attempt to develop a chilling accumulation model was the Chilling Hours model or below 7.2 °C model (Weinberger, 1950). The Chilling Hours model was developed with peach twigs in Georgia, USA, and it assumes that all temperatures between 0 and 7.2 °C are equally effective towards chilling accumulation. It calculates chilling accumulation in Chilling Hours (CH), so that one hour at any temperature between 0 and 7.2 °C is equivalent to one CH (Weinberger, 1950). It is unclear how this temperature range was chosen as no experiments

comparing the effectiveness of different temperatures were carried out; however, probably due to its simplicity, the Chilling Hours model is still frequently used by many fruit growers.

Another commonly used model is the Utah model, also known as the High-chill model, which was developed for two peach cultivars in the United States by Richardson, Seeley and Walker (1974). This is the first model to account for the effectiveness of different chilling temperature towards releasing endodormancy, and to incorporate the negative effect of warm temperatures during endodormancy, as they can reduce bud burst (Richardson et al., 1974; Thompson et al., 1975; Anzanello et al., 2014). The Utah model quantifies chilling accumulation in Chill Units (CU); one Chill Unit is equivalent to one hour of exposure at 6 °C, believed to be the optimum temperature for chill accumulation in peach. Exposure to temperatures above or below 6 °C contribute to chilling with a proportion of a CU. Temperatures above 15 °C have a negating chilling effect, reaching a maximum of -1 CU at 22 °C and no contribution to chilling is accounted for temperatures below 0 °C (Richardson et al., 1974).

Although the Utah model successfully predicted rest completion for the peach cultivars used to develop the model, it was not accurate when Gilreath and Buchanan (1981) applied it to low-chill varieties of nectarines. Following a range of controlled environment experiments, these authors developed a variation of the Utah model, called the Low-chill model, which incorporated a greater contribution to chilling by temperatures between 8 and 18 °C. The Low-chill model performed better than the Utah model when applied to cultivars grown under mild winter conditions, where winter temperatures are likely to be within the 8-18 °C range (Gilreath and Buchanan, 1981).

The North Carolina model was the first model developed for apple, using excised shoots of the cultivar “Starkrimson Delicious” (Shaltout and Unrath, 1983). It is based on the principles of the Utah model (Richardson et al., 1974) but with temperature parameters calibrated for “Starkrimson Delicious”. The optimum temperature for chill accumulation in the North Carolina model is 7.2 °C and the maximum chilling negation is higher than with the Utah model, reaching -2 CU at 23.3 °C (Shaltout and Unrath, 1983). Similar to the Low-chill model (Gilreath and Buchanan, 1981), a greater importance is also given to the chilling contribution of temperatures between 7.2 and 19.5 °C (Shaltout and Unrath, 1983).

A more recent adaptation of the Utah model, named CUapple, was developed in Canada for “Gala” apple (Guak and Neilsen, 2013). Two main differences to the Utah model were defined, which greatly improved the performance of CUapple, especially for predicting bud break during warmer years: (i) chilling started accumulating after harvest and (ii) sub-zero temperatures were considered to contribute to chilling. When chilling temperatures between -4 and -2 °C were included in the model, bud break predictions significantly improved (Guak and Neilsen, 2013). This suggests a potentially important contribution of negative temperatures towards chill accumulation, which had

been disregarded in previous models (Weinberger, 1950; Richardson et al., 1974; Gilreath and Buchanan, 1981; Shaltout and Unrath, 1983).

Table 2.1 – Summary of existing chilling models; including: name of the model, units used to calculate chilling, crop and plant material used to develop the model, temperature range contributing to chilling, optimum temperature, temperature range that negates previous chilling accumulated and reference of the study where the model was developed.

Model	Units	Crop and plant material used	Temperature range	Optimum temp	Negating effect of warm temp	Reference
Chilling Hours or below 7.2 °C	Chilling Hours	Peach Excised shoots	0 – 7.2 °C	NA	NA	(Weinberger, 1950)
Utah	Chill Units	Peach Excised shoots	0-15 °C	6 °C	15 – 22 °C	(Richardson et al., 1974)
Low-chill	-	Nectarines Rooted cuttings	8 – 18 °C	7	18.5 - 21.5 °C	(Gilreath and Buchanan, 1981)
North Carolina	Chill Units	Apple ("Starkrimson Delicious") Excised shoots	0 – 16.5 °C	7.2 °C	16.5 - 23.3 °C	(Shaltout and Unrath, 1983)
CU apple	-	Apple ("Gala") Excised shoots	-4 - 13 °C	3.5 °C	NA	(Guak and Neilsen, 2013)
Dynamic	Chill Portions	Peach Potted trees	0 - 13 °C (13 - 15 °C to fix chilling accumulated)	6 °C	16 - 24 °C	(Fishman et al., 1987)

A particular sequential model is the Dynamic model (Fishman et al., 1987). Whilst chilling and heat also occur sequentially, the Dynamic model is based on the idea that chilling accumulation can be partially reversed. The model was developed after carrying out numerous controlled environment experiments with potted trees of peach (Erez and Lavee, 1971; Erez et al., 1979; Couvillon and Erez, 1985); it considers the same optimum temperature as the Utah model (6 °C) but chilling accumulates in Chill Portions (CP), one hour at 6 °C is equivalent to one CP (Fishman et al., 1987). The dynamic model assumes a two-step process, the first step is chilling accumulation, promoted by low temperatures (0-13 °C) and reversible by high temperatures (16-24 °C). The second step, triggered by moderate temperatures (13-15 °C), fixes chilling accumulated which becomes irreversible. In other words, high temperatures can negate chilling accumulated up to a certain level, after which high temperatures have no chilling-negation effect (Erez and Couvillon, 1987). The model was developed after observing that alternating chilling temperatures (4-15 °C) were more effective for chilling accumulation and that the impact of higher temperatures differed depending on the amount of previous chilling accumulated (Erez and Lavee, 1971; Erez et al., 1979; Couvillon and Erez, 1985). This two-step process is based on the concept that there is a "thermally unstable precursor" (not yet identified) that promotes or prevents the transition from one step to the other (Fishman et al., 1987). However, these assumptions lack experimental evidence.

2.4.2.2 Parallel and Alternating models

In comparison to Sequential models, Parallel and Alternating models consider chilling and heating can accumulate simultaneously. However, with the Parallel model chill and heat start accumulating at the same time (Landsberg, 1974), whilst a minimum amount of chill is required for heat to start accumulating in Alternating models (Cannell and Smith, 1983; Chuine et al., 1999).

The Parallel (Landsberg, 1974) and Alternating models (Cannell and Smith, 1983) consider the same temperature thresholds, between 0 and 5 °C for chilling accumulation, and above 5 °C for heat accumulation. In both models, temperatures between 0 and 5 °C are considered equally effective towards chilling accumulation, although the authors recognized that 5 °C was arbitrarily chosen (Landsberg, 1974). The Parallel model was developed with potted trees of several apple cultivars (Landsberg, 1974) whilst Cannell and Smith's model (1983) was developed for *Picea sitchensis*. The latter included a mechanism so that the amount of heat required for blooming decreased with longer chilling accumulated (Cannell and Smith, 1983), a phenomenon which has been observed in other studies (Ruiz et al., 2007; Darbyshire et al., 2013; Guo et al., 2014).

In recent years, a Partial Least Squares (PLS) Regression approach has been proposed as a tool to delineate the chilling and heat accumulation phases (Luedeling and Gassner, 2012; Luedeling et al., 2013). All studies using this approach have observed that, from the start of dormancy, periods of chilling and heat accumulation appear to alternate (Guo et al., 2014, 2015a, 2015b; Drepper et al., 2020), as proposed in the earlier Alternating model (Cannell and Smith, 1983).

2.4.2.3 Overlap models

A growing number of studies are supporting the hypothesis of an overlap between the chilling and heat accumulation phases (Pope et al., 2014; Guo et al., 2015a; Darbyshire et al., 2017; Drepper et al., 2020). They use the same chilling and heat accumulation models as Sequential models, but consider a complete or partial overlap between phases (Pope et al., 2014; Darbyshire et al., 2017). Whilst the physiological mechanisms behind this idea are not proven, these studies suggest a more gradual transition from chilling to heat accumulation.

In a study with “Golden Delicious” phenology data from 11 locations from the northern hemisphere and three locations from the southern hemisphere, Darbyshire et al., (2017) compared the performance of sequential and overlap models. The Chill Overlap model performed better than any Sequential model tested, for predicting both early and full bloom. However, all models performed poorly under mild winters (Darbyshire et al., 2017).

The performance of Sequential and Overlap models to predict blooming time of a range of almond cultivars was compared by Pope et al., (2014), considering three levels of overlap between chilling and heat accumulation (25, 50 and 75%). In this study, chilling accumulation was calculated with the

Dynamic model (Fishman et al., 1987) and heat accumulation with the Growing Degree Days model (Anderson et al., 1986) (see section *2.4.3 Heat accumulation: the Growing Degree Hours Model (GDH)*). Across all cultivars, the best model was the one that accounted for a 75% overlap (Pope et al., 2014), suggesting that Sequential models may be neglecting important physiological dormancy mechanisms due to an oversimplification of the chilling and heat accumulation process.

2.4.3 Heat accumulation: the Growing Degree Hours Model (GDH)

Whilst a range of chilling models exist, most studies predicting blooming time in fruit trees use the Growing Degree Hours (GDH) model (Anderson et al., 1986) to quantify heat accumulation. The GDH model is often combined with different chilling models in a Sequential (Legave et al., 2008; Drepper et al., 2020) or Overlap (Pope et al., 2014; Darbyshire et al., 2017) approach.

The GDH model can be understood as a type of Thermal time model that describes the relationship between warm temperature and their effectiveness towards heat accumulation as a sine-wave shape, instead of a linear relationship (Anderson et al., 1986). It assumes temperatures between 4 (base temperature) and 36 °C (critical temperature) are effective towards heat accumulation, with an optimum temperature at 25 °C. A fraction of a heat unit is proportionally accumulated at temperatures above and below the optimum, with two cosine equations defining this relationship (Anderson et al., 1986).

Other studies have investigated different functions to estimate heat accumulation, including exponential, triangular and linear relationships; but model performance was not better than with the GDH model (Legave et al., 2008, 2013).

2.4.4 Selecting starting dates for chilling and heat accumulation

A critical time point that must be determined for any model is the date when chilling accumulation starts. In the case of Two-phase models, the starting date for heat accumulation (endodormancy release) is also required (Chuine et al., 2016). These parameters are critical for accurate modelling as they have a great effect on the amount of chilling and heat accumulated.

In most models, starting dates are predefined and based on arbitrary choices. In the Dynamic model, chilling is set to start accumulating from the 1st of September (Fishman et al., 1987). In the Utah model and all its variations (except the CUapple), the starting date for chilling accumulation is the day when no more negative CU are observed (Richardson et al., 1974). In other words, negative CU accumulate if warm temperatures occur during dormancy, the starting date for chilling accumulation is the day when no further warm temperatures are recorded (Richardson et al., 1974; Gilreath and Buchanan, 1981; Shaltout and Unrath, 1983). In the Alternating model (Cannell and Smith, 1983), the 1st of November was questionably selected in the northern hemisphere as a starting date for chilling accumulation, assuming that before this date, little chilling occurs and buds are not dormant.

Some models have interpreted abrupt changes in the weather, such as the first winter frost, as signs for the start of chilling accumulation (Landsberg, 1974); and others reported better blooming predictions by accumulating chilling and heat from the end of harvest (CUapple model (Guak and Neilsen, 2013)).

Different statistical approaches have been used to find these starting dates (Nendel, 2010; Luedeling et al., 2013; Jarvis-Shean et al., 2015). In a grapevine study, the starting date selected was that producing a model with the lowest coefficient of variation (standard deviation divided by the mean) (Nendel, 2010). They compared all dates between 2 January and 31 March, and found the optimum date varied between the locations studied, ranging from the 14 February to the 12 March (Nendel, 2010). This study suggested that the starting date for heat accumulation should coincide with the time when the vertical soil temperature gradient is inverted, which is linked with timing of sap flow inversion and therefore could affect bud break (Nendel, 2010). Using non-parametric regressions, Jarvis-Shean *et al.*, (2015) found October 1st to be the optimum date for starting chilling accumulation in almonds in California; and January 1st for starting heat accumulation.

2.5 The importance of model selection for winter chilling projections under climate change scenarios

Appropriate model selection is fundamental to predict how cultivars will perform in different growing regions and climates. Overall, there is no consensus on the best model to use and great variability has been reported depending on the cultivars, locations studied and on the specific chilling and heat models selected (Legave et al., 2008, 2013; Luedeling et al., 2009a; Chuine et al., 2016; Darbyshire et al., 2017). Whilst existing chilling and heat accumulation models provided accurate flowering time predictions in the cultivars and location used to develop them (Richardson et al., 1974), results are highly variable when they are applied to other areas or species, particularly in warm regions (Luedeling and Brown, 2011). Models should be re-calibrated for each specific cultivar and location, but unfortunately, this step is usually omitted, and predictions are generated for species and regions different than those used to create the model (Guo et al., 2014; Darbyshire et al., 2016b; Parkes et al., 2020).

Two-phase models provide better predictions than one-phase models (Legave et al., 2008, 2013) and a growing number of studies suggests that overlap models might be a better approximation than sequential models to describe the chilling and heat accumulation processes (Pope et al., 2014; Guo et al., 2015a; Darbyshire et al., 2017; Drepper et al., 2020). Regarding the chilling model, the Dynamic model (Fishman et al., 1987) has been suggested to generate better predictions, particularly in warmer growing regions (Ruiz et al., 2007; Luedeling et al., 2009b). However, the

physiological basis behind its hypothesis has not been proven yet, and its complex formula has probably hindered a broader uptake by fruit growers.

Winter chilling projections under climate change scenarios highlight the disconnect between existing chilling accumulation models (Luedeling and Brown, 2011). Although winter chill reductions are predicted with all models, the severity of these impacts is diverse and highly dependent on the chilling model used, as well as on the species and locations studied (Luedeling and Brown, 2011; Darbyshire et al., 2016b). In California, Luedeling et al., (2009) estimated chilling requirements in a range of fruit and nut trees and then projected changes in chill accumulation under different climate change scenarios. Whilst reductions in winter chill were predicted with all models, the magnitude of this reduction varied greatly, with a predicted decline of 33% with the Chilling Hour Model, 26% with the Utah Model, and 14% with the Dynamic model (Luedeling et al., 2009a). A higher number of greenhouse gas emission scenarios was considered in a related study, where more severe reductions in winter chill were also projected with the Chilling Hour Model compared to the Dynamic model (Luedeling et al., 2009c). But differences between chilling models are not only restricted to climate change predictions; in the UK, Sunley et al., (2006) calculated the amount of chilling accumulated between 1950 and 2002 using the Utah and the below 7.2 °C model. They observed a decline in chilling accumulation with the below 7.2 °C model but not with the Utah model, indicating again significant differences between models and highlighting the importance of model selection. These results showed significant differences in model sensitivity to changes in the climate, suggesting that inadequate model selection could under or overestimate climate change effects on winter chill accumulation.

The disparity of results obtained between models reflects the diverse temperature dependent relationships defined in chilling accumulation models (Table 2.1). Furthermore, these models did not consider climate change scenarios when they were created, hindering the development of accurate predictions under these new conditions. Another limitation is that two-phase models have to estimate chilling requirements, as the only phenological observation they incorporate are blooming dates. These estimations are often unreliable and including endodormancy release dates for each variety would likely increase model accuracy, especially under future warmer climates (Chuine et al., 2016).

2.6 Physiological processes regulating dormancy

Although no apparent external changes occur in the buds during endodormancy, many physiological processes take place internally, including changes in the balance of hormones (Michalczuk, 2005; Cooke et al., 2012), in the composition of the cell membrane (Rinne et al., 2001; Horvath et al., 2003) and in the concentration of different carbohydrates (Ito et al., 2012; Fernandez et al., 2019). Some of these key changes are summarised in the following sections.

2.6.1 Hormones

The theory that dormancy is induced, terminated and regulated by changes in the balance of growth inhibitors and promoters, known as the Linear hormonal hypothesis, was one of the first attempts to try to explain the dormancy process in seeds (Amen, 1968). Changes in the balance of hormones are also associated with the bud dormancy process (Olsen et al., 1995b; Li et al., 2003a; Ruttink et al., 2007; Cooke et al., 2012; Liu and Sherif, 2019) but a direct regulatory effect has not yet been demonstrated.

Changes in the concentration of gibberellins (GA), auxin (indole-3-acetic acid, IAA), cytokinins (CK), Abscisic Acid (ABA) and ethylene (ET) have been linked to different stages of the dormancy cycle. GA, IAA and CK are known plant growth promoters whilst ABA and ET are growth inhibitors.

Gibberellins (GA)

Gibberellins (GA) are known growth promoters involved in many plant processes, including shoot elongation and seed germination (Hedden and Sponsel, 2015). Changes in the concentration of various GA types have been reported in the literature at different dormancy stages; overall, GA levels decrease during growth cessation, and increase before bud break (Cooke et al., 2012; Liu and Sherif, 2019).

During short-day induced growth cessation in bay willow (*Salix pentandra)*, gibberellins are down-regulated before growth stops (Junttila, 1990; Olsen et al., 1995a, 1997a). Whilst no changes in GA levels have been observed during dormancy maintenance, these increase before bud break and growth initiation (Olsen et al., 1997b). Exogenous application of GA has also been shown to reinitiate shoot elongation of bay willow (Junttila and Jensen, 1988) and to restore growth of birch (*Betula pendula*) and hybrid aspen (*Populus tremula x tremuloides*) seedlings under short-day conditions (Mølmann et al., 2003). A GA_4 treatment also induced earlier bud break in Japanese apricot (*Prunus mume* Sieb. et Zucc) and promoted changes in proteins associated with energy metabolism and oxidation-regulation (Zhuang et al., 2013).

The specific mechanisms by which GA affects dormancy are not clearly defined but in underground buds of leafy spurge, GA have a key role in inducing the expression of genes involved in the cellular synthesis phase as well as those associated to the G1-S phase transition (Horvath et al., 2002). Rinne et al., (2011) showed that GA_4 can restore plasmodesmata connectivity after endodormancy release in Populus (see section *2.6.2 Cytological changes*), and GA has also been linked to increasing levels of reactive oxygen species (ROS) in apricot (Zhuang et al., 2013), which are key for bud break (Beauvieux et al., 2018).

Existing studies are mostly focused on species where photoperiod has a key dormancy-regulating role (Olsen et al., 1997a; Mølmann et al., 2003; Zhuang et al., 2013), but no information has been

found for apple or pear, where dormancy is regulated by temperature (Garner and Allard, 1923; Heide and Prestrud, 2005).

Abscisic Acid (ABA)

Abscisic Acid (ABA) is a plant growth inhibitor which regulates many plant processes such as cell division and seed germination, and plays a key role in plants' responses to environmental stresses like drought and cold (Finkelstein, 2013).

ABA levels fluctuate during dormancy in an opposite pattern to GA; its concentration increases during growth cessation, reaches a peak in dormant buds and decreases with dormancy release (Rinne et al., 1994a; Li et al., 2003b; Wen et al., 2016). Studies have associated the increase in bud ABA levels with short-day induced dormancy (Rinne et al., 1994a; Ruttink et al., 2007). Furthermore, application of exogenous ABA not only reduced shoot elongation and induced growth cessation in "Fuji" apple nursery plants but it also enhanced dormancy development (Guak and Fuchigami, 2001). In this same study, ABA significantly accelerated cold acclimation, although the final level of cold resistance reached was the same in trees with and without exogenous ABA application (Guak and Fuchigami, 2001).

ABA and GA appear to have antagonistic roles in dormancy regulation (Wen et al., 2016; Liu and Sherif, 2019); in Japanese apricot flower buds, ABA and GA concentrations followed opposite patterns throughout dormancy progression (Wen et al., 2016). Furthermore, low-chill cultivars showed lower ABA and higher GA_3 levels, compared to high-chill varieties; suggesting a link with temperature-regulated dormancy break (Wen et al., 2016). Another study with tea showed that exogenous GA application during dormancy affected the expression of genes related with the metabolism and signalling pathways of ABA; and the same occurred with GA genes when an ABA treatment was applied; indicating a clear link between the concentration of both hormones (Yue et al., 2018).

Some studies have suggested that ABA might regulate dormancy by controlling plasmodesmata opening (Tylewicz et al., 2018). During endodormacy, plasmodesmata become blocked with deposition of callose (1,3-β-D glucan) (Rinne et al., 2001) (see section *2.6.2 Cytological changes*). In a study with hybrid aspen, ABA-insensitive transgenic trees were used to study the role of ABA during dormancy (Tylewicz et al., 2018). A short-photoperiod induced growth cessation equally in transgenic and non-transgenic plants; however, when transferred to long-day conditions, buds from wild type plants remained dormant whilst transgenic plants reinitiated growth. It was observed that the frequency of closed plasmodesmata after 10 weeks of short-days in transgenic plants, was close to 0%, whilst it had reached 83.6% in the wild type, indicating a key role of ABA mediating plasmodesmata closure as a response to short photoperiods (Tylewicz et al., 2018).

ABA also appears to have a key role in regulating the cell cycle (Gutierrez et al., 2002; Horvath et al., 2003). Cells in dormant buds appear to be mostly at the G1 stage, and ABA stops further cell development by preventing DNA replication (Gutierrez et al., 2002) (see section *2.6.2 Cytological changes)*.

Ethylene (ET)

Ethylene (ET) is also involved in many plant processes, such as fruit ripening and vegetative growth (Smalle and Van Der Straeten, 1997; Bleecker and Kende, 2000).

ET appears to be involved in dormancy induction and bud formation (Ruttink et al., 2007). In a study with transgenic ethylene-insensitive birch trees, Ruonala *et al.*, (2006) showed that under short-day conditions, mutant lines stopped growth like the wild-type; however, they did not form terminal buds and the development of dormancy was significantly delayed. A link between ABA and ethylene levels was also observed as no changes in ABA concentration were detected in apical buds of mutant trees when transferred to short-days, and application of exogenous ABA did not inhibit bud burst as it did in the wild-type (Ruonala et al., 2006). Mutants exhibited reduced apical dominance, producing three times more branches than the wild type and a bush-like appearance (Ruonala et al., 2006). These observations suggest an interaction not only between signalling pathways of ethylene and ABA but also with auxins, known to be involved in the control of apical dominance (Cline, 1991, 2000).

Auxins (IAA) and cytokinins (CK)

Auxin indole-3-acetic acid (IAA) is the most common hormone of the auxin class and, as well as gibberellins, it is also thought to be involved in shoot elongation and growth cessation. IAA levels seem to follow a similar pattern to GA during dormancy, decreasing with growth cessation under short days and increasing with long photoperiods until bud break (Olsen et al., 1997a, 1997b; Li et al., 2003b). Cytokinins (CK) are a type of phytohormone involved in cell growth and promote cell division (Zürcher and Müller, 2016).

IAA and CK have been widely studied in relation to paradormancy as they regulate apical dominance (Cline, 1991, 2000); however, their role during endodormancy is less well understood. The apical bud is a major site for auxin production, from where it moves basipetally towards lateral buds (Woolley and Wareing, 1972; White et al., 1975). Application of exogenous auxin at the top of decapitated stems is able to repress growth of lateral buds, indicating an important function of apically produced auxin in inducing apical dominance (Thimann and Skoog, 1933; Cline, 2000). Auxin regulates branching by affecting the levels and transport of other substances involved in growth promotion, such as cytokinins (Bangerth, 1994). After decapitation of the apical bud, CK levels in xylem sap of bean plants increased dramatically (Bangerth, 1994), and auxin application on decapitated shoots

reduced CK concentration (Bangerth et al., 2000), indicating a clear connection in the balance of these hormones.

But CK might also play a role during dormancy release (Faust et al., 1997); in excised apple shoots, the concentration of CK in xylem sap increased before bud break (Cutting et al., 1991). In this same study, application of Hydrogen Cyanamide (a chemical used to release dormancy in areas with insufficient chilling (Jackson and Bepete, 1995; Griesbach, 2007)) increased the amount of CK in xylem sap and advanced bud break (Cutting et al., 1991), suggesting an important role of this hormone at the later dormancy stages.

2.6.2 Cytological changes

Various cytological changes have been observed at different stages of the dormancy cycle (Rinne et al., 2001; Gutierrez et al., 2002; Horvath et al., 2003). One of the key attributes of dormancy is the suspension of cell division and elongation (Horvath et al., 2003). In eukaryotes, the cell cycle has four phases: G1, S, G2, and M. The synthesis phase (S) is when DNA replication takes place, and the mitosis phase (M) is when the cell divides. Phases G1 and G2 are gap phases. During the first gap phase the cell expands and prepares for DNA replication whilst during G2 the cell prepares for mitosis. During dormancy, cells appear to be arrested between phases G1 and S (Gutierrez et al., 2002), and genes that act at the G1-S transition phase seem to be upregulated after dormancy break (Devitt and Stafstrom, 1995).

Changes in cell-to-cell communications through plasmodesmata have also been reported (Jian et al., 1997; Rinne et al., 2001). In a study with poplar (*Populus deltoids*), Jian *et al.*, (1997) observed a decrease in the frequency of plasmodesmata in cell walls of apical buds during the development of dormancy, as well as a reduction in the diameter of the pores. A study with birch observed that during dormancy induction, plasmodesmata became blocked by the presence of newly formed deposits of 1,3-β-D glucan (callose). This resulted in isolation of the cells until the apical meristem was exposed to chilling conditions required to release dormancy (Rinne et al., 2001). These studies suggest an important role of plasmodesmata communication regulating dormancy, as the opening of these pathways allows the movement of certain hormones and molecules that could have a role in dormancy control.

At a cellular level, changes in the water status in the buds during dormancy development have also been reported (Faust et al., 1991), shifting from a bound state during endodormancy, to free water when ecodormancy is reached. Bound water is primarily restricted to the cell wall matrix whilst free water is mainly intracellular. This suggests that satisfaction of chilling requirement is linked with a conversion from bound to free water (Faust et al., 1991; Malagi et al., 2015), relating bound water to cold resistance during dormancy and free water to growth resumption.

In a study with “Delicious” apple buds, Wang and Faust (1990) observed important changes in the composition of lipids in cell membranes during dormancy development due to chilling exposure. The concentration of polar lipids increased as buds were formed and dormancy developed, peaking at the end of April during bud expansion (Wang and Faust, 1990). As the major increase was found in linoleic acid, Erez (2000) suggested the interaction between the enzymes responsible of regulating linoleic acid (oleate desaturase and linoleate desaturase) as a key dormancy control mechanism, due to the different temperature ranges they required to be activated.

2.6.3 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are by-products of the metabolism of oxygen, produced during photosynthesis and respiration in different organelles (Huang et al., 2019). ROS are involved in plant development (Considine and Foyer, 2014) and stress responses such as disease resistance (Grant and Loake, 2000). However, high ROS concentrations are also linked to DNA damage and cell death (Redza-Dutordoir and Averill-Bates, 2016).

Beauvieux et al., (2018) highlighted a key role of ROS during dormancy, as they are involved in many metabolic processes regulating growth and hormone signalling pathways (Zhuang et al., 2013; Considine and Foyer, 2014). An increase in the concentration of Hydrogen peroxide (H_2O_2), a ROS, was observed in dormant grapevine flower buds before endodormancy release (Pérez and Burgos, 2004; Pérez and Lira, 2005). Similarly, H_2O_2 content in Japanese pear flower buds increased gradually with chilling accumulation, and decreased with endodormancy break (Kuroda et al., 2002). Furthermore, H_2O_2 levels remained low in buds that did not receive any chilling (Kuroda et al., 2002).

Studies indicate that Hydrogen Cyanamide, induces bud break by increasing ROS in buds (Pérez and Burgos, 2004; Pérez and Lira, 2005), suggesting a link between ROS and dormancy break.

2.6.4 Carbohydrates

Temperate woody perennials, such as apple, accumulate carbohydrates before winter, usually in the form of starch. Bud development in spring depends on carbohydrates stored during the previous season, which are also essential to survive winter months (Sauter et al., 1996). Carbohydrates play a crucial role during frost hardiness and cold acclimation (Sauter et al., 1996) by increasing freezing tolerance and supporting embolism restoration (Améglio et al., 2000, 2004).

As temperatures rise in spring, stored carbohydrates are degraded into soluble sugars and transported to different areas of the plant (Bonhomme et al., 2010; Tixier et al., 2017). Phloem transport is highly restricted during winter, so xylem carbohydrate transport becomes crucial for bud growth (Loescher et al., 1990; Decourteix et al., 2008; Ito et al., 2012). In apple xylem sap, sorbitol levels increase with colder temperatures (Raese et al., 1977) and studies in pear have observed low sorbitol concentration in trees receiving insufficient chilling (Ito et al., 2013).

Many studies have shown a correlation between seasonal carbohydrate dynamics and dormancy progression (Bonhomme et al., 2010; Ito et al., 2012; Kaufmann and Blanke, 2017; Fadón et al., 2018), with some suggesting changes in carbohydrate concentrations could be used as physiological markers to distinguish cultivars with different chilling requirements (Fernandez et al., 2019). Studies have investigated carbohydrate concentrations in different areas of the plant, including buds, stems, xylem sap, and various floral organs (Bonhomme et al., 2005, 2010; Ito et al., 2013; Kaufmann and Blanke, 2017). The type and concentration of sugars varies between species; but overall, soluble carbohydrate levels in floral buds peak during endodormancy and decrease before bud break (Bonhomme et al., 2005; Ito et al., 2012; Fernandez et al., 2019), whilst starch levels remain low during winter (Bonhomme et al., 2010; Ito et al., 2012; Kaufmann and Blanke, 2017). Peaks of different carbohydrates during endodormancy have also been reported in xylem sap (Bonhomme et al., 2010; Ito et al., 2012, 2013).

The bud break inducing effect of Hydrogen Cyanamide is also linked to carbohydrate concentrations as it increases starch degradation and concentration of soluble sugars, resulting in higher ROS levels in the buds (Pérez and Lira, 2005; Beauvieux et al., 2018).

2.7 The genetics of bud dormancy

It has been demonstrated that bud break and flowering time are heritable traits (Labuschagné et al., 2002); but the influence of environmental factors is also highly significant (Celton et al., 2011), and dormancy's close link with other physiological processes such as cold acclimation make it difficult to ensure changes observed in gene expression are linked to dormancy itself and not to other factors. An understanding of the genetic determinism of dormancy regulation and, particularly, chilling requirements, would improve apple breeding programmes aiming to select suitable varieties for the climate of each growing region.

Although no genetic markers for chilling requirement have yet been elucidated, important advances in our understanding of the genetics behind dormancy have taken place during the last decade and various studies have identified candidate genes for dormancy regulation in apple (Mimida et al., 2015; Wisniewski et al., 2015; Wu et al., 2017). In particular, one group of genes have received most attention, the *DORMANCY-ASSOCIATED MADS-box (DAM)* genes, which are sometimes referred to as SVP-like genes as they are phylogenetically related to the SHORT VEGETATIVE PHASE (SVP) genes from *Arabidopsis thaliana* (Mimida et al., 2015; Porto et al., 2016; Falavigna et al., 2019).

DAM genes were first discovered in a peach mutant called *EVERGROWING (EVG)*, which showed continuous growth and an inability to enter dormancy (Bielenberg et al., 2004, 2008). Since then, they have been identified in many fruit trees, including apple (Celton et al., 2011; Mimida et al., 2015; Wisniewski et al., 2015; Porto et al., 2016). Several *DAM* genes have been described and in

apple there is a debate on the terminology to use as authors have assigned different names for each gene (Mimida et al., 2015; Wisniewski et al., 2015; Porto et al., 2016). This review will use the nomenclature proposed by Porto *et al.*, (2016).

The expression of DAM genes changes throughout dormancy (Mimida et al., 2015; Porto et al., 2016; Wu et al., 2017). Falavigna et al., (2019) summarised these changes in three overall expression patterns: (i) DAM genes which increase expression during bud set, at the beginning of autumn; (ii) DAM genes showing the peak of expression during endodormancy and decreasing after fulfilling chilling requirements; and (iii) DAM genes with the highest level of expression before bud break. These three patterns have been observed in apple (Mimida et al., 2015; Porto et al., 2016; Wu et al., 2017).

In the cultivar "Jonathan", Mimida et al., (2015) monitored the expression of four DAM genes throughout the season in trees exposed to natural chilling in the field. Expression of *MdDAM1* peaked during bud set and then decreased, whilst *MdDAM2* was high in summer and decreased with dormancy induction. Similar results were observed under artificial chilling for the cultivars "Fuji Standard", "Royal Gala" and "Castel Gala" (Porto et al., 2016). *MdDAM2* transcription peaked in the summer whilst peaks during winter were observed for *MdDAM1, MdDAM3* and *MdDAM4*. All cultivars followed similar patterns but *MdDAM1, MdDAM3* and *MdDAM4* expression in "Castel Gala" decreased quicker, indicating a cultivar-specific gene expression linked to chilling requirements as "Castel Gala" is a low-chill variety (Porto et al., 2016). In a study with the cultivar "Sciros", high levels of *MdDAMb* expression were detected before bud break, and transgenic trees with *MdDAMb* overexpression showed delayed time of bud break, suggesting an important role in growth inhibition (Wu et al., 2017).

Overexpression of a peach *CBF* (C-Repeat Binding Factor) gene, related with cold tolerance, increased cold hardiness in apple, delayed bud break, reduced growth and induced early dormancy (Wisniewski et al., 2011; Artlip et al., 2014). Transgenic lines also showed a different expression of DAM genes *MdoDAM1* and *MdoDAM3* compared to non-transformed trees (Wisniewski et al., 2015), indicating again these genes could be important candidates for dormancy regulation.

Overall, results from existing studies suggest a link between temperature regulated dormancy and DAM gene expression (Mimida et al., 2015; Porto et al., 2016; Wu et al., 2017; Falavigna et al., 2019). Other studies have provided key information on the molecular changes that take place during dormancy development, including changes in the expression of genes associated with light signal transduction pathways, sugar biosynthesis and cell proliferation genes (Ruttink et al., 2007). Many studies have highlighted the importance of epigenetic mechanisms regulating dormancy and the expression of DAM genes (Horvath, 2009; Falavigna et al., 2019). Whilst molecular techniques provide a great tool to improve our understanding of the dormancy process, it will take decades for

this information to enter breeding programs. Meanwhile, it is key to use other methodologies to anticipate how existing cultivars will perform under different climates.

2.8 Research objectives

Winter chilling has declined in the UK over the last decades, and further reductions are predicted (Atkinson et al., 2004; Luedeling et al., 2011). Warmer spring temperatures will advance blooming times and increase the risk of spring frost, already causing losses to UK apple growers (DEFRA et al., 2017).

From the literature review conducted here, it is clear that chilling accumulation and the relationship between temperature and bud break is cultivar-specific. However, existing chilling accumulation models were developed for different fruit species and have not been re-calibrated for apple cultivars. Furthermore, they were developed without considering the climate variability expected with global warming, do not incorporate the correlation between the chilling and heat accumulation processes, and lack a link to biological principles as the physiological mechanisms behind dormancy break are not well understood. These limitations are most likely hindering the formulation of accurate predictions.

The aims of this research are therefore (i) to investigate the cultivar-specific relationship between temperature and bud break in a range of commercial apple varieties, incorporating the climatic variability predicted with climate change; (ii) to improve our understanding of the interaction between chilling and heat accumulation, (iii) and to investigate possible physiological markers that could help develop more accurate models.

Chapter 3

Dormancy progression of eight apple cultivars and the effect of warm temperatures during ecodormancy

3.1 Introduction

Time of bud break in apple is regulated by two temperature-driven factors, chilling accumulation in winter and heat accumulation in the spring (see section *2.2 Environmental regulation of apple dormancy*). Because of the combined effect of these two processes, the predicted impacts of climate change on apple production include not only poor bud break as a consequence of insufficient winter chilling (Campoy et al., 2011a; Atkinson et al., 2013), but also a higher risk of frost damage due to an earlier start to the growing season (Harding et al., 2015; Pfleiderer et al., 2019).

Although declines in winter chilling are predicted in the UK as a consequence of climate change (Atkinson et al., 2004), current levels of winter chill are sufficient to meet the chilling requirements of most apple cultivars (Hauagge and Cummins, 1991a). However, a more immediate impact on apple production could be caused by an earlier start to the growing season and the associated risk of frost damage (Harding et al., 2015). Earlier blooming dates have already been observed in different apple growing regions worldwide as consequence of warmer temperatures (Fujisawa and Kobayashi, 2010; Legave et al., 2013; Drepper et al., 2020), and significant production losses due to spring frost have been reported in the UK in recent years (DEFRA et al., 2017).

When winter chilling is not a constraint, time of bud break is mostly determined by heat accumulation in spring (Drepper et al., 2020). Whilst a range of chilling models exist, most studies calculate heat accumulation according to the Growing Degree Hours (GDH) model (Anderson et al., 1986) (see section *2.4.3 Heat accumulation*). The model was developed with sour cherry, but it is used to quantify heat accumulation in most fruit crops, without any re calibration (Ruiz et al., 2007; Guo et al., 2015a; Darbyshire et al., 2017). Since heat requirements are also cultivar-specific (Hauagge and Cummins, 1991a), more research is needed to identify specific heat accumulation needs in commercial cultivars.

To anticipate how different apple cultivars will perform under warming climates, it is important to understand not only their responses to mean warmer temperatures, but also to extreme

fluctuations in temperature during different phenological stages, as this will determine the likely impact on fruit production. Blooming and bud break stages are highly vulnerable to frost damage (Augspurger, 2013; Pfleiderer et al., 2019), and periods of unusually warm temperatures after endodormancy release have the potential to induce earlier bud break, as heat requirements could be met quicker. An increase in the frequency of these extreme events is predicted with climate change and they have already been observed in recent years (Murphy et al., 2018; Kendon et al., 2020); in February 2019, temperatures above 18 °C were recorded for seven consecutive days in the UK (Kendon et al., 2020). Understanding the potential of these events to induce bud break at different dormancy stages could help develop effective management practises to reduce the risk of frost damage.

This chapter is structured in two parts; first, dormancy progression of eight apple cultivars has been studied to differentiate dormancy stages according to those defined by Lang et al., (1987) (see section *2.1 Dormancy: definition and phases*). Secondly, the effect of warm temperatures on time of bud break during ecodormancy was investigated. The eight apple cultivars included in this study were chosen based on commercial relevance (Jackson, 2003; DEFRA, 2021) but also to include a range of flowering times (observed in long-term phenology datasets from NIAB EMR) and chilling requirements (Hauagge and Cummins, 1991a, 2000; Jackson, 2003). Preliminary results for cultivars "Braeburn Mariri Red" and "Galaxy Gala" were presented at the XII International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems (2021), and the corresponding publication was accepted and its awaiting publication. Cider varieties were included as requested by one of the funding organisations of this research. Whilst other sports of the chosen varieties might be commercially more important, those studied were prioritised as trees were growing in the same field and under the same management practises, ensuring comparable results between cultivars.

3.2 Materials and methods

One-year-old excised apple shoots from eight apple cultivars were used for this experiment: "Bramley", "Braeburn Mariri Red", "La Vera Cox", "Fuji Aztec", "Galaxy Gala", "Jonagold Robijn", and two cider varieties, "Dabinett" and "Kingston Black".

Shoots were collected from an orchard at NIAB EMR, south-east England (51.287089, 0.445985) (Figure 3.1); all trees were planted in 2014 on "M9" rootstocks. Forty-to fifty-centimetre-long shoots were sampled between 1 and 1.8 m above the ground and placed in plastic bags to avoid desiccation during transport to the laboratory. During two consecutive years, shoots were collected at different time points from the start of chilling accumulation until bud break (26 October 2018 to 14 March 2019 in Year 1, and 23 October 2019 to 7 March 2020 in Year 2). The cultivars "Dabinett" and "Kingston Black" were only studied in the second year (2019/2020). Seven shoot collections were

completed during winter 2018/19 and six during the second year of study (2019/20) (Table 3.1). Hourly temperature in the experimental orchard was recorded by an Adcon system with telemetry installed on site and data were acquired with addVANTAGE Pro 6.4 Software.

Figure 3.1 – Experimental orchard at NIAB EMR where shoots were collected during both years of study. Photo taken on 11/02/2021.

On the day of collection, shoots were cut to 30 cm lengths, individually labelled, and placed in four growth cabinets (Versatile Environmental Test Chamber MLR-352H, Panasonic Healthcare Co., Ltd., Japan) to force bud break at four different temperatures: 13, 16, 19 and 22 °C (± 0.5 °C), 90% relative humidity and a 16 h photoperiod. Light was supplied with fluorescent lamps (FL40SSENW37), providing a photosynthetic photon flux density of approximately 300 $\mu molm^{-2}s^{-1}$. Ten shoots were taken per collection and forcing temperature treatment for all cultivars except "Fuji Aztec", "Jonagold Robijn", "Dabinett" and "Kingston Black", for which only 5 shoots were used due to limited availability of wood. During forcing treatments, shoots were stood with their basal ends in 2.5 litre buckets (Figure 3.2) containing a mixture of tap water and bleach at 5 ml/litre of water (Cook and Jacobs, 1999; Campoy et al., 2011b). Once a week, the water mix was changed and 1 cm of the base of each shoot was cut to avoid vessel occlusion.

In both years, the total number of buds per shoot was counted at the beginning of the experiment and bud break was assessed twice a week for 50 days. Bud-break was recorded when green tip (Figure 3.3) (Stage 3 of development, as defined by Chapman and Catlin (1976)) was observed.

Table 3.1 –Shoot collection dates for the two years of study, and chilling accumulated (Chill Units, CU (Richardson et al., 1974)) at each collection date.

Year of study	Collection number	Collection date	Chill Units accumulated
2018/19	1	26 Oct 2018	108.5
2018/19	2	22 Nov 2018	538.0
2018/19	3	14 Dec 2018	900.5
2018/19	4	4 Jan 2019	1277.0
2018/19	5	28 Jan 2019	1697.5
2018/19	6	21 Feb 2019	2090.0
2018/19	7	14 Mar 2019	2420.0
2019/20	1	23 Oct 2019	152.0
2019/20	2	13 Nov 2019	490.5
2019/20	3	12 Dec 2019	960.5
2019/20	4	9 Jan 2020	1519.5
2019/20	5	6 Feb 2020	2026.5
2019/20	6	7 Mar 2020	2559.0

Chilling accumulation

For each collection date (Table 3.1), the number of Chill Units (CU) (Richardson et al., 1974) and Growing Degree Hours (GDH) (Anderson et al., 1986) accumulated was calculated using the R package *chillR* (Luedeling, 2021). The basis of these models is explained in *section 2.4 Phenological models: predicting time of bud break and flowering.*

The start date of chilling accumulation was the first day after which no negative CU were recorded, as established by the Utah model (Richardson et al., 1974). This occurred on 17 October in 2018 and on 7 October in 2019. GDH accumulation was calculated in two ways: starting to accumulate on the same day as chilling, as assumed in parallel models (Landsberg, 1974), and starting to accumulate after chilling requirements are met, as in sequential models (Richardson et al., 1974) (see sections *2.4.2.1 Sequential models* and *2.4.2.2 Parallel and Alternating models*).

Figure 3.2 – Shoots were individually labelled after being collected. Photo taken 28/01/2019 after the fifth shoot collection.

Figure 3.3 – Green tip stage as defined by Chapman and Catlin (1976). Photo taken on 20/03/2018.

3.2.1 Data analyses

All data analyses and graphs were carried out using R statistical software (R Core Team, 2021). Time and percentage of bud break were used to assess the effect of chill accumulation and forcing temperature on the apple cultivars studied here. Time of bud break (*Time to first bud break (Days)*) was defined as number of days until the first bud reached green tip (Chapman and Catlin, 1976) in a shoot; and *Maximum bud break (%)* was the percentage bud break measured at the end of the forcing period.

Cultivar-specific General Linear Mixed Models (GLMM) of days to first bud break were developed with the *lme4* R package (Bates *et al.*, 2015), using data from the first year of study (2018/19). Models included three fixed terms: CU accumulated, forcing temperature and the interaction between both variables; and a random term to deal with pseudoreplication as some shoots were collected from the same tree (i.e., the bundle of shoots from a cultivar-collection-forcing temperature was considered the experimental unit). Model assumptions, including homogeneity of variance and normality of residuals, were visually assessed for each model. The total number of buds per shoot was included in each model to account for the variability in number of buds. For modelling

purposes, a value of 50 days (maximum number of days bud break was assessed over) was applied to shoots where no bud break was observed, as done previously in similar studies (Guak and Neilsen, 2013).

Models were developed with 2018/19 data and validated with data from the second year of study (2019/20). Only the last four collections, corresponding to the ecodormancy phase (see section *3.4 Discussion*), from each year were used for fitting and validating the models. For cultivars "Dabinett" and "Kingston Black", data from 2019/20 was used to fit the models, which could not then be validated as only one year of data was available.

To better understand the potential effect of periods of unusually warm weather (hereafter referred to as a "heatwave") from mid-winter to late spring (ecodormancy) on time of bud break; cultivar-specific models were used to generate predictions. The number of days it would take for the first buds to open in each apple cultivar was estimated after a range of chilling accumulations (1,500 – 2,250 CU) and assuming four heatwave scenarios at mean temperatures of 14, 18, 22 and 26 °C.

3.3 Results

Chill and heat accumulation during the years of study

Chill accumulation started 10 days later in 2019 but the rate of accumulation was higher, and more chilling had been recorded by January 2020, compared to the first year of study (Figure 3.4). Whilst GDH accumulation from the start of chilling increased more rapidly in 2018, less than 7,000 GDH had accumulated by March 2019. In comparison, over 8,000 GDH had been recorded by March 2020 (Figure 3.4). Regarding GDH accumulated after fulfilling CR, it also increased quicker in 2019/20, with almost 4,000 GDH recorded in three months, compared to less than 3,000 GDH in the previous year (Figure 3.4).

In 2018/19, the cumulative percentage change in CU and GDH calculated from CR fulfilment followed a similar trend, increasing steadily from the first collection (Figure 3.5). GDH accumulated from CR fulfilment increased more rapidly than chilling in 2019/20. In both years of study, GDH values calculated from the start of chilling increased more slowly than did chilling (Figure 3.5).

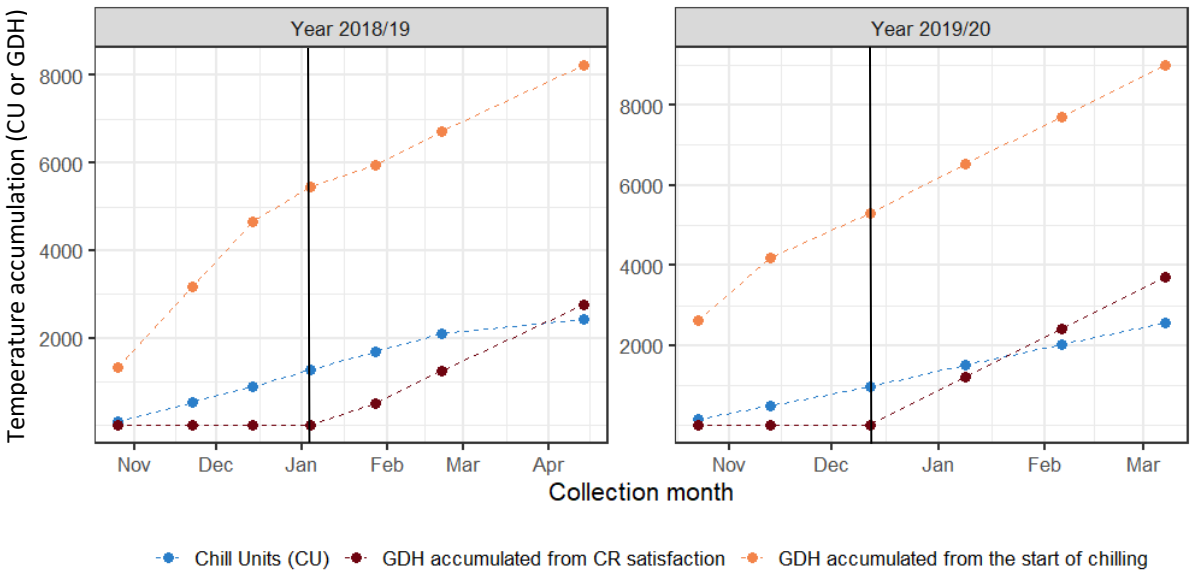


Figure 3.4 – Chilling and heat accumulation during the two years of study. Different colour dots indicate the temperature accumulated at the time of each shoot collection: Chill Units (Richardson et al., 1974) (blue) and GDH (Anderson et al., 1986) (orange) started accumulating on 7 October in 2018/19 and 17 October in 2019/20. Dark red dots represent GDH accumulating from satisfaction of chilling requirements. Vertical black lines indicate the start of ecodormancy.

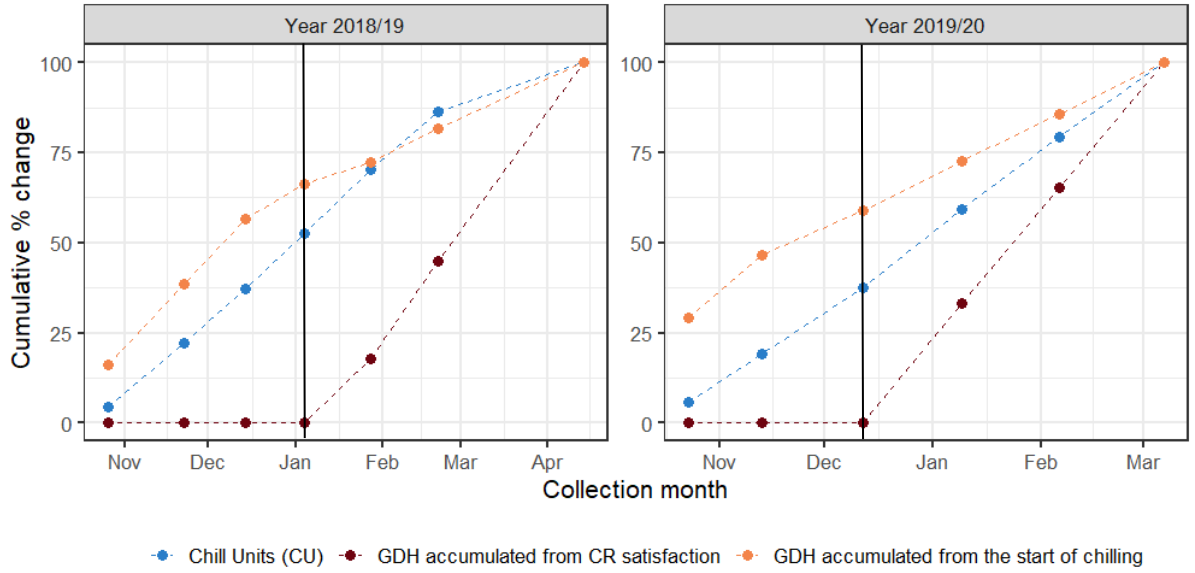


Figure 3.5 – Cumulative percentage change in chilling and heat accumulated throughout both years of study. Different colour dots indicate the cumulative percentage change at the time of each shoot collection: Chill Units (Richardson et al., 1974) (blue) and GDH (Anderson et al., 1986) (orange) started accumulating on 7 October in 2018/19 and 17 October in 2019/20. Dark red dots represent GDH accumulating from satisfaction of chilling requirements. Vertical black lines indicate the start of ecodormancy.

Dormancy progression

Dormancy progression was assessed by observing the time it took for the first bud to open in a shoot (Figure 3.6) and the maximum percentage of bud break at the end of the forcing period (Figure 3.7); but only shoots forced at 22 °C, represented with **dark red** dots in Figures 3.6 and 3.7, were considered (see section *3.4 Discussion*).

Overall, in most cultivars and for both years of study, the number of days to first bud break was small in autumn, when less than 200 CU had accumulated (Figure 3.6). After the first collection, time to bud break increased, reaching a peak between collections three and four (538 - 900 CU), depending on the variety (Figure 3.6). The peak was followed by a sharp decrease as more chilling accumulated, and the least number of days to first bud break was observed after the last collection for all cultivars studied (Figure 3.6).

The number of days to first bud break after forcing at 22 °C was lowest for “Braeburn Mariri Red”, “Fuji Aztec” and “Jonagold Robjin” in both years of study; and highest in “Galaxy Gala”, “La Vera Cox” and “Bramley” (Figure 3.6). In 2018/19, at its highest value, time to first bud break after forcing at 22 °C was approximately 40 days or more in “Galaxy Gala”, “La Vera Cox” and “Bramley”, whilst it was less than 30 in “Fuji Aztec” and “Jonagold Robjin”; and less than 20 in “Braeburn Mariri Red” (Figure 3.6). At the end of the experiment, buds opened in less than 5 days in shoots of all varieties (Figure 3.6).

In most cultivars, time to first bud break at 22 °C followed a similar pattern between years (Figure 3.6). Some differences were observed at the beginning of autumn, with a slight delay reaching the peak of days to bud break in shoots of “Fuji Aztec”, “Jonagold Robijn” and “Braeburn Mariri Red” in the second year. In “Bramley”, time to bud break at its highest point was less than 30 days in 2019/20, compared to more than 40 in the previous year (Figure 3.6). Differences between years were minimal after 1,000 CU (Figure 3.6).

Maximum percentage bud break after forcing at 22 °C followed a similar pattern in both years of study, being lowest in autumn and increasing as more chilling accumulated (Figure 3.7); however, this increase was more evident in some cultivars than others. In “Bramley”, “Jonagold Robjin” and “Fuji Aztec”, percentage of bud break remained low (<50%) throughout the experimental period in both years of study. In “Galaxy Gala”, “Braeburn Mariri Red” and “La Vera Cox”, a clear increase in percentage of bud break was observed after 900 CU, with maximum bud break (>75%) occurring after the last collection (Figure 3.7). Compared to time to bud break, greater variability was observed between replicates in this parameter.

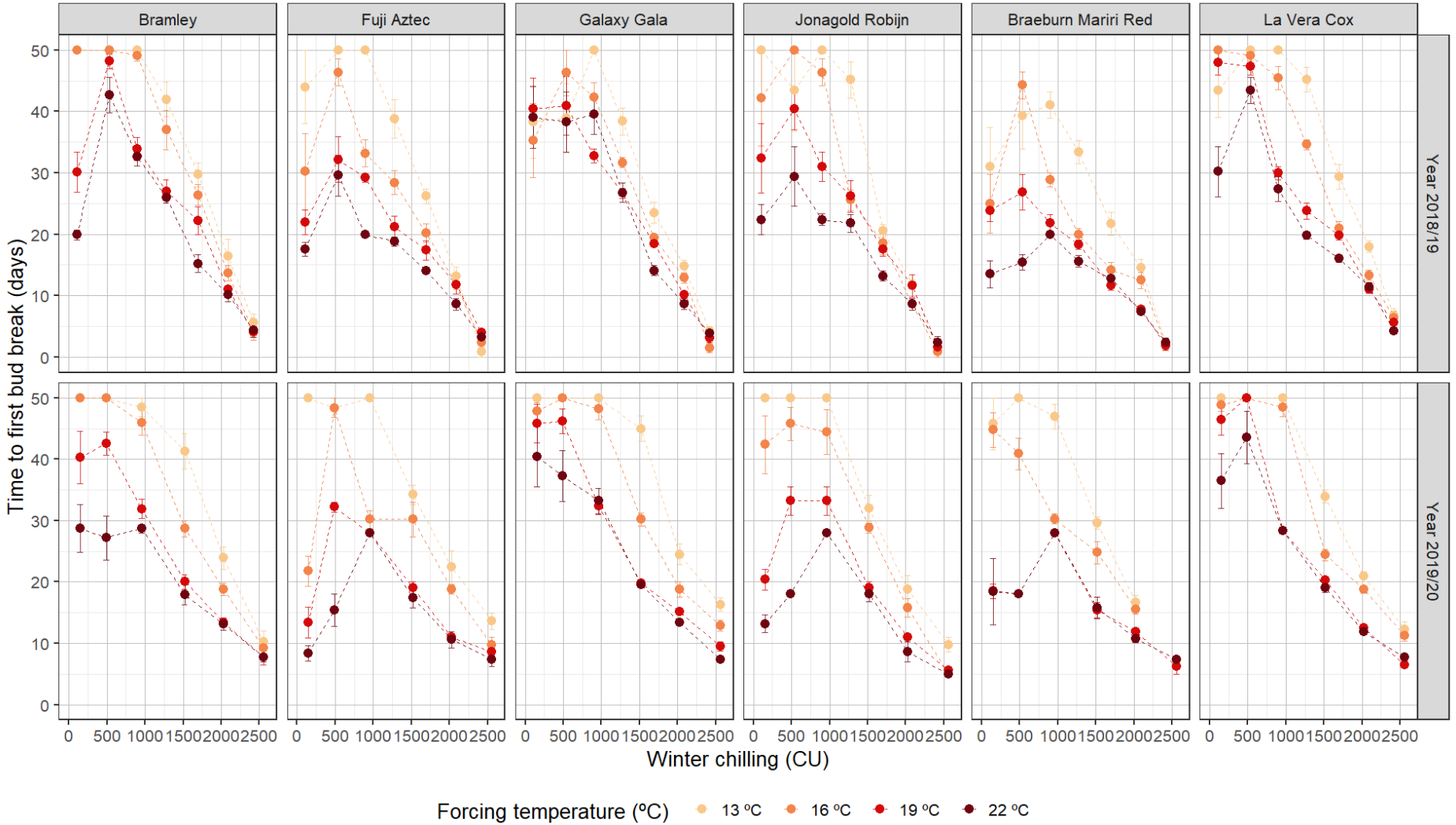


Figure 3.6 – Mean days to first bud break ± SE, of one-year old shoots of "Bramley", "Fuji Aztec", "Galaxy Gala", "Jonagold Robjin", "Braeburn Mariri Red" and "La Vera Cox", after receiving different amounts of chilling in the field (Winter chilling, CU) and being forced at different temperatures (13, 16, 19 and 22 °C) as indicated by different colour dots. Data presented from both years of study, 2018/2019 (top) and 2019/20 (bottom).

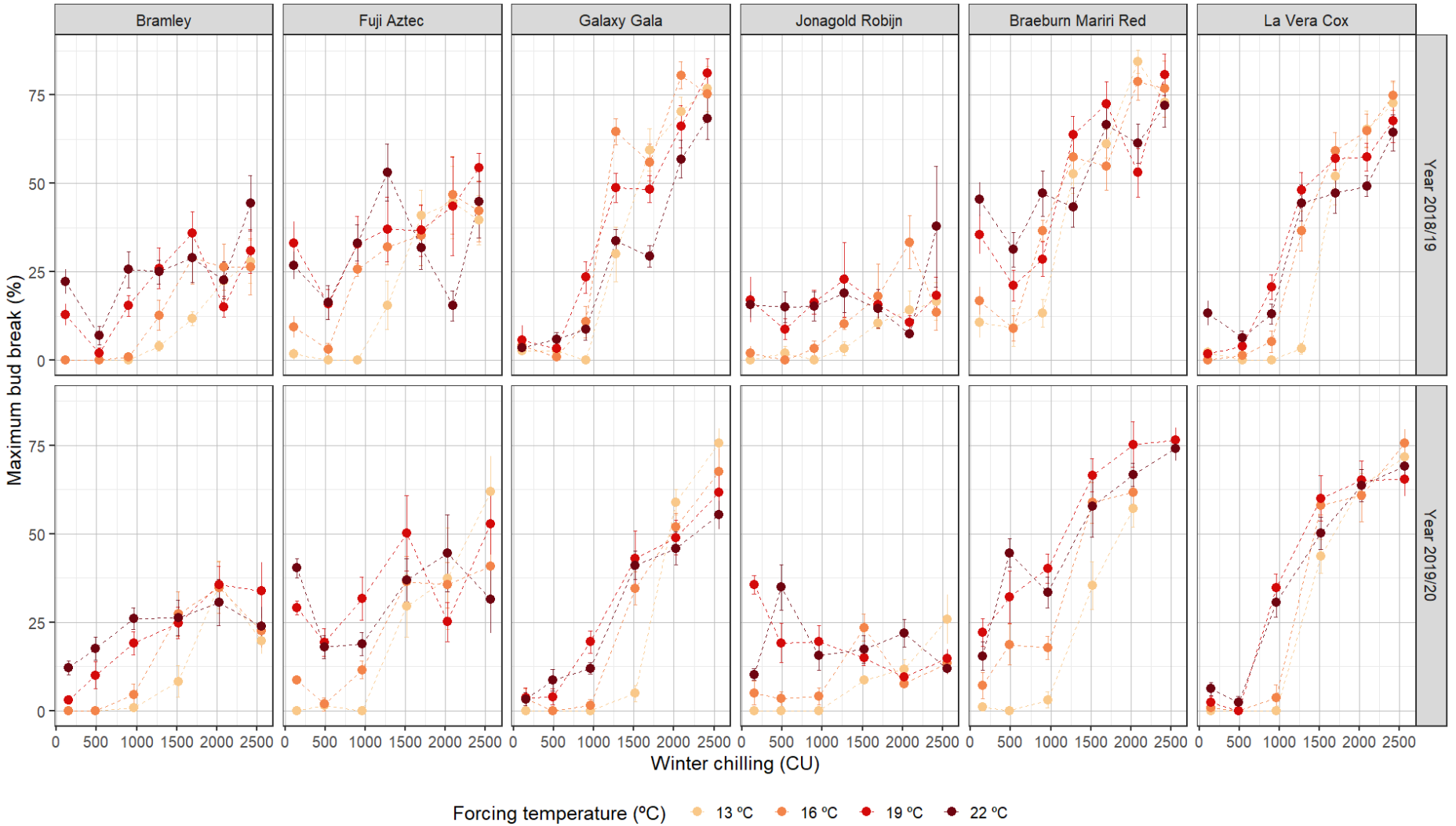


Figure 3.7 – Mean maximum percentage of bud break at the end of forcing ± SE. Results from one-year old shoots of "Bramley", "Fuji Aztec", "Galaxy Gala", "Jonagold Robjin", "Braeburn Mariri Red" and "La Vera Cox", after receiving different amounts of chilling in the field (CU) and being forced at different temperatures (13, 16, 19 and 22 °C) as indicated by different colour dots. Data presented from both years of study, 2018/2019 (top) and 2019/20 (bottom)).

Regarding the cider apple cultivars, time to first bud break in shoots of "Dabinett" and "Kingston Black" forced at 22 °C followed a similar pattern to that measured in dessert apple varieties; reaching a peak around 900 CU and then decreasing to a minimum after the last collection (Figure 3.8). But compared to dessert cultivars (Figure 3.6), time to bud break was shorter and less variable throughout the time of the study, ranging from 10 to less than 30 days in both varieties (Figure 3.8).

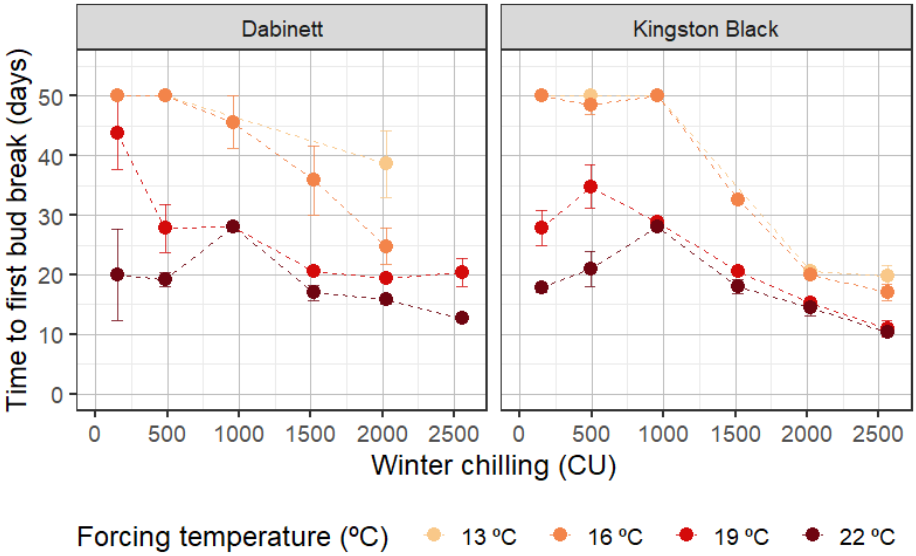


Figure 3.8 Mean days to first bud break ± SE, of one-year old shoots of "Dabinett" and "Kingston Black", collected in 2019/20 after receiving different amounts of chilling in the field (Winter chilling, CU) and being forced at different temperatures (13, 16, 19 and 22 °C) as indicated by different colour dots.

In "Dabinett", percentage of bud break remained very low (less than 20%) throughout the study and did not increase with longer accumulated chilling (Figure 3.9). "Kingston Black" followed a similar pattern to dessert cultivars (Figure 3.7), with percentage bud break increasing significantly after 900 CU, reaching a maximum of more than 75% after the last collection (Figure 3.9).

The effect of different forcing temperatures on bud break

Throughout dormancy, bud break occurred sooner in shoots forced at 22 °C compared to 13 °C, but differences between forcing temperatures varied amongst cultivars and these decreased as chilling accumulated (Figures 3.6 and 3.8). In all cultivars, differences in time of bud break between forcing temperatures were more pronounced during autumn and the beginning of winter (<1,000 CU) but were less evident in the last four collection dates (>1,000 CU) (Figure 3.6 and 3.8).

When depth of dormancy was greatest, in cultivars such as "Braeburn Mariri Red" or "Jonagold Robjin", buds opened 20 days sooner at 22 °C compared to 13 °C; whilst a difference of only 10 days was observed in "Galaxy Gala" or "La Vera Cox" (Figure 3.7). In dessert varieties, almost no differences were observed between forcing temperatures at the end of the dormancy period, with buds opening within 8 days at any forcing temperature (Figure 3.7). In contrast, in the cider cultivars, differences were still evident after more than 2,500 CU, when buds opened 10 days sooner at 22 °C compared to 13 °C (Figure 3.8).

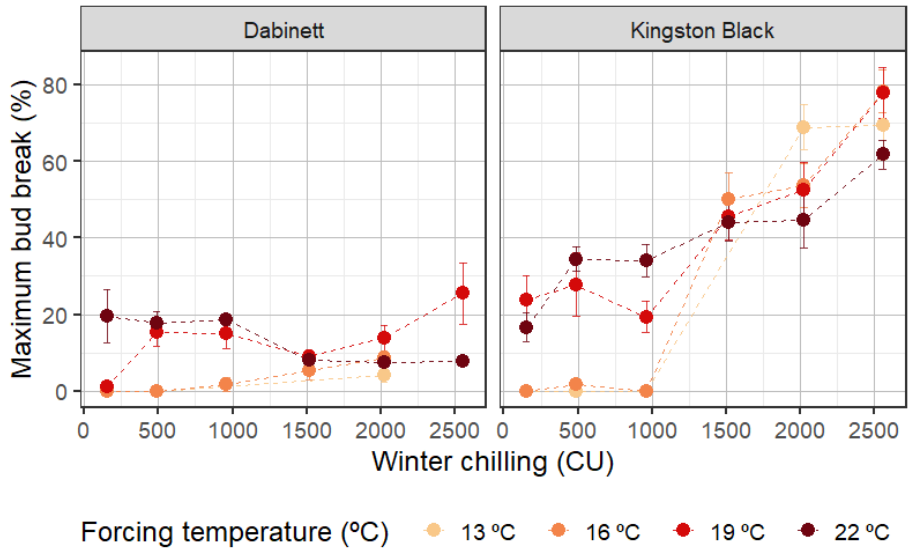


Figure 3.9 - Mean maximum percentage of bud break at the end of forcing ± SE of one-year old shoots of "Dabinett" and "Kingston Black", collected in 2019/20 after receiving different amounts of chilling in the field (Winter chilling, CU) and being forced at different temperatures (13, 16, 19 and 22 °C) as indicated by different colour dots.

For each cultivar, differences in percentage bud break between forcing temperatures did not follow a clear pattern (Figure 3.7 and 3.9). As dormancy progressed, the highest percentage of bud break was not always observed at the same forcing temperature. In all dessert varieties except "Braeburn Mariri Red", percentage of bud break at 13 °C was negligible until more than 900 CU had accumulated; and the same trend was observed at 16 °C in all varieties except "Fuji Aztec" and "Galaxy Gala" where 10-15% bud break was observed in the first collection (Figure 3.7). In "Dabinett", percentage of bud break after forcing at 13 and 16 °C was negligible throughout dormancy, whilst an increase after 900 CU was observed in "Kingston Black" (Figure 3.9).

At all forcing temperatures, "Bramley", "Dabinett" and "Jonagold Robijn" showed a low percentage of bud break whilst it was higher in "Braeburn Mariri Red", "Galaxy Gala" and "Kingston Black" (Figures 3.7 and 3.9).

Effect of chilling accumulation and warm temperature during ecodormancy

Changes in the break points of curves of time to bud break (Figures 3.6 and 3.8) were used as an indicator of endodormancy release and entry into ecodormancy (Lang et al., 1987) (see section *3.4 Discussion*). Cultivar-specific linear mixed models were developed with 2018/19 data to investigate the effect of forcing temperature, chilling accumulation, and their interaction on time to first bud break. Results are presented in Table 3.2 (dessert cultivars) and 3.3 (cider cultivars) and graphical representations for each dessert cultivars in Figure 3.10. Model results for "Braeburn Mariri Red" and "Galaxy Gala" were presented in Gonzalez-Noguer et al, (*in press*).

In all dessert apple cultivars, warmer forcing temperature and increased accumulation of chilling significantly reduced time to bud break (Table 3.2). A positive interaction between forcing

temperature and chilling accumulation was also significant, indicating that the effect of forcing temperature declined as more chilling accumulated, evidenced by the change in the slope of the green surfaces in Figure 3.10. In “Bramley”, “Fuji Aztec” and “Jonagold Robijn”, if all other variables remained constant, a 1 °C increase in forcing temperature could advance bud break by more than four days ($p < 0.001$), and by more than 5 days in “La Vera Cox” ($p < 0.001$). A more significant effect of chilling accumulation was observed in all cultivars, with an additional 100 CU advancing bud break by 5.4 and 5.1 days in “Bramley” and “Fuji Aztec” respectively, and almost 6 days in “Jonagold” and “La Vera Cox” (Table 3.2).

Table 3.2 – Results from Cultivar-specific mixed models on days to first bud break for dessert apple cultivars. Models include 3 variables: forcing temperature, Chill Units (CU) accumulated and the interaction between both. Results presented: variable estimates, standard error (SE), t-value and p-value indicating significance level of each variable. Analysis of Variance Table with Satterthwaite's method. Models trained with 2018/19 data from collections 4-7.

Cultivar	Variable	Estimate	SE	t-value	P-value
Bramley	Intercept	1.240e+02	1.171e+01	10.594	
	Forcing temperature	-4.386e+00	6.518e-01	-6.728	3.693e-10 ***
	CU accumulated	-5.429e-02	6.200e-03	-8.756	6.950e-15 ***
	Forcing temp * CU	1.801e-03	3.451e-04	5.217	6.167e-07 ***
Fuji Aztec	Intercept	1.18e+02	1.28e+01	9.193	
	Forcing temperature	-4.589e+00	7.073e-01	-6.489	3.599e-05 ***
	CU accumulated	-5.166e-02	6.963e-03	-7.42	3.147e-06 ***
	Forcing temp * CU	1.863e-03	3.818e-04	4.878	0.0003307 ***
Jonagold Robijn	Intercept	1.26e+02	2.00e+01	6.314	
	Forcing temperature	-4.678e+00	1.108e+00	-4.221	0.0009777 ***
	CU accumulated	-5.712e-02	1.074e-02	-5.319	8.972e-05 ***
	Forcing temp * CU	2.013e-03	5.935e-04	3.391	0.0042975 **
La Vera Cox	Intercept	1.32e+02	1.09e+01	12.075	
	Forcing temperature	-5.20e+00	6.14e-01	-8.465	2.092e-06 ***
	CU accumulated	-5.73e-02	5.70e-03	-10.045	3.435e-07 ***
	Forcing temp * CU	2.09e-03	3.20e-04	6.528	2.793e-05 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Regarding cider cultivars, forcing temperature and chilling also reduced time to bud break in “Kingston Black”, although the effect of these variables was lower compared to dessert apple cultivars (Table 3.3). A 1 °C increase in forcing temperature advanced bud break by almost two days ($p<0.01$) and 100 CU by approximately 1.5 days ($p<0.01$). The interaction between forcing temperature and chilling was not significant. In “Dabinett”, only forcing temperature influenced time to bud break ($p<0.001$), whilst chilling accumulation was not significant (Table 3.3).

Table 3.3 – Results from Cultivar-specific mixed models on days to first bud break for cider apple varieties. Initial models included 3 variables: forcing temperature, Chill Units (CU) accumulated and the interaction between both. Only significant variables were kept and are presented in the table. Results presented: variable estimates, standard error (SE), t-value and p-value indicating significance level of each variable. Analysis of Variance Table with Satterthwaite's method. Models trained with 2019/20 data from collections 3-6.

Cultivar	Variable	Estimate	SE	t-value	P-value
Dabinett	Intercept	6.29e+01	9.15e+00	6.877	
	Forcing temperature	-2.68e+00	4.51e-01	-5.942	0.0004719 ***
Kingston Black	Intercept	6.72e+01	9.37332	7.168	
	Forcing temperature	-1.69e+00	0.39549	-4.281	0.00147 **
	CU accumulated	-1.51e-02	0.00319	-4.722	0.04488 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

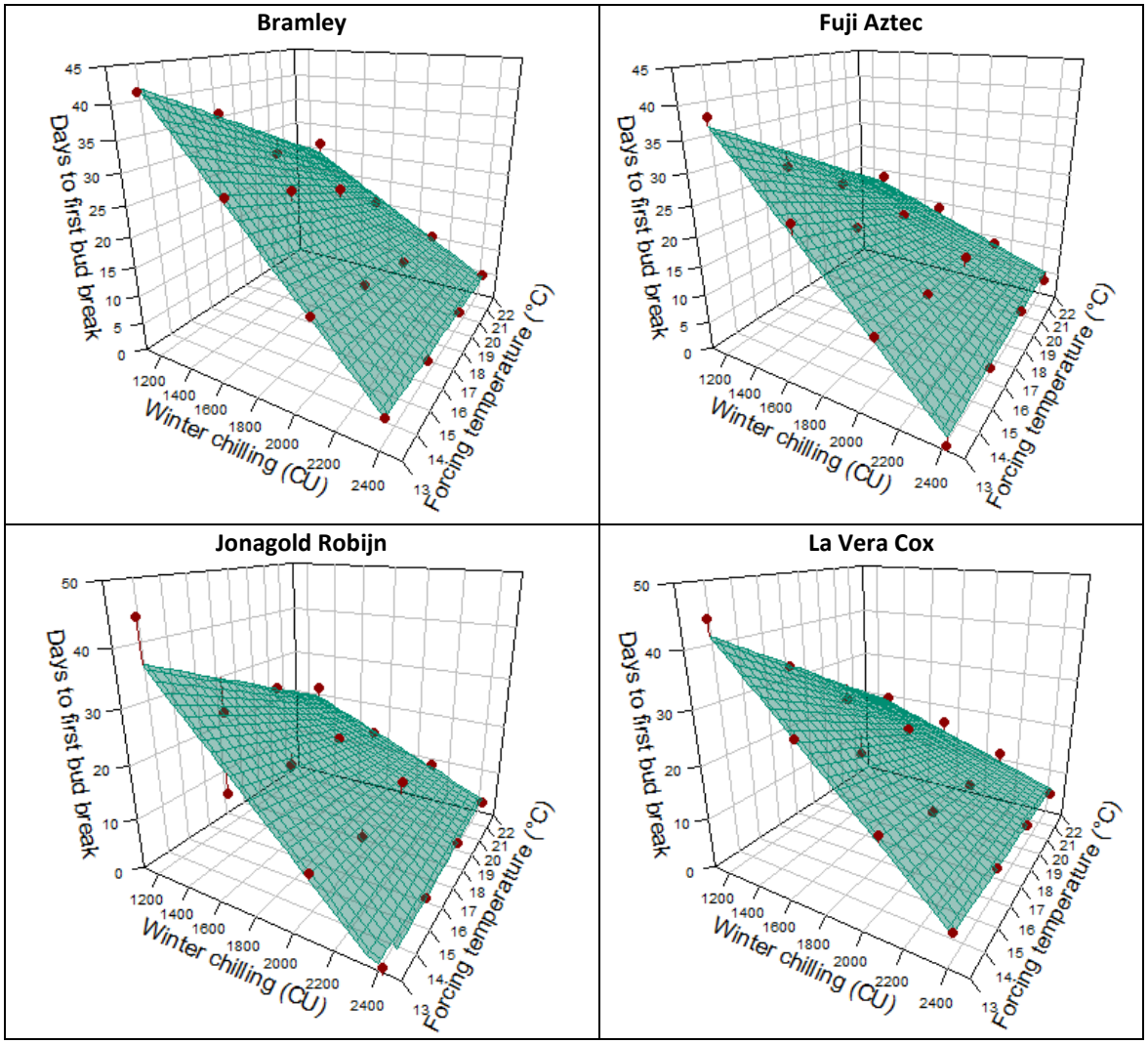


Figure 3.10 – Cultivar-specific ("Bramley", "Fuji Aztec", "Jonagold Robijn" and "La Vera Cox") 3D graphs representing the observed and modelled bud break response to winter chilling accumulated (calculated in Chill Units) and forcing temperatures during the ecodormancy period in 2018/19. (●): Observed values. Green surface: modelled values.

Testing the accuracy of the models

Models were tested on data from the following year (2019/20) to investigate how accurately time to first bud break could be predicted under the forcing temperatures and chilling accumulations studied. For all cultivars, time to bud break was almost always predicted sooner than observed in 2019/20, and predictions became more accurate with longer chilling and warmer forcing temperatures (Figure 3.11). In "Bramley", time to bud break was predicted with three days accuracy for all forcing temperatures after 2,400 CU. In "Fuji Aztec", significantly less accurate predictions were observed for 13 °C, ranging approximately from 6 to 13 days. In both "Jonagold Robjin" and "La Vera Cox", after 1,200 CU, predictions for all forcing temperatures remained within less than 6 days from the observed values (Figure 3.11). The "Kingston Black" model could not be tested as only data from one year was available for this cultivar. The "Dabinett" model was not used for further analyses due to poor model fit and the reduced percentage of bud break observed in this cultivar.

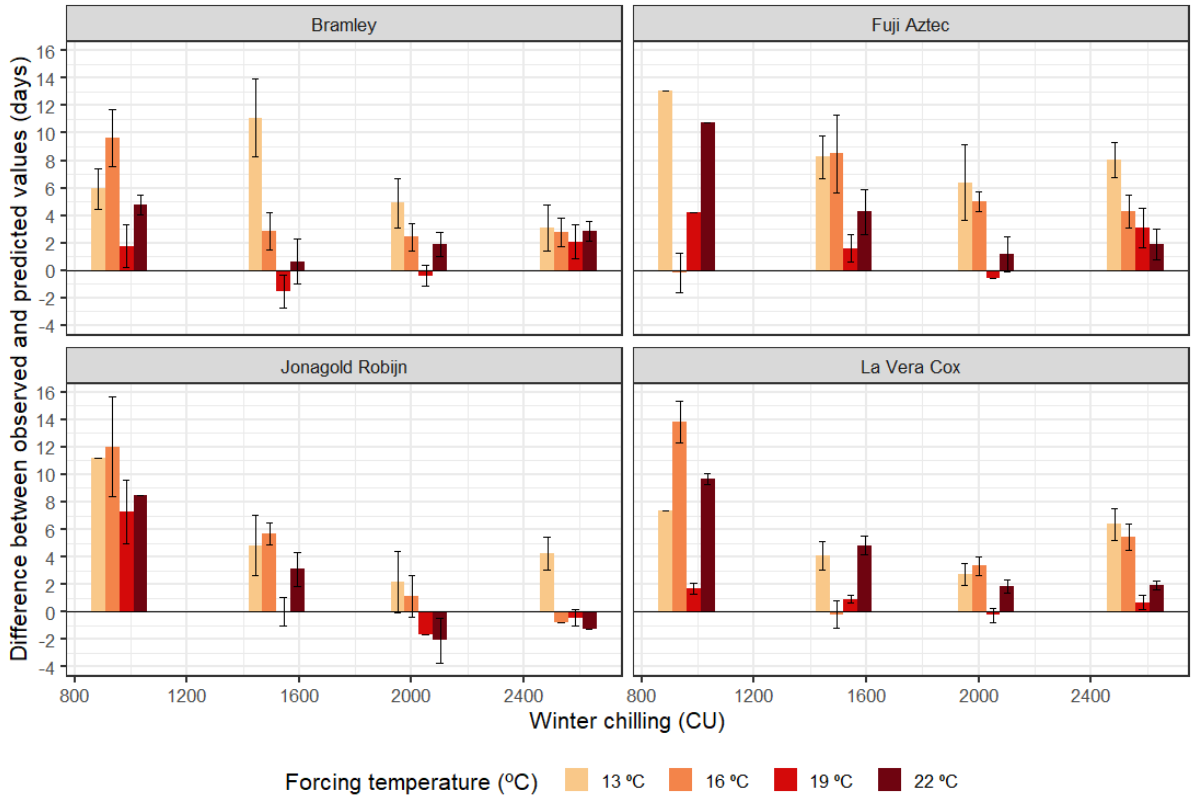


Figure 3.11 – Differences between observed and predicted days to first bud break ± SE of shoots that had accumulated different amounts of chilling in the field (Winter chilling, CU) and were forced at a range of forcing temperatures (13, 16, 18 and 22 °C). Each box represents an apple cultivar: "Bramley", "Fuji Aztec", "Jonagold Robijn" and "La Vera Cox". Models were calibrated with 2018/19 data and tested with 2019/20 data.

Model predictions

Cultivar-specific models were used to investigate the potential behaviour of these cultivars in the event of a heatwave (Figure 3.12). Time to first bud break was predicted for all varieties at four mean temperatures (14, 18, 22 and 26 °C) and for chilling accumulations between 1,500 and 2,250,

at increases of 50 CU. In all cultivars, differences between heatwave scenarios declined with longer chilling; with all scenarios inducing bud break within 12 days after 2,250 CU (Figure 3.12).

In “La Vera Cox” and “Kingston Black”, differences between heatwave scenarios were more apparent than in other cultivars, a heatwave of 26 °C could induce bud break in less than 10 days at the end of January (1,500 CU) whilst approximately 35 days at 14 °C would be needed. A smaller difference between the warmest and coldest heatwave scenarios was observed at the same chilling accumulation in “Jonagold Robijn” and “Fuji Aztec”, ranging from 10-29 days and 8-29 days respectively. The effect of 26 °C on time to first bud break remained almost constant in “Fuji Aztec”, “Jonagold Robijn”, “Kingston Black” and “La Vera Cox”; whilst in “Bramley” it significantly reduced time to bud break as more chilling accumulated. In “Bramley” and “Kingston Black”, differences between heatwave scenarios were still observed after 2,250 CU; when 14 °C could induce bud break after 12 and 21 days, respectively, but only 5 and 6 days would be required at 26 °C (Figure 3.12).

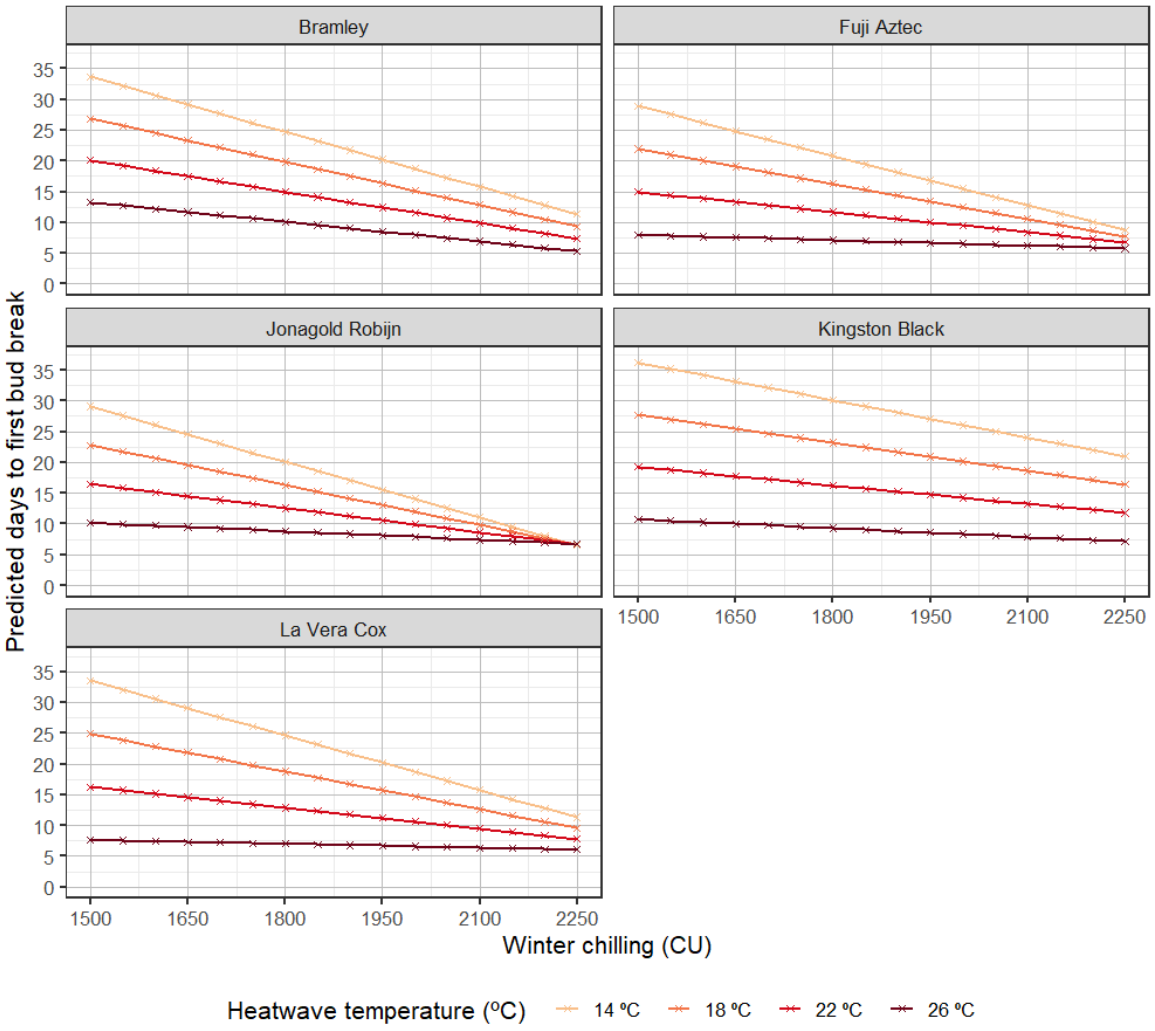


Figure 3.12 – Predicted number of days to first bud break of five apple cultivars (“Bramley”, “Fuji Aztec”, “Jonagold Robijn”, “Kingston Black” and “La Vera Cox”) after receiving different amounts of chilling (1500-2250 CU) and in the event of fours possible heatwaves at 14, 18, 22 or 26 °C.

3.4 Discussion

Assessing dormancy progression

To better understand dormancy progression in the cultivars under study, the effects on two parameters were quantified, time to first bud break and percentage of bud break after 50 days under forcing conditions at 22 °C. Changes in the timing of both parameters were assessed, instead of focusing on the time when a predefined bud break percentage threshold was met, as was done in several published studies to define chilling requirements (Cook and Jacobs, 2000; Ruiz et al., 2007; Parkes et al., 2020). Although the aim of this experiment was not to define cultivar-specific chilling requirements, by investigating the trend of time to first bud break and percentage of bud break, key phases in the dormancy cycle (Lang et al., 1987) were identified.

Most studies investigating the chilling requirements of fruit trees are based on experiments with excised shoots using forcing temperatures of 20-25 °C (Hauagge and Cummins, 1991c, 1991b; Cook and Jacobs, 2000; Guak and Neilsen, 2013; Anzanello et al., 2014), as this temperature is considered higher than the chilling accumulation temperature range in all existing models (Richardson et al., 1974; Fishman et al., 1987). The specific methodologies vary greatly between studies, including the age and size of the shoots, temperature and media used during forcing, and the frequency at which data on bud break was collected. These disparities make comparison between studies difficult (Dennis, 2003), but a common characteristic is that chilling requirements are assumed to be reached when an arbitrary percentage of bud break (from 20 to more than 50%) is achieved (Hauagge and Cummins, 1991b; Cook and Jacobs, 2000; Ruiz et al., 2007; Parkes et al., 2020).

In the present experiment, the percentage bud break in some cultivars was consistently low (Figure 3.7 and 3.9), even after trees had received more than 2,000 CU, which is sufficient to meet the chilling requirements of all known apple varieties (Hauagge and Cummins, 1991a). These results suggest that a threshold of a certain percentage of bud break after 10 days might not be a good indicator of dormancy break in some cultivars, and therefore other parameters should be considered. The low percentage of bud break observed could be a consequence of strong apical dominance, which can inhibit bud break in the remaining axillary buds of a shoot (Faust et al., 1995; Cook and Jacobs, 1999). To overcome low bud break percentage due to the effect of apical dominance, dormancy studies have used different approaches such as removing the apical bud (Naor et al., 2003; Anzanello et al., 2014) or positioning potted trees in a horizontal position (Naor et al., 2003). However, the wound effect caused by apical removal could also induce early bud break (Naor et al., 2003), thereby producing unreliable results. Positioning trees horizontally has raised the percentage of bud break in some studies (e.g. Naor et al., 2003), but creates an artificial environment by changing the natural direction of growth.

An inherent assumption when using the current parameter for establishing satisfaction of chill requirement (i.e. a specific percentage of bud break) (Hauagge and Cummins, 1991b) is that all buds within a shoot have the same probability to open. This assumption could be misleading not only because different cultivars possess different levels of apical dominance (Faust et al., 1995; Figure A1 - Appendix A), but also because it was observed during the present experiments that shoots tended to dry once the first buds within a shoot opened, even when high humidity conditions were maintained throughout the study. This could be a consequence of an increase in transpiration as buds opened, or also a side effect of adding bleach to the water. Future experiments could attempt to mimic the composition of apple xylem sap in the water to improve the system. Shoots gradually drying would have had a negative effect on the ability of the remaining buds to open, impacting the final percentage of open buds. The arguments presented above guided the choice of days to first bud break as a parameter for assessing dormancy intensity; and changes in the observed response curve have been used to distinguish dormancy phases (Lang et al., 1987).

Dormancy progression and dormancy depth of the studied cultivars

Time to first bud break at 22 °C followed a similar pattern in all cultivars studied, increasing at the beginning of autumn, indicating trees were entering dormancy, reaching a peak around 900 CU, and then decreasing until the last collection. Maximum percentage of bud break curves did not follow such a clear trend in some cultivars that showed very low percentage of bud break throughout the time of study, such as “Bramley”, “Dabinett” and “Jonagold Robjin”. This again suggests that, at least in some cultivars, percentage bud break is not a good indicator of dormancy break, possibly due to the experimental limitations highlighted above. However, even for cultivars with low percentage of bud break, the pattern of the time to bud break curves followed a similar trend to those published by Hauagge and Cummins, (1991d). They investigated dormancy depth of 90 genotypes of apple and related *Malus spp.*, dividing them into three groups according to the maximum number of days it took to reach 50% terminal bud break under forcing conditions at 22 °C. As days to first bud break was used in the present study instead of days to 50% bud break, the number of days was lower than those reported by Hauagge and Cummins, (1991d). However, based on the similarities in the dormancy progression curves (Figure 3.6, dark red dots), the criteria proposed by Hauagge and Cummins (1991d) were adapted to classify cultivars, such as: deep dormancy cultivars (time to first bud break is more than 40 days at its highest point), intermediate dormancy (20-40 days) and shallow dormancy (less than 20 days).

“Galaxy Gala” and “Braeburn Mariri Red” were classified as deep and shallow dormancy respectively (Gonzalez-Noguer et al., *in press)*. There is no scientifically robust information about the chill requirements of any of the cultivars studied, with the exception of “Galaxy Gala” (Parkes et al., 2020). Some information on closely related cultivars is presented below. The results presented here considering time to first bud break when shoots were forced at 22 °C indicate that “Jonagold”, “Fuji

Aztec", "Dabinett" and "Kingston Black" are intermediate dormancy cultivars, whilst "La Vera Cox" and "Bramley" followed a deep dormancy pattern.

Time to bud break in "Jonagold" remained below 30 days throughout these experiments. However, fruit quality and productivity in this cultivar were significantly reduced when grown in areas with less than 800 h below 7.2 °C (Hauagge and Cummins, 2000); and in a low-chill area of Zimbabwe, production required the application of dormancy-breaking agents (Jackson, 2003). Whilst the aim of these experiments was not to accurately define chilling requirements, the results showed that time to bud break in "Jonagold" began to decrease after 538 CU had accumulated, suggesting a lower chill requirement for this variety.

"Dabinett" and "Kingston Black" also showed medium dormancy depth, but little variability in time of bud break was observed throughout dormancy in these cultivars (Figure 3.8). Even after more than 2,500 CU, it took more than 10 days for the first buds to open. Whilst no formal information on CR has been found for these cultivars, field observations on time of bud break (Table B1 - Appendix B) show buds of both varieties opened later than any of the other cultivars studied, suggesting a higher chill requirement. In "Kingston Black", percentage bud break increased significantly after 900 CU, indicating CR could have been met. In "Dabinett" however, percentage of bud break was exceptionally low for shoots forced at all temperatures and throughout the whole experiment. As described in the *3.2.1 Data Analyses* section, for modelling purposes, shoots where no bud break was observed were given a value of 50 days. Results on time of bud break in this cultivar might have been skewed by this approach and therefore should be interpreted with caution. Reduced bud break in "Dabinett" suggests the need to use a different experimental approach to study dormancy in some varieties (see Chapter 5). "Dabinett" is a key cultivar for the UK cider industry and the National Association of Cider Makers (NACM) and several growers have highlighted concerns regarding uneven flowering in recent years (Loraine Boddington, NACM, pers. comm.).

The change in the dormancy progression curve observed in "Fuji Aztec" (Figure 3.6) is in agreement with previous studies which estimated the CR of "Fuji" to be around 1,077 CU (Hauagge and Cummins, 1991a). In both years of study, an intermediate dormancy depth was noted in this cultivar, as buds opened in less than 40 days, compared to more than 100 days (deep dormancy) observed in "Fuji" by Hauagge and Cummins, (1991a). Whilst this disparity could be due to differences in the experimental approach, it could also be a consequence of different genetics, even though they are highly related cultivars. For example, "Fuji Frey", a bud-mutation of Fuji, has been reported to require only 450 CU (Hauagge and Cummins, 2000).

"La Vera Cox", "Bramley" and "Galaxy Gala" showed deeper dormancy intensity throughout the time of study; at the deepest point, it took more than 40 days for the first buds to open (Figure 3.6). In "La Vera Cox", changes in time of bud break occurred after 538 CU, but a significant increase in maximum percentage of open buds was only observed after 900 CU. Previous studies with the

cultivar “Cox Orange Pippin” identified highly significant reductions in productivity and fruit quality parameters when grown in Brazil, in an area with less than 800 h below 7.2 °C (Hauagge and Cummins, 2000). No published studies appear to have examined dormancy in “Bramley”. This cultivar is usually harvested earlier than “Cox” in the UK (Jackson, 2003), but the present field data on time of bud break shows similar timing in 2019, although in 2018 “Bramley” reached 50% bud break 10 days after “La Vera Cox” (Table B1 – Appendix B). A significant reduction in “Bramley”’s time of bud break was observed after 538 CU (Figure 3.6), which could indicate a lower CR. Strong apical dominance has previously been identified in this cultivar (Pratt, 1988), which could explain the low percentage of bud break observed throughout the experimental period. An experiment carried out during the first year of this PhD, showed that apical buds of “Bramley” opened, on average, 10 days earlier than the rest of the buds (Figure A1 - Appendix A). Furthermore, field observations indicated that more than 20 days elapsed between the first apical buds opening and when > 50% bud break was observed (Table B1 – Appendix B).

Whilst most studies focus on determining the exact time point when CR is met (Parkes et al., 2020; Ruiz et al., 2007), the present results on time and percentage of bud break at 22 °C, indicate that growth resumption occurs gradually and therefore a precise measure of CR is unrealistic. Whilst, in most cultivars, time of bud break began to decrease after 538 CU, it wasn’t until the third collection (900.5 CU) that a reduction was evident. Although there is no accurate information on the chilling requirements of the cultivars studied here, the present results indicate that dormancy intensity might not necessarily be linked to chilling requirements, contrary to previous suggestions (Hauagge and Cummins, 1991d). For example, dormancy intensity was intermediate in “Jonagold” and “Fuji Aztec”, although previous studies suggested high chilling requirements in closely related cultivars (Hauagge and Cummins, 2000; Hauagge and Cummins, 1991c).

It is generally agreed that when chilling requirements are met, trees enter ecodormancy (Lang et al., 1987). At this stage, warmer temperatures are needed to induce bud break. Using the natural break point in the time to bud break and bud break percentage curves at 22 °C provided a better indicator of the transition from endodormancy to ecodormancy than attempting to estimate CR. These results showed that all varieties had entered ecodormancy after accumulation of 900 CU and this was used as a threshold for further analyses on the effect of warmer temperatures during this phase. In future work, more frequent collections between 700 and 1,200 CU could provide valuable data to accurately identify the break point when the slope of the curve changes and therefore the transition from one phase to the other.

Modelling dormancy progression dynamics

Whilst a range of chilling models exist (Weinberger, 1950; Richardson et al., 1974; Fishman et al., 1987), most studies use the Growing Degree Hours model for quantifying heat accumulation during ecodormancy (Anderson et al., 1986). The GDH model maintains the same parameters as heat

accumulates, assuming the thermal time relationship remains constant as buds develop (Anderson et al., 1986). However, the present cultivar-specific models showed a significant interaction between forcing temperature and chilling accumulation, with the effect of warmer temperatures declining as more chilling accumulated (Figure 3.10). This suggests that the thermal time relationship changes as ecodormancy progresses; indicating that a dynamic heat accumulation model where responses to temperature change as buds develop might better represent reality. During development of the GDH model, Anderson et al., (1986) identified the need to re-calibrate for each species; the differences observed here in the effect of forcing temperatures between cultivars also suggest that models should be calibrated at a cultivar level.

Whilst the GDH model assumes a baseline temperature of 4 °C (Anderson et al., 1986), other studies have suggested 10 °C (Gianfagna and Mehlenbacher, 1985) and 0 °C (Parker et al., 2011) as preferable baseline temperatures. It was observed that, in most cultivars, percentage of bud break in shoots forced at 13 °C was below 25% at the beginning of ecodormancy, but it increased to a similar percentage as in shoots forced at warmer temperatures as more chilling accumulated (Figure 3.7). Equal time to bud break was also observed between all forcing temperatures by the last collection point. The present results suggest that whilst 13, 16, 19 and 22 °C appear to contribute equally towards heat accumulation by the end of ecodormancy, this was not true after chill requirements were just satisfied. A possible explanation for these results is that the baseline temperature for heat accumulation decreases with ecodormancy progression, supporting the hypothesis of a changing thermal time relationship as buds develop.

Cultivar-specific models demonstrated that time of bud break during ecodormancy is determined by the forcing temperature treatments and chilling accumulated in the field (Table 3.2 and 3.3). It could be argued that the observed effect of chilling accumulation is instead a consequence of warmer temperatures occurring in the field between collection points four (4 January 2019) and seven (14 March 2020). Bud break models often calculate GDH after CR fulfilment (Cannell and Smith, 1983; Hänninen, 1990), although other studies assume simultaneous accumulation (Landsberg, 1974; Pope et al., 2014; Darbyshire et al., 2017). In 2019/20, GDH and CU accumulation in the field during ecodormancy showed a similar cumulative percentage increase between collections (Figure 3.5). Whilst heat accumulation is needed for bud break after chilling requirements are met, further chilling accumulation has also been shown to reduce time to bud break (Hauagge and Cummins, 1991d). It is not possible to determine with this experiment whether it is further chilling or heat accumulation in the field, after CR, that contribute towards inducing bud break. However, to establish a true forcing temperature effect in determining time of bud break, cultivar-specific models using GDH accumulated, instead of CU, were developed (Table B2 – Appendix B). These confirmed a significant effect of both forcing temperature treatments and the interaction with heat accumulated

in the field (Table AB – Appendix B). These models were validated with 2019/20 data (Figure A2 - Appendix A) and accuracy was very similar to that obtained using CU (Figure 3.11).

The impact of warmer temperatures during ecodormancy

The accuracy of the models presented here is similar to other studies that have predicted date of blooming or bud break (Chuine et al., 2016; Drepper et al., 2020). Here, models were used to predict time to first bud break in the event of four heatwave scenarios at 14, 18, 22 and 26 °C, occurring at different stages throughout dormancy (Figure 3.12). "Braeburn Mariri Red" appeared to be the most responsive cultivar to warmer temperatures (Gonzalez-Noguer et al., *in press*), but varieties such as "La Vera Cox" and "Kingston Black" were also highly responsive. However, in these latter two cultivars, differences between heatwave scenarios were larger, suggesting that only extremely high unseasonal temperature events would induce bud break. On the other hand, in "Fuji Aztec" and "Jonagold Robijn", smaller differences between heatwave scenarios were predicted, even at the start of ecodormancy. These findings suggest that although a longer warm period would be required for buds to open in these cultivars, relatively warm temperatures could be as efficient as hotter temperatures at inducing bud break.

Important limitations inherent in the experimental approach must be considered when interpreting the results presented here, such as the use of excised shoots and the unrealistic constant daily temperature in the imposed heatwave events. However, despite these caveats, the predictions show significantly different responses between cultivars to warm temperatures during ecodormancy. This provides key information to improve our understanding of how these varieties could respond to unseasonally warm temperatures and, to help to estimate the potential risk of frost damage.

Chapter 4

Chilling accumulation in two apple cultivars

4.1 Introduction

Fruit trees require cold temperatures during winter (accumulation of chilling) to exit endodormancy; the amount of chilling required is known as the Chilling Requirement (CR) and varies between species and cultivars (Hauagge and Cummins, 1991a). Chilling Requirement is a key parameter to consider during planting decisions as fruit production will be compromised if cultivars are grown in areas beyond their climatic requirements (Campoy et al., 2011a; Atkinson et al., 2013). Insufficient chilling can reduce or delay bud break, cause non-uniform flowering, and an overall reduction in fruit quality (Petri and Leite, 2004). With the predicted increase in temperatures due to climate change, both present and future region-specific climate should be considered.

Declines in winter chilling in the UK have been predicted for the next few decades, even under low emission scenarios (Atkinson et al., 2004; Luedeling and Brown, 2011). However, the magnitude of these predicted declines in winter chilling is highly dependent on the model used to quantify chilling accumulation (Luedeling et al., 2009c, 2009a). Several chilling models exist (summarised in section *2.4.2.1 Sequential models);* they all require hourly air temperature as a predictor variable but vary in the temperature range considered effective for chilling accumulation, as well as in the relative contribution to chilling accumulation assumed for each temperature. Models also differ in the experimental approaches followed and parameters considered to estimate the contribution of each temperature towards chilling accumulation. A lack of well-established protocols makes estimation of CR difficult (Dennis, 2003) and hinders comparison between different models; a consequence is that different CR are often reported in the literature for the same cultivar (Hauagge and Cummins, 1991a; Naor et al., 2003; Darbyshire et al., 2017).

Two of the most commonly used chilling models are the Utah model (Richardson et al., 1974) and the Dynamic model (Fishman et al., 1987); which quantify chilling in Chill Units (CU) and Chill Portions (CP), respectively. Both models were developed for peach varieties. The Utah model considers temperatures within the 0-15 °C range and assumes an optimum temperature of 6 °C (Richardson et al., 1974). The Dynamic model is based on the idea that chilling accumulation is a two-step process: chilling accumulation, promoted by low temperatures (0-13 °C), can be reversed by higher temperatures (16-24 °C) and requires moderate temperatures (13-15 °C) to be fixed (Erez and Couvillon, 1987; Fishman et al., 1987). Other models developed for apple include the North

Carolina model (Shaltout and Unrath, 1983) and the CUapple model (Guak and Neilsen, 2013) (see section *2.4.2.1 Sequential models*).

Although both chilling and heat accumulation are required for bud break, it remains unclear how these two temperature-driven processes are inter-linked. Most studies assume that they occur sequentially, with chilling accumulating until CR satisfaction, and heat accumulation starting afterwards (Richardson et al., 1974). But in some reports, bud break predictions have been improved by considering a partial overlap between chilling and heat accumulation (Pope et al., 2014; Darbyshire et al., 2017), suggesting that the sequential approach may be an oversimplification of the chilling and heat accumulation process (see section *2.4.2.3. Overlap models*).

CR and the relationship between temperature and bud break differs between fruit tree species and cultivars (Hauagge and Cummins, 1991a). Although the need for model calibration at the cultivar level was highlighted during model development (Anderson et al., 1986), existing chilling models are used in combination with heat accumulation models to predict time of bud break in varieties and species different than those used to develop them (Ruiz et al., 2007; Campoy et al., 2012; Luedeling et al., 2013; Guo et al., 2014; Darbyshire et al., 2016a; Parkes et al., 2020). Predictions of time of bud break with existing models are often inaccurate, partly due to this lack of calibration but also because models were developed without considering climate change scenarios such as warmer winters and heatwaves (Legave et al., 2008; Luedeling and Brown, 2011).

Research reported in this Chapter investigated chilling accumulation in two apple cultivars: “Braeburn Lochbuie” and “Discovery”. “Braeburn Lochbuie” was selected as it is a sport of “Braeburn”, one of the most important varieties grown in the UK (DEFRA, 2021) and an early flowering cultivar (Jackson, 2003). The choice of “Discovery” was imposed by the limited availability of other cultivars. Whilst “Discovery” was an important cultivar for the UK market until 2016 (DEFRA, 2021), a very short shelf-life has reduced its production in favour of other varieties (Jackson, 2003). Nevertheless, phenology records on time of bud break from NIAB EMR indicate later bud break in “Discovery” than “Braeburn” in south-east UK.

The work reported here aimed to establish the temperature range and optimum temperature for chilling accumulation in the two studied cultivars, and to improve our overall understanding of the relationship between temperature and bud break in these varieties. This information is key to develop more accurate chilling accumulation models that can inform commercial planting decisions over the next few decades.

4.2 Materials and methods

4.2.1 Plant material

One-year-old bare-rooted trees of “Braeburn Lochbuie” or “Discovery” scions grafted on to M9 rootstocks were purchased from Lodder-Unterlagen GmbH nursery (Dülmen, Germany) (Figure 4.1). Trees were grafted in winter 2018 and grown on near Freiburg im Breisgau (South-West Germany 47.9938, 7.8273) for approximately a year (Figure 4.2), and were transported for 10 days in a non-refrigerated lorry to the University of Reading (UK). All trees measured between 95 and 110 cm height on arrival. They were potted into 3 L pots containing a peat-based compost (Sinclair pro compost mix consisting of bark (10%), lime (1.5kg in 900 L), coarse and medium peat (90%), slow-release fertiliser (8-9 M (months longevity), 1 kg per 900 L, N12-P14-K24) and wetter) with added slow release fertiliser (Osmocote Extact Standard 8-9 M , N15-P9-K11+2MgO+TE, 4 kg per 900 L) and a granular biological insecticide (Met 52, 1 kg per 900 L).

Figure 4.1 - Bare root trees on arrival to the University of Reading. Photo taken on 04/11/2019

Figure 4.2 - Trees growing in the field before being lifted. Photo taken on 09/12/2019

4.2.2 Treatments

Before imposing treatments, trees were acclimated in a glasshouse compartment at 15 ± 3 °C under ambient light and photoperiod for 16 days. Each tree was labelled with a unique identifier number. After acclimation, when most trees were moved into different chilling treatments, four replicates of each variety (Control group) were left in the glasshouse, where the temperature was increased to 18 ± 4 °C. These trees did not receive any chilling and were used to assess the initial depth of dormancy of the imported trees.

Chilling treatments

Chilling treatments were imposed using controlled environment growth cabinets (Fitotron 3 Weiss Technik UK Ltd., UK) at the Crops and Environment Laboratory, University of Reading (Figure 4.3). Seven different constant temperature treatments were used: -4, -2.5, -1, 1.75, 4.5, 7.25, 10 °C and a day/night temperature of 7/2 °C (12/12h). Deviations from the desired temperature occurred at different scales in each treatment; and some technical problems with the growth cabinets resulted in an increase in temperature in some treatments:

- -4 ± 1.5 °C. The cabinet was stopped for a day (10 - 15 °C) for a defrost cycle after approximately 45 days of treatment. After two months, temperature increased 4 °C on two non-consecutive days.
- -2.5 ± 1.5 °C
- -1 ± 1.5 °C
- 1.75 ± 1 °C. On three non-consecutive days, temperature increased 4 °C.
- 4.5 ± 1 °C. On three consecutive days, temperature increased 6 °C.
- 7.25 ± 0.5 °C
- 10 ± 0.5 °C
- 7/2 ± 1 °C

Figure 4.3 - Start of chilling treatment at -2.5 °C. Photo taken on 21/11/2019

All treatments received a 12/12h photoperiod using 4ft warm-white T5 fluorescent tubes (TL5 39W Master, Philips, UK) providing a radiation intensity of approximately 100 µmol/m^2s at 60 cm height, measured with a PAR quantum sensor (Skye Instruments Ltd., UK). Humidity was not controlled during chilling due to the limited capability of the growth cabinets at low temperatures. Trees were randomly distributed inside the chambers and soil moisture content was monitored every 2 weeks using a WET Sensor with an HH2 Moisture Meter (Delta-T Devices, UK) to ensure that soil water availability was uniform across all treatments. Plants were hand watered with tap water when Volume Water Content (VMC) was below 0.350m^3/m^3. In all growth cabinets, temperature and humidity were recorded hourly with dataloggers (Tinytag Plus 2 TGP-4500, Gemini Data Loggers, UK) to provide an independent record of environmental conditions within each growth cabinet.

Four trees from each variety and temperature were moved from the growth cabinet into a glasshouse compartment to force bud break at three different times, to investigate the effects of different durations of chilling:

- 1,080 hours of chilling (Removal 1)
- 1,776 hours of chilling (Removal 2)
- 2,424 hours of chilling (Removal 3)

Forcing treatment

All trees were forced in a glasshouse compartment (the same as containing the control group of plants) at the University of Reading (Figure 4.4) at a temperature of 18 ± 4 °C. Artificial lighting of approximately 100 µmol/m^2s (SON-T HPS lamps, 400W, Philips, UK) was provided between 06:00-

08:30 and 16:00–18:30 to extend the natural photoperiod from November 2019 to March 2020. Trees were randomly positioned inside the glasshouse compartment.

Temperature and humidity were recorded hourly with a Decagon VP-4 temperature and humidity sensor at canopy height, connected to a Decagon Em50G remote datalogger (both from Meter Group, Inc. USA (previously called Decagon Devices Ltd). Trees were drip irrigated with tap water for 3 minutes twice a day, using emitters (Netafim, USA) with a flow rate of 2 L/h.

4.2.3 Data collection

To determine the effect of different temperature treatments on the dormancy status of the trees, data were collected on tree growth and bud break. For each tree, the total number of terminal and axillary buds were counted at the beginning of the chilling treatment. After two months in the glasshouse, new growth (length of growth from terminal buds, measured from the base of the bud to the tip of the furthest leaf) and homogeneity of bud break were assessed. New growth was averaged per terminal bud to account for the different number of terminal buds in each tree. Homogeneity was measured on a scale from 1 to 5, with five representing equal bud break in all parts of the canopy (Figure 4.5).

Time to bud break was assessed twice a week in all trees, open buds (buds at green tip stage 3 of development, as defined by Chapman and Catlin (1976), Figure 3.3) were recorded.

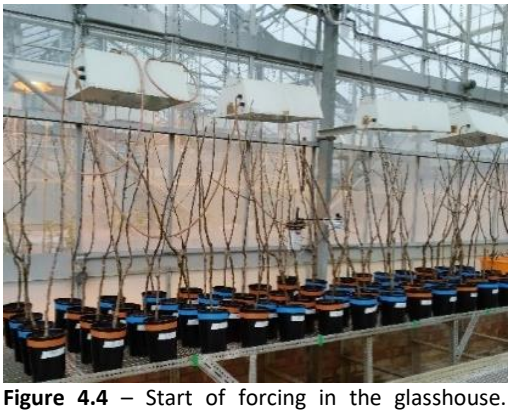


Figure 4.4 – Start of forcing in the glasshouse. Photo taken on 07/01/2020

Figure 4.5 - Assessment of bud break homogeneity. Lowest level (1) on the left and highest homogeneity (5) on the right. Photo taken on 13/03/2020

4.2.4 Data analyses

The overall effect of chilling temperature and chilling duration (in hours) on bud break was investigated by examining (1) the rate of progress to bud break ($days^{-1}$) and (2) the maximum percentage of bud break achieved.

Cultivar-specific linear models were created in R (R Core Team, 2021); initial models included three fixed effects: chilling temperature, chilling duration, and their interaction. The total number of buds per tree was incorporated in the model to account for the initial natural variability between trees. Manual backwards-stepwise model refinement based on p-values was used to delete non-significant terms (Thomas et al., 2017). A significance level of 0.05 has been used.

Rate of progress to bud break was used to identify the optimum temperature at each duration of chilling, defined here as the temperature at which the maximum rate of progress to bud break occurred. To find the optimum temperature at each chilling duration, chilling temperatures were converted to *Effective chilling temperature* (temp) (Figure 4.6) so that if:

- Chilling temp = Optimum temp, then *Effective temp* = 0 °C
- Chilling temp < Optimum temp, then, *Effective temp* = (Optimum temp – Chilling temp)
- Chilling temp > Optimum temp, then, *Effective temp* = - (Optimum temp – Chilling temp)

By converting real chilling temperatures to *Effective temperatures*, the optimum temperature was fixed at 0 in the x-axis, and a linear regression was then fitted between *Effective temperature* and the observed rate of progress to bud break (Figure 4.6). For each cultivar and duration of chilling, the optimum temperature was found with the least-square approximation method (Heumann et al., 2016), by testing a range of optimum temperatures at intervals of 0.1 °C (Table B3 – Appendix B).

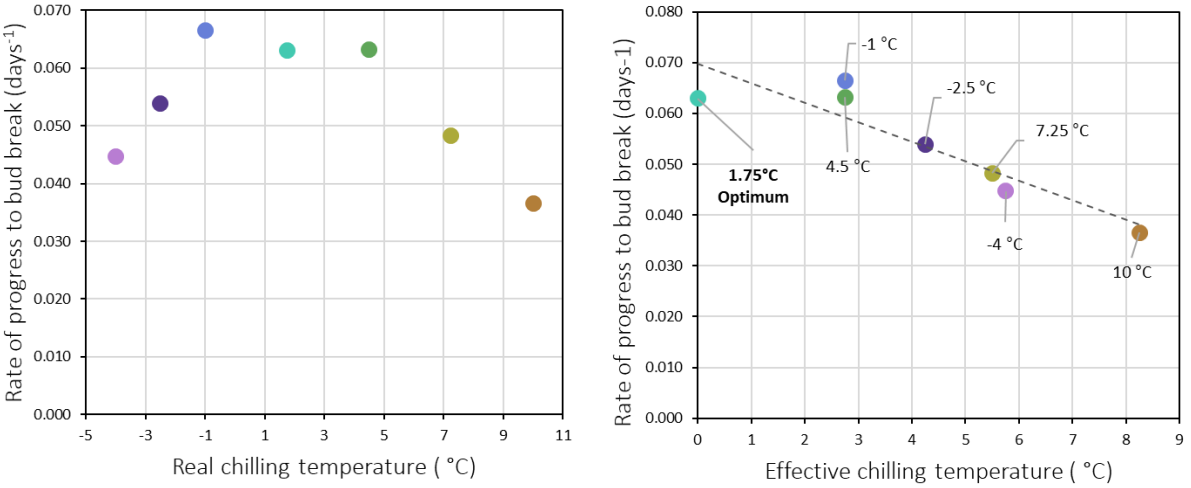


Figure 4.6 – Graphical representation of the methodology followed to find the optimum temperature. Left: rate of progress to bud break at each chilling temperature. Right: chilling temperatures converted to *Effective chilling temperatures* assuming an optimum temperature of 1.75 °C. A regression line was fitted. The process was repeated considering different optimum temperatures until the lowest R^2 value was found (Table B3 – Appendix).

At each duration of chilling, a one-way ANOVA followed by a post-hoc Dunnett test (Dunnett, 1955) was used to compare rate of progress to bud break between the control group (trees that did not receive any chilling) and trees chilled at different temperatures. And a Mann-Whitney test (Thomas et al., 2017) was used to explore differences in rate of progress to bud break at each chilling duration between trees chilled at 4.5 and the 7/2 °C treatment.

4.3 Results

Effect of chilling duration and temperature on time and rate of progress to bud break

Differences in the number of days to bud break were observed between cultivars and overall, buds of "Braeburn Lochbuie" trees opened more quickly than those of "Discovery" at most chilling temperatures and durations (Figure 4.7). For "Discovery", buds from trees chilled at -2.5 °C opened sooner throughout the experiment, taking less than 20 days to burst at any chilling duration. On the other hand, trees chilled at 10 °C took the longest time, from more than 60 days after 1,080h of chilling to approximately 30 days after 2,424 h of chilling (Figure 4.7). A less consistent pattern was observed with "Braeburn Lochbuie", but buds of trees chilled at 1.75 °C opened quicker than other chilling temperatures after all chilling durations (Figure 4.7).

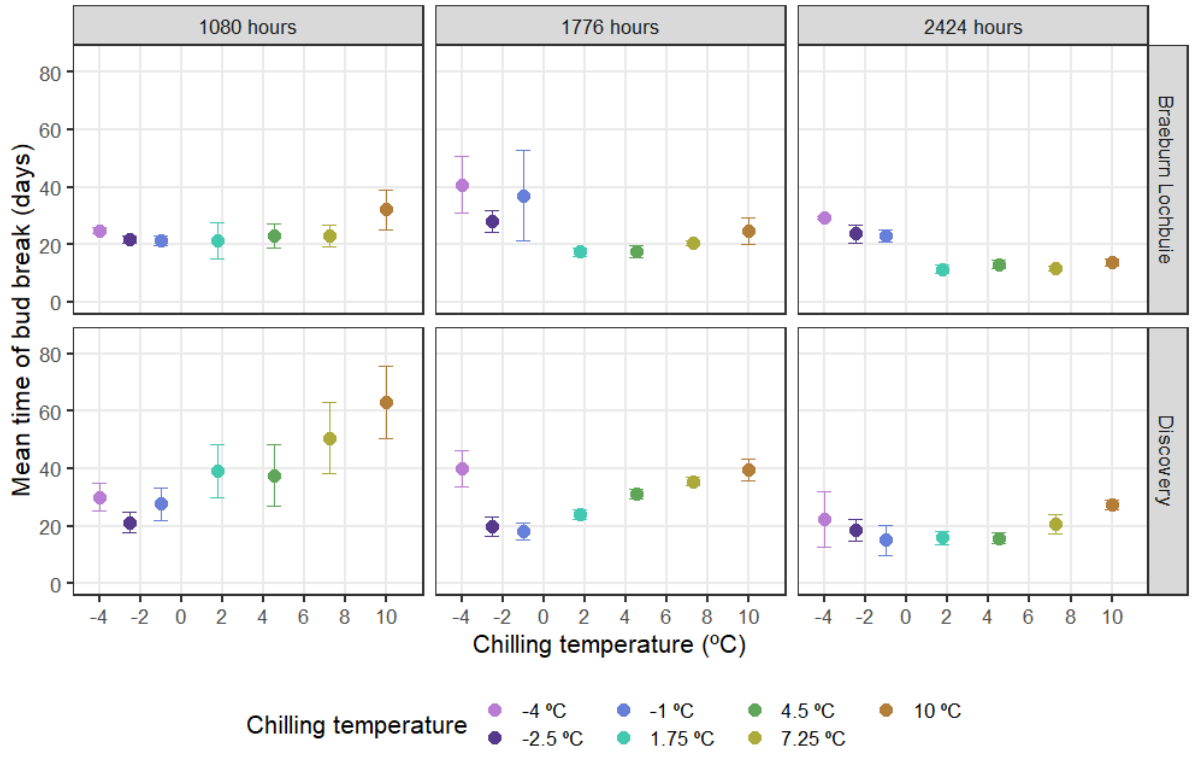


Figure 4.7 – Mean number of days to bud break ± SE, of "Braeburn Lochbuie" (top) and "Discovery" (bottom) trees chilled at different temperatures and for three durations (1,080; 1,776 and 2,424 h). Results are the average of 3-5 replicates.

Cultivar-specific linear models were developed to quantify the effect of chilling temperature and chilling duration on rate of progress to bud break (Table 4.1). For both cultivars, warmer chilling temperatures reduced the rate of progress to bud break whilst the opposite effect was observed with increased chilling duration, a longer chilling period increased rate of progress to bud break ($p<0.001$) (Table 4.1, Figure 4.7). The interaction between chilling temperature and chilling duration was not significant and therefore it was removed from all models. In "Discovery", chilling temperature and chilling duration explained almost 80% of the variability in rate of progress to bud

break; and both variables were equally significant (p<0.001). In "Braeburn Lochbuie", both variables accounted for almost 60% of the variability but chilling duration appeared more significant than chilling temperature (Table 4.1).

Table 4.1 – Cultivar-specific linear models on rate of progress to bud break, including chilling temperature and chilling duration (hours). Results presented: variable estimates, standard error, t-value, p-value indicating significance level of each variable and overall model parameters including the adjusted R^2, overall model significance, F-value and degrees of freedom of numerator, denominator in brackets.

Cultivar	Variable	Estimate	Std. Error	t value	P-value	Model parameters
Discovery	Intercept	1.67e-03	2.58e-03	0.647	0.52	Adj. R^2: 0.799 p-value: < 2.2e-16 F (2, 77) = 158
	Chilling temp	-1.69e-03	2.64e-04	-6.413	6.354e-05 ***	
	Chilling duration	2.47e-05	1.43e-06	17.267	< 2.2e-16 ***	
Braeburn Lochbuie	Intercept	2.08e-02	4.51e-03	4.602	1.99e-05 ***	Adj. R^2: 0.5908 p-value: 9.141e-14 F (2, 65) = 49.36
	Chilling temp	-6.24e-04	4.11e-04	-1.519	0.03847 *	
	Chilling duration	2.35e-05	2.42e-06	9.709	2.848e-14 ***	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

In "Discovery", rate of progress to bud break was higher in the -2.5 and -1 °C temperature treatments for all durations of chilling indicating an optimum temperature between these temperatures (Figure 4.8). An optimum temperature was less evident in "Braeburn Lochbuie", and in this cultivar, rate of progress to bud break in freezing treatments was significantly lower than in trees chilled at temperatures above zero, particularly after 2,424 h of chilling (Figure 4.8).

Comparison with control trees (no chilling)

To identify chilling accumulation temperature thresholds, and to assess the dormancy status of the trees at the beginning of the experiment, rate of progress to bud break in trees that did not receive any chilling (control) was compared with that of trees chilled for 1,080, 1,776 and 2,424 h at each different chilling temperature (Figure 4.8).

Overall, chilling temperature significantly affected rate of progress to bud break at all chilling durations. Mean rate of progress to bud break in control trees was significantly lower (p<0.001) than in trees chilled for 1,080 h at any temperature treatment in "Braeburn Lochbuie", although it was just slightly lower than that of trees chilled at 10 °C (p = 0.021). In "Discovery" trees chilled for 1,080 h, no differences were recorded between the control and the 7.25 and 10 °C temperature treatments (Figure 4.8).

In "Braeburn Lochbuie", no differences between control trees and those chilled at -4 and -1 °C were observed after 1,776 h; and between the control and the -4 °C treatment after 2,424 h. In Discovery, rate of progress to bud break was significantly faster in all chilling treatments compared to the control after 1,776 and 2,424 h of chilling (p<0.01) (Figure 4.8).

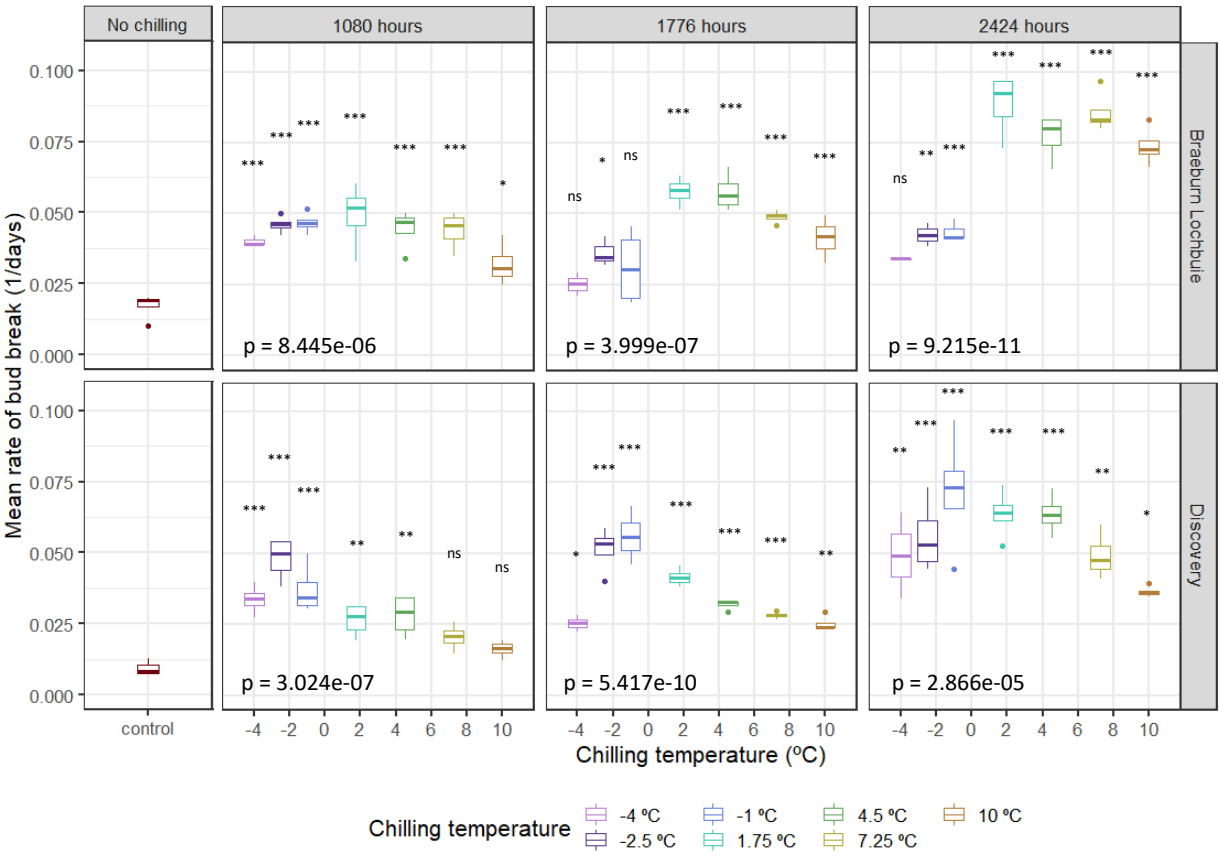


Figure 4.8 – Mean rate of progress to bud break (days^{-1}) of "Braeburn Lochbuie" (top) and "Discovery" (bottom) trees chilled at different temperatures and for three chilling durations. Control indicates trees that did not receive any chilling. A one-way ANOVA followed by a post-hoc Dunnett test was done for each duration and cultivar. Overall test significance is indicated with a p-value in each box. '*' above boxplots indicate significant difference between the "Control" and each temperature treatment. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 'ns' non-significant differences. Boxplots show median, interquartile range, whiskers from maximum to minimum values and outliers are represented as single dots.

Optimum chilling temperature

The optimum chilling temperature (temperature with the highest rate of progress to bud break) at each duration of chilling was estimated (Figure 4.9) following the methodology described in *Section 4.2.4. Data analyses*. Results of multiple linear regressions for 0.1 °C above and below the optimum are given in Table B3 (Appendix B).

Overall, the optimum chilling temperature was higher in 'Braeburn Lochbuie' compared to 'Discovery' (Figure 4.9). In both cultivars, the optimum chilling temperature increased with longer chilling, from 0.8 to 6.1 °C in "Braeburn Lochbuie", and from -2.5 to 1.4 °C in "Discovery". In 'Discovery', optimum chilling temperature remained below zero until more than 1,776 h of chilling had accumulated (Figure 4.9).

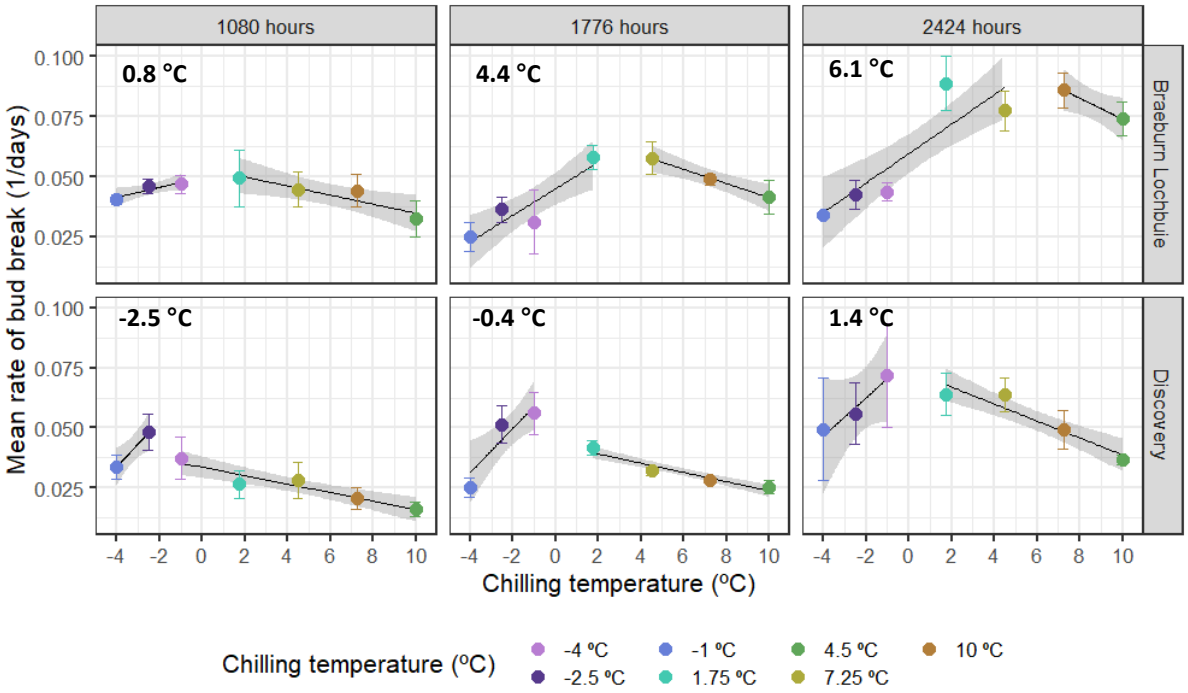


Figure 4.9 – Piecewise linear regressions between Chilling temperature and Mean rate of progress to bud break (days^{-1}), for "Braeburn Lochbuie" (top) and "Discovery" (bottom) at each chilling duration (1,080; 1,776 and 2,424 h). Error lines indicate ± 1 SE. Optimum chilling temperature for each chilling duration and cultivar is indicated inside each box. Optimum chilling temperatures were calculated as described in *section 4.2.4. Data analyses* and results from the linear regressions are shown in Table B3 (Appendix B).

Comparing 4.5 °C chilling temperature with day/night (12/12h) 7/2 °C treatment

The rate of progress to bud break at the day/night (12/12h) alternating 7/2 °C chilling treatment was compared with the constant 4.5 °C temperature at each duration of chilling. Statistical analyses indicated no significant differences occurred between treatments at any of the chilling durations studied (Figure 4.10).

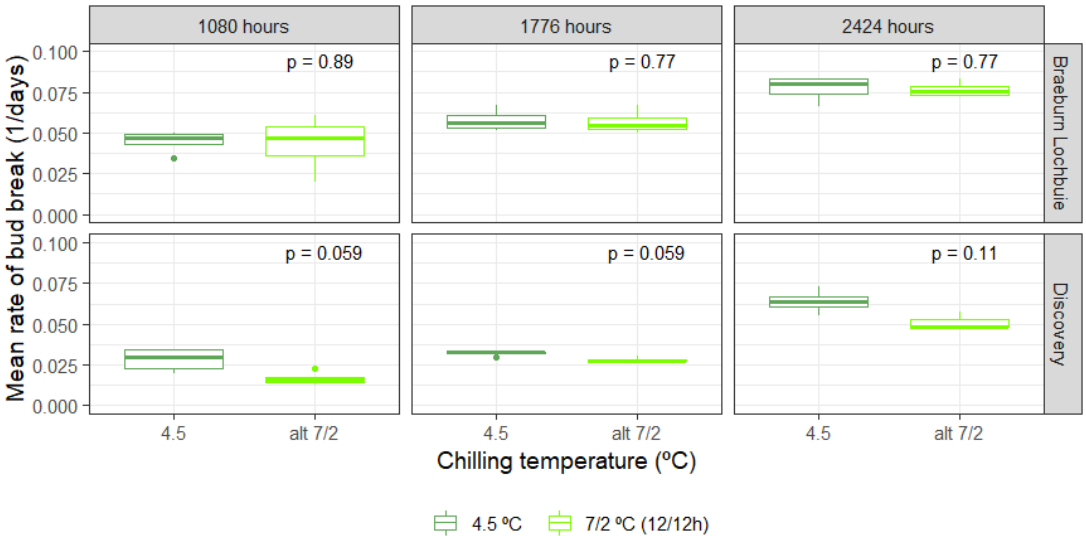


Figure 4.10 - Mean rate of progress to bud break (days^{-1}) of "Braeburn Lochbuie" (top) and "Discovery" (bottom) trees chilled at 4.5 °C and 7/2 °C for three chilling durations. A Mann-Whitney test was performed for each cultivar and chilling duration, p-value indicated in the top-right corner of each box. Boxplots show median, interquartile range, whiskers from maximum to minimum values and outliers are represented as single dots.

Effect of chilling duration and temperature on percentage of bud break

Maximum percentage of bud break increased with longer periods of chilling, but more variability was observed in percentage data (Figure 4.11), compared to rate of progress to bud break (Figure 4.8). Overall, the effect of temperature was small at all chilling durations and for both cultivars except in "Braeburn Lochbuie" after 2,424 h, where large differences were observed particularly between below and above zero temperatures (Figure 4.11). In "Discovery", percentage of bud break was low (<20 %) after 1,080 h of chilling; and a similar pattern was observed in "Braeburn Lochbuie", except in freezing temperature treatments, where it exceeded 50% (Figure 4.11).

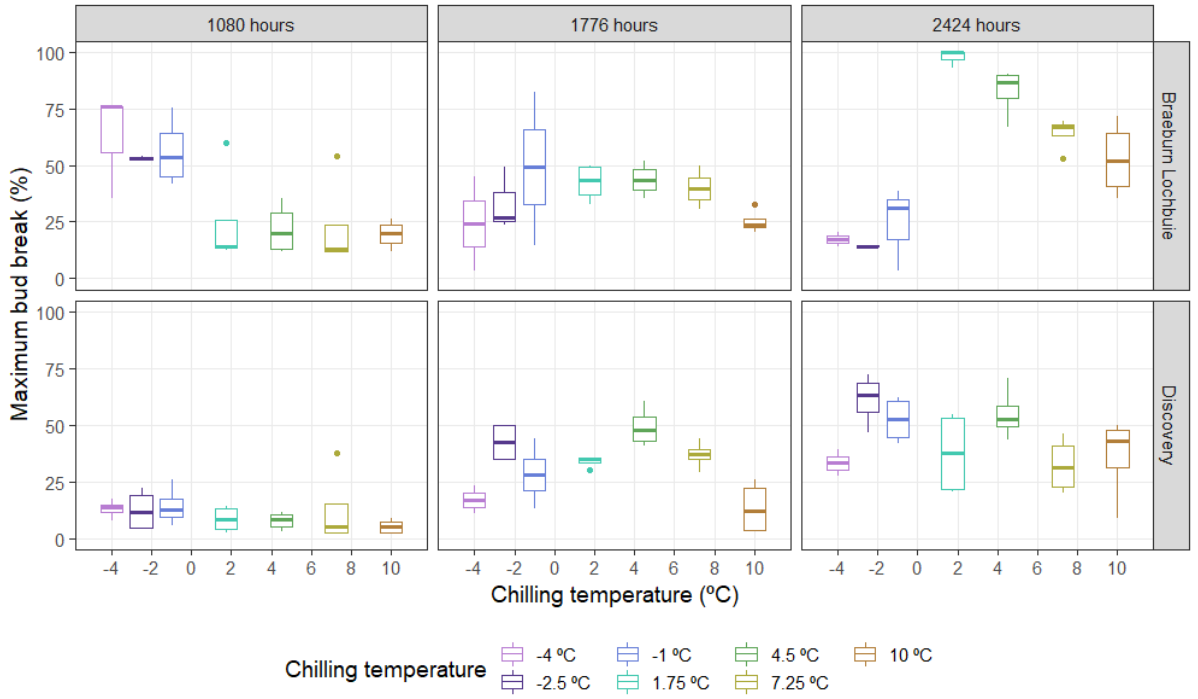


Figure 4.11 – Maximum percentage of bud break (%) of "Braeburn Lochbuie" (top) and "Discovery" (bottom) trees chilled at different temperatures and for three durations (1,080; 1,776 and 2,424 h). Boxplots show median, interquartile range, whiskers expand from maximum to minimum values and outliers are represented as single dots.

Cultivar-specific linear models indicated that in "Discovery", chilling temperature did not appear to have a significant effect on the percentage of bud break, but chilling duration alone explained 58% of the variability observed ($p<0.001$), and percentage of bud break increased with a longer chilling period ($p<0.001$) (Table 4.2). In "Braeburn Lochbuie", lower temperatures increased the percentage of bud break whilst a longer duration of chilling had the opposite effect ($p<0.001$) (Table 4.2).

Table 4.2 - Cultivar-specific linear models on maximum percentage of bud break, determined after 60 days under forcing conditions. Results presented: variable estimates, standard error (Std. Error), t-value, p-value indicating significance level of each variable and overall model parameters including the adjusted R^2, overall model significance, F-value and degrees of freedom of numerator, denominator in brackets.

Cultivar	Variable	Estimate	Std. Error	t value	p-value	Model parameters
Discovery	Intercept	-15.2936	4.4117	-3.467	0.00086 ***	Adj. R^2: 0.5839 p-value: < 2.2e-16 F (1, 78) = 111.8
	Chilling duration	0.02565	2.e-03	10.576	< 2e-16 ***	
Braeburn Lochbuie	Intercept	-6.5239	6.1025	-1.069	2.89E-01	Adj. R^2: 0.6708 p-value: 2.36e-16 F (2, 63) = 67.22
	Chilling temp	0.03958	0.003561	11.115	< 2e-16***	
	Chilling duration	-3.2336	0.5987	-5.401	1.07e-06***	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

New growth and homogeneity of bud break

Homogeneity of bud break and new growth were also compared between chilling temperatures at each duration of chilling (Figures 4.12 and 4.13); but some experimental issues significantly affected these parameters which did not accurately represent tree growth (see section *4.4 Discussion*).

In "Discovery", homogeneity of bud break increased with a longer duration of chilling at all temperatures. After 1,080 h, trees chilled at temperatures above 1.75 °C had less even bud break compared to trees chilled at freezing temperatures. After 2,424 h, most trees had a homogeneity score higher than 3, except temperatures 7.25, 10 and -4 °C (Figure 4.12). A similar pattern was observed in "Braeburn Lochbuie", with trees chilled at sub-zero temperatures also showing more even bud break after 1,080 h, but not after 1,776 and 2,424 h. After 2,424 h, the most homogeneous bud break was observed at temperatures above freezing (Figure 4.12).

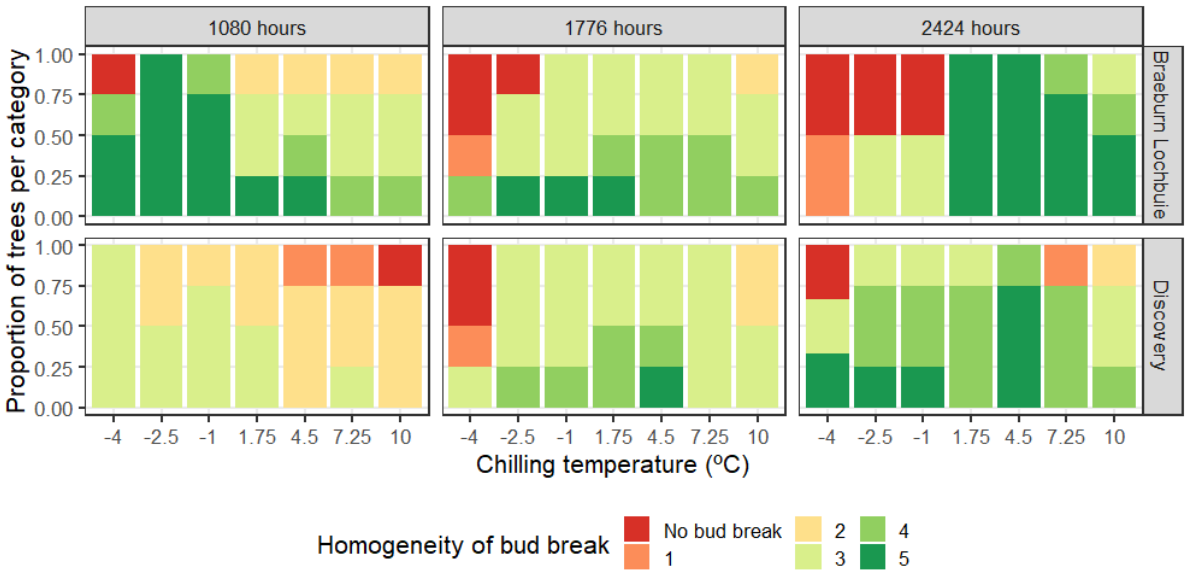


Figure 4.12 - Proportion of trees in each homogeneity of bud break category scored after 2 months under forcing conditions and different chilling treatments (7 temperatures and three chilling durations: 1,080; 1,776 and 2,424 h). Homogeneity of bud break was scored from 1 to 5, with 5 being equal bud break in the whole tree. Trees where no bud break was observed are shown in red.

In both cultivars, growth from the terminal bud increased with longer chilling in temperatures above zero (Figure 4.13). A different pattern was observed in trees chilled at freezing temperatures, in "Braeburn Lochbuie" new growth in these treatments was minimal throughout the time of study, and reduced growth was observed in "Discovery" after 1,446 and 2,424 h of chilling (Figure 4.13).

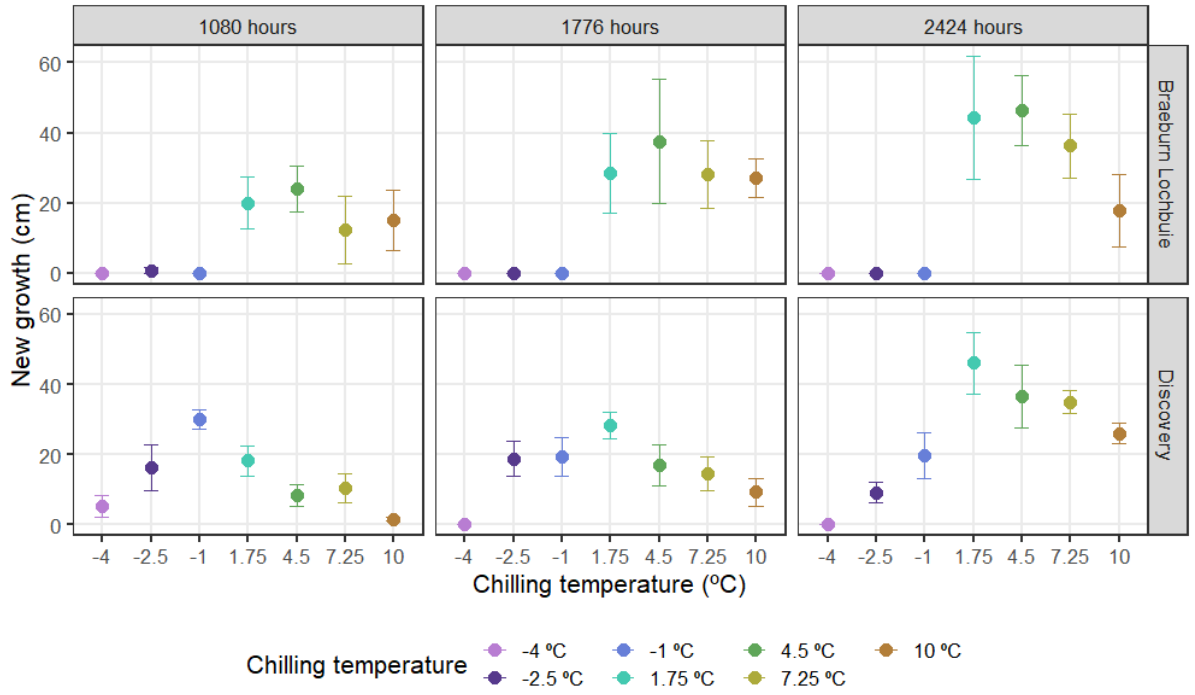


Figure 4.13 – Mean length of new growth (cm) per terminal bud ± SE in "Braeburn Lochbuie" (top) and "Discovery" (bottom) trees chilled at different temperatures and durations (1,080; 1,776 and 2,424 h).

4.4 Discussion

A lack of standardised protocols for assessing dormancy break (Dennis, 2003) and the diversity of approaches used to develop existing chilling models (Richardson et al., 1974; Fishman et al., 1987; Guak and Neilsen, 2013), make comparisons with and between published results challenging. However, results from the present experiment show important differences between the cultivars studied in their response to chilling temperature and duration, and highlight the need to investigate these responses at a cultivar level.

Problems associated with using percentage of bud break to assess dormancy

In several published studies investigating chilling accumulation, percentage of bud break or length of new growth have been used as parameters to assess dormancy release (Thompson et al., 1975; Shaltout and Unrath, 1983; Naor et al., 2003), instead of time of bud break. Here, a continuous increase in percentage of bud break and new growth was observed with longer periods of chilling; but the present results show more variability in percentage of bud break compared to time of bud break, as reported in previous studies (Guak and Neilsen, 2013). This could be explained by experimental issues encountered during this experiment, which make percentage of bud break and length of new growth less reliable to assess bud break.

For example, some of the trees chilled at sub-zero temperatures for 1,667 and 2,424 h suffered severe low temperature damage, including frozen branches, and twelve trees died during the experiment. Damage was greatest at the tips of branches, which subsequently affected new growth from terminal buds. As new growth was measured only from terminal buds, this parameter in trees chilled at freezing temperatures generated results that did not accurately reflect true patterns of growth. Low temperature damage affected the two cultivars differently; cold acclimation is highly linked to dormancy and differences in low temperature tolerance have previously been reported between cultivars (Salazar-Gutiérrez et al., 2016).

Most studies did not explore the chilling efficiency of sub-zero temperatures (Thompson et al., 1975; Shaltout and Unrath, 1983; Naor et al., 2003), and therefore the experimental issues described above were not encountered. Results reported here indicate that, compared to percentage of bud break or length of new growth, the rate of progress to bud break provides a more realistic measure of the contribution of different chilling temperatures to chilling accumulation since it overcomes technical problems associated with imposing colder treatments.

Using rates of development (day^{-1}) is a common approach in thermal time studies (Hadley et al., 1983), and here, this improved model fit compared to time of bud break, and ensured that model assumptions were met.

Optimum temperature for chilling accumulation

Most chilling studies omit the chilling model calibration step (Ruiz et al., 2007; Campoy et al., 2012; Luedeling et al., 2013; Guo et al., 2014; Darbyshire et al., 2016a; Parkes et al., 2020) and so authors assume that the chilling temperature range and the optimum chilling temperature for any given cultivar are the same as those in the cultivars used to develop the original models. The results described here show significant differences in the optimum chilling temperature estimated for each cultivar, as well as important differences in how "Discovery" and "Braeburn Lochbuie" responded to temperature. In "Discovery", time to bud break was lowest at freezing temperatures, whilst in the case of "Braeburn Lochbuie" the optimum temperature was 0.8 °C after 1,080 h of chilling. There do not appear to be any published reports estimating the optimal temperature for chilling accumulation in the two cultivars studied.

Previous studies with apple identified a range of optimum temperatures for chilling accumulation: the "North Carolina Model", developed with long shoots of "Starkrimson Delicious", suggested temperatures from 7.2 - 12.9 °C to be optimum as they maximised the percentage of bud break (Shaltout and Unrath, 1983). Much lower temperatures were reported by Naor et al., (2003) in an experiment investigating the 0 - 20 °C temperature range with potted trees of "Golden Delicious". They observed maximum bud break after chilling at 0 °C, which is similar to the 0.8 °C estimated optimum chilling temperature for "Braeburn Lochbuie" in this experiment. The CUapple model, developed with excised shoots of "Gala", reported that temperatures between -2 (the lowest temperature treatment imposed) and 5.5 °C to be best at reducing the number of days to bud break, with a minimum observed at 3.5 °C (Guak and Neilsen, 2013). In potted trees of "Jonathan" apple, Thompson et al., (1975) reported that 2 °C was the most effective temperature in the 2 - 10 °C range in terms of the number of growing buds and extent of new growth.

The reported variability in optimum temperature is likely to be due to important differences in methodology, such as the types of plant material used, the cultivar investigated, experimental approaches, parameters considered for assessing bud break, and the temperature ranges investigated. These differences make comparisons difficult, but a clear common thread between previous studies is that the most efficient temperature was always the lowest one studied (Thompson et al., 1975; Naor et al., 2003; Guak and Neilsen, 2013), implying that the real optimum could be lower, as identified in "Discovery" in the experiment described here. The experimental challenges associated with imposing sub-zero temperature treatments could explain why previous studies have not reported optimum temperatures as low as in the present study. On the other hand, the optimum chilling temperature estimated for "Braeburn Lochbuie" is similar to that reported in previous studies (Naor et al., 2003), indicating than in some cultivars the optimum temperature could be higher.

Studies investigating optimum temperature for chilling accumulation also differ in the number of chilling hours that had accumulated by the time chosen to estimate the optimum temperature. Naor et al., (2003) reported that trees had accumulated between 1,500-2,400 h; Guak and Neilsen, (2013) reported 1,320 h in the CUapple model and Thompson et al. (1975) reported between 500 - 1,850 h. Contrary to findings presented here, in studies where a range of chilling durations were investigated, the same temperature treatment appeared optimum throughout all durations of chilling (Thompson et al., 1975; Naor et al., 2003; Guak and Neilsen, 2013). The present results indicate that optimum temperature rises as chilling accumulated from 1,080 to 2,424 h, increasing from -2.5 to 1.4 °C in "Discovery", and from 0.8 to 6.1 °C in "Braeburn Lochbuie".

Differences between the present results and those from previous studies could be a consequence of the methodology used. In Thompson et al. (1975), only three chilling temperatures were considered (2, 6 and 10 °C), and so the reduced number of treatments and the choice of widely separated temperatures could have masked an increase in optimum temperature. More temperature treatments were investigated by Naor et al., (2003), but a narrower range of chilling durations was considered (1,500-2,400 h). Other possible explanations for this disparity are explored below.

Simoultaneous chilling and heat accumulation

The increase in optimum chilling temperature as longer chilling accumulated could be explained if warmer temperatures were accumulating simultaneously in a thermal time relationship (Hadley et al., 1983; Trudgill et al., 2005). Whilst it is well accepted that chilling and warmer temperatures are required for bud break, it is less clear if these two processes occur sequentially (Hänninen, 1990) or if there is an overlap (Landsberg, 1974; Harrington et al., 2010; Campoy et al., 2011a; Darbyshire et al., 2017).

In addition to the reported increase in optimum chilling temperature, two more observations from the present study support the overlap hypothesis. Firstly, trees continued to be receptive to chilling temperatures even after CR fulfilment, as shown by an increase rate of progress to bud break at all chilling temperatures after 1,776 h. Secondly, a higher increase in the rate of progress to bud break was observed in warmer chilling temperatures (i.e., > 1.75 °C) from 1,776 to 2,424 h, compared to colder temperatures; suggesting that trees could already be receptive to warmer temperatures after 1,776 h.

Regarding the first observation, there are no published reports on the CR of the two studied varieties, however, "Braeburn" is one of the first cultivars in spring to reach bud burst in the UK (Table B1 - Appendix B), and high productivity under low-chill winters in Zimbabwe has been previously reported (Jackson 2003), suggesting a low chill requirement in this variety. In the case of "Discovery", no formal information on its CR exists, but long-term phenology data from NIAB EMR indicates earlier bud break compared to "Golden Delicious", which requires approximately 1,000 CU

(Hauagge and Cummins, 1991a). Based on these data, a logical assumption is that in our experiments, trees had fulfilled their chilling requirement after 1,776 h, and in some chilling temperatures, after 1,080 h. It can, therefore, be assumed that trees were ecodormant (Lang et al., 1987) between the second and third measurement timepoints. The observed continuous effect of chilling temperature on increasing rate of progress to bud break after CR satisfaction suggests that trees were actively responding to chilling temperatures during the ecodormancy phase, supporting the hypothesis that chilling and heat accumulate simultaneously (Landsberg, 1974; Pope et al., 2014; Darbyshire et al., 2016b).

Contrary to the present results, Guak and Neilsen (2013) using the CUapple model did not report a reduction in time of bud break after 1,000 h of chilling in the 300 – 1,500 h range. This discrepancy could be due to the use of excised shoots in their study (Guak and Neilsen, 2013). As detached shoots accumulated chilling hours, the potential impact of their excision on the ability of the buds to open likely increased.

The present results suggest that some of the reported effects of high/low temperature cycles during chilling accumulation (Erez and Couvillon, 1987; Naor et al., 2003) could also be the consequence of chilling and heat accumulating simultaneously, at least after partial chilling accumulation (Pope et al., 2014). The Dynamic model was developed from a series of experiments comparing percentage of bud break in trees chilled at a constant low temperature with trees chilled in a low/high temperature cycle (Erez et al., 1979; Erez and Couvillon, 1987). Percentage of bud break was higher after a low/high temperature cycle when the high temperature was between 11-18 °C, but lower if the high temperature was above 18 °C (Erez and Couvillon, 1987). A similar effect was observed by Naor et al., (2003) after 2,100 h of chilling, with percentage of bud break being higher after a 6/13 °C cycle compared to constant 6 °C, but lower after chilling at 6/17 °C and 6/20 °C. An experiment investigating the effect of the 4/15 °C temperature cycle at different stages of dormancy, compared to constant 4 °C, showed that it reduced percentage of bud break if applied at the start of chill accumulation, but it increased it if applied in the middle or at the end of rest (Erez and Couvillon, 1987). The observed negative effect of the warmest temperatures in the high/low temperature cycles and of the 4/15 °C cycle at the start of dormancy (Erez and Couvillon, 1987; Naor et al., 2003); could be due to these warmer temperatures hindering dormancy induction, rather than its exit as previously interpreted (Erez and Couvillon, 1987). And the positive effect of low/high temperature cycles when the higher temperature is less warm (Erez and Couvillon, 1987; Naor et al., 2003); might be a consequence of chilling and heat accumulating simultaneously.

It can be hypothesised that during the first removal (1,080 h), chilling was also contributing to dormancy entry. After that, chilling and heat accumulated, and contributed to dormancy release, as shown by an increased rate of progress to and percentage of bud break at all chilling temperatures, including 10 °C. The present results support the overlap hypothesis, whilst chilling accumulation still

significantly reduced time of bud break after 1,777 h, the simultaneous accumulation of heat is likely to be responsible for the observed increase in optimum chilling temperature. Most studies predicting time of bud break in fruit trees assume a baseline temperature of 4 °C for heat accumulation, based on the Growing Degree Hours model developed with sour cherry by Anderson et al., (1986). However, the increase in rate of progress to bud break at all chilling temperatures from 1,776 to 2,424 h (except in frost damaged trees) suggests that the baseline temperature for heat accumulation might be much lower.

Upper and lower temperature thresholds for chilling accumulation

Different studies have highlighted the importance of investigating the upper and lower temperature thresholds of chilling accumulation, particularly to improve predictions under climate change scenarios (Harrington et al., 2010; Luedeling, 2012). With regard to the lowest temperature threshold, most commonly used models assume 0 °C is the lowest temperature for chilling accumulation, although sub-zero temperatures were not investigated when developing the models (Richardson et al., 1974; Shaltout and Unrath, 1983; Erez and Couvillon, 1987). Some studies have highlighted the potential role of freezing temperatures in chilling accumulation (Naor et al., 2003; Harrington et al., 2010; Guak and Neilsen, 2013), but the practical difficulties of investigating this temperature range has perhaps limited further research. The experiment that lead to the development of the CUapple model included a treatment at -2 °C; results obtained at -2 °C were extrapolated to a -4 °C temperature, which significantly improved bud break predictions (Guak and Neilsen, 2013). In the parallel bud break model developed with Douglas-fir (Harrington et al., 2010), incorporating freezing temperatures to -4.7 °C generated the best predictions.

The results for “Discovery” indicate that the lower limit for chilling accumulation might be colder than -4 °C, as the rate of progress to bud break at this temperature was higher than in other treatments. Rate of progress to bud break at -4 °C was also higher than in trees that did not receive any chilling (control), suggesting that chilling is effectively accumulated at this temperature. In “Braeburn Lochbuie”, the same was observed after 1,080 h of chilling but no significant differences occurred in trees chilled for longer. This was likely to have been influenced by the frost damage described above. As the rate of progress to bud break at -4 °C was higher than in trees that did not receive any chilling, further experiments looking at the lower temperatures are needed to define the lower value of variety-specific chilling temperature accumulation curves.

Different upper temperature thresholds for chilling accumulation have also been reported in the literature: 13 °C in the Dynamic model (Erez and Couvillon, 1987), 15 °C in the Utah model (Richardson et al., 1974) and in Harrington et al., (2010) parallel model. The Utah and Dynamic models also include a negating chilling effect for higher temperatures, but this potential mechanism was not investigated here. In their study with “Gala” apple, Guak and Neilsen (2013) reported no chilling effect above 13 °C whilst Naor et al. (2003) observed limited bud break (<15%) in trees

chilled between 10-20 °C, but only after more than 2,000 h of chilling. In the present study, trees chilled at 10 °C had the lowest rate and percentage of bud break throughout the experiment (except in frost damaged trees). When comparing with the control, responses at 10 °C in "Discovery" were not significantly different after 1,080 h, and only slightly higher than the control in "Braeburn Lochbuie", suggesting that this temperature might not contribute to chilling accumulation. However, as the chilling period increased, rate of progress to bud break at 10 °C appeared significantly higher than the control. The lack of differences with control trees at 1,080 h and the low bud break percentage below 1,776 h suggest that 10 °C might not be within the temperature range for chilling accumulation. However, percentage of bud break in trees chilled at 10 °C doubled from the second to the third chilling duration. This increase in bud break percentage together with the observed difference in rate of progress to bud break between 10 °C and the control as more chilling accumulates, support the hypothesis that chill and heat might be accumulating simultaneously after 1,080 h of chilling.

Using daily mean temperatures instead of hourly temperatures

An alternating day/night temperature treatment (7/2 °C) was included in this study to gain insight into how precise chilling models need to be regarding units of time and temperature. Existing chilling models require hourly temperatures (Richardson et al., 1974; Fishman et al., 1987), a parameter difficult to obtain for long-term phenological studies which often extrapolate hourly values from daily maximum and minimum temperatures.

No differences in rate of progress to bud break were observed between trees chilled at 4.5 °C and trees chilled at 7/2 °C. Further experiments are required to determine whether the lack of differences between constant and fluctuating temperatures is consistent across the whole chilling temperature range. However, results suggest that, when hourly temperatures are within the chilling accumulation temperature range, using mean daily temperature for chilling models could be as accurate as using hourly temperatures.

Overall, results obtained show that "Discovery" has a lower optimum chilling temperature than previously reported for any other apple cultivars (Thompson et al., 1975; Shaltout and Unrath, 1983; Naor et al., 2003); whilst the observed results in "Braeburn Lochbuie" indicate a similar optimum chilling temperature to other apple varieties (Naor et al., 2003). Differently than in published studies (Thompson et al., 1975; Naor et al., 2003; Guak and Neilsen, 2013), the optimum chilling temperature increased in both varieties as longer chilling accumulated. This result, in combination with the observed rate of progress to bud break responses to chilling temperature and duration, support the hypothesis that both chilling and heat accumulate simultaneously, at least after partial chilling accumulation (Pope et al., 2014; Darbyshire et al., 2016b). Although further experiments

with a wider range of day/night fluctuating temperatures are required, the lack of differences observed between the 4.5 °C and 7/2 °C temperature treatments indicate that chilling accumulation models could be simplified by using daily temperatures, instead of hourly temperatures as required by existing models (Richardson et al., 1974; Fishman et al., 1987).

Chapter 5

A new approach for modelling the combined effect of chilling and heat accumulation

5.1 Introduction

As a consequence of climate change, winter chilling in the UK is predicted to decline, but warmer temperatures in spring will contribute to higher levels of heat accumulation (Atkinson et al., 2004; Luedeling et al., 2009a; Luedeling and Brown, 2011). Because both temperature processes are interlinked (Cannell and Smith, 1983; Guo et al., 2014; Pope et al., 2014; Kaufmann and Blanke, 2019), it is vital to investigate their combined effect on time of bud break in an attempt to better understand and predict how cultivars will perform in a climate change context.

Thermal time models are widely used in crop sciences to measure developmental processes driven by temperature (Hadley et al., 1983; Ritchie and Nesmith, 1991; Trudgill et al., 2005). Thermal time models are based on the hypothesis that plant development ceases at a certain temperature (the so-called base temperature, *Tb*), and that the rate of development above this temperature increases linearly with warmer temperatures (Hadley et al., 1983; Trudgill et al., 2005). Thermal time models are often used to predict the rate of development in many plant processes such as seed germination (Steinmaus et al., 2000), leaf development (Ellis et al., 1986; Granier and Tardieu, 1998) or flower emergence in herbaceous plants (Hadley et al., 1983). However, they are not commonly used alone to predict bud break of perennial trees (but see Linkosalo et al., 2008), as bud burst in these plants is also dependent on chilling accumulation (Vegis, 1964; Lang et al., 1987; Heide and Prestrud, 2005).

Instead, current bud break models combine a chilling model (i.e. Fishman et al., 1987; Richardson et al., 1974; Weinberger, 1950) to predict endodormancy release, and a thermal time model (Anderson et al., 1986) to quantify the progress to bud break or blooming as a function of heat accumulation during ecodormancy. Whilst better predictions are achieved with the combination of a chilling and a heat accumulation model compared to using only thermal time models (Darbyshire et al., 2013), these are still inaccurate when models are extrapolated to species or areas different from those used to develop them (Legave et al., 2008; Chuine et al., 2016).

Another aspect that hinders accurate predictions is that most commonly used models in apple were originally developed independently, focusing only on chilling (i.e. Fishman et al., 1987; Guak and Neilsen, 2013; Richardson et al., 1974) or heat accumulation (Anderson et al., 1986). A correlation

between these processes exists, as longer chilling can reduce heat requirements for blooming (Ruiz et al., 2007; Darbyshire et al., 2013; Guo et al., 2014), however, models were developed without accounting for a possible interaction or compensation between the two temperature-dependent processes.

There are some exceptions, such as the Parallel and Alternating models (see section *2.4.2.2 Parallel and Alternating models*), in which it is assumed that heat and chilling accumulate simultaneously (Landsberg, 1974; Cannell and Smith, 1983; Harrington et al., 2010). Cannell and Smith's (1983) model, developed for *Picea sitchensi*, included a compensating mechanism so that the amount of heat required for blooming declined exponentially as more chilling accumulated. However, this model considered 5 °C as the upper and lower threshold for chilling and heat accumulation, respectively, a value chosen arbitrarily (Cannell and Smith, 1983). The same temperature thresholds were established in Landsberg's (1974) model for apple, but their approach required a minimum chilling to accumulate before heat accumulation began. It has been extensively shown that not all temperatures contribute to chilling equally (Erez and Lavee, 1971; Richardson et al., 1974; Naor et al., 2003), which could explain why these models have not performed well in predictive studies (Linkosalo et al., 2008).

Whilst a range of chilling models exist (i.e. Fishman et al., 1987; Richardson et al., 1974; Weinberger, 1950), studies with fruit trees mainly rely on one model for quantifying heat accumulation, the Growing Degrees Hour Model (GDH) (Anderson et al., 1986). The GDH model is a thermal time model that involves three parameters, a base temperature of 4 °C, an optimum temperature (25 °C) at which maximum heat accumulation occurs, and a critical temperature (36 °C) above which there is no heat accumulation (Anderson et al., 1986). The model is based on two cosine equations that define the relationship between GDH accumulated, and the three temperature parameters (Anderson et al., 1986). Whilst many studies use the original GDH model parameters, thereby omitting calibration at a species level (Ruiz et al., 2007; Guo et al., 2015b; Kaufmann and Blanke, 2019), some have shown more accurate blooming date predictions using different *Tb* values (Parker et al., 2011; Zhang et al., 2015). Estimating *Tb* correctly in a thermal time model is important as heat will accumulate more quickly or more slowly depending on this parameter. A range of methods exist to calculate *Tb*, including the development rate or x-intercept method (Arnold, 1959), which was used here.

Investigating the combined effect on bud break of different chilling and heat accumulations is key to understand how cultivars will respond to predicted changes in the UK climate. The effect of a range of chilling and forcing temperatures on time and percentage of bud break of three apple cultivars is considered here. Varieties studied included sports of two commercially important apple cultivars in the UK, “Braeburn Lochbuie” and “Galaxy Gala” (DEFRA, 2021), and an extensively grown apple cider

variety, “Dabinett”; UK growers have raised concerns about uneven flowering in “Dabinett” during recent years (Loraine Boddington, NACM, pers. comm.).

Data obtained here and in Chapter 4 were used to develop a new chilling model (*Malus model*), which was subsequently combined with forcing temperatures, in a thermal time approach (Arnold, 1959) to calculate heat accumulation according to different chilling accumulations. This *Malus model* was used to investigate whether it is possible to compensate insufficient chilling with higher heat accumulation (Ruiz et al., 2007; Darbyshire et al., 2013; Guo et al., 2014).

5.2 Materials and methods

5.2.1 Plant material

Trees grafted on M9 rootstock with three different scion varieties (“Braeburn Lochbuie”, “Dabinett” and “Galaxy Gala”) were used for this experiment.

In February 2020, one-year-old shoots from the three scion varieties were collected from trees planted in 2014, grafted on “M9” rootstocks in an experimental apple orchard at NIAB EMR (south-east England). On the day of collection, bud wood was wrapped in plastic bags and stored in a cold store at 2 °C for one month. In March, shoots were removed from the cold store and grafted onto “M9” rootstocks (Frank P Matthews Ltd, Tenbury Wells, Worcestershire, UK) previously established in 3 L pots containing a peat-based compost (Sinclair pro compost mix consisting of bark (10%), lime (1.5kg in 900 L), coarse and medium peat (90%), slow-release fertiliser (8-9 M (months longevity), 1 kg per 900 L, N12-P14-K24) and wetter) with added slow release fertiliser (Osmocote Extact Standard 8-9 M, N15-P9-K11+2MgO+TE, 4 kg per 900 L) and a granular biological insecticide (Met 52, 1 kg per 900 L). Composite trees were grown on in a polytunnel at NIAB EMR to help to ensure successful grafting and to promote scion growth (Figure 5.1).

Figure 5.1 - Grafts growing in a polytunnel at NIAB EMR. Photo taken on 11/03/2020.

Figure 5.2 - Trees being transported to Reading. Photo taken on 17/10/2020.

On 17 October 2020, trees were transported in a non-insulated van (Figure 5.2) to the University of Reading; each was labelled with a unique number before being placed randomly in a glasshouse compartment at 15 ± 3 °C to ensure no chilling accumulated before the experiment. Trees remained in the glasshouse compartment for a month due to Covid-19 related delays with previous experiments. In the glasshouse compartment, air temperature and humidity were recorded hourly with a Decagon VP-4 temperature and humidity sensor positioned at canopy height and

connected to a Decagon Em50G remote datalogger (both from Meter Group, Inc. USA (previously called Decagon Devices Ltd), 2365 NE Hopkins Ct., Pullman, WA 99163, USA).

On 12 November 2020, air temperature in the glasshouse compartment was reduced to 10 ± 3 °C to begin acclimation before imposing chilling treatments (Figure 5.3).

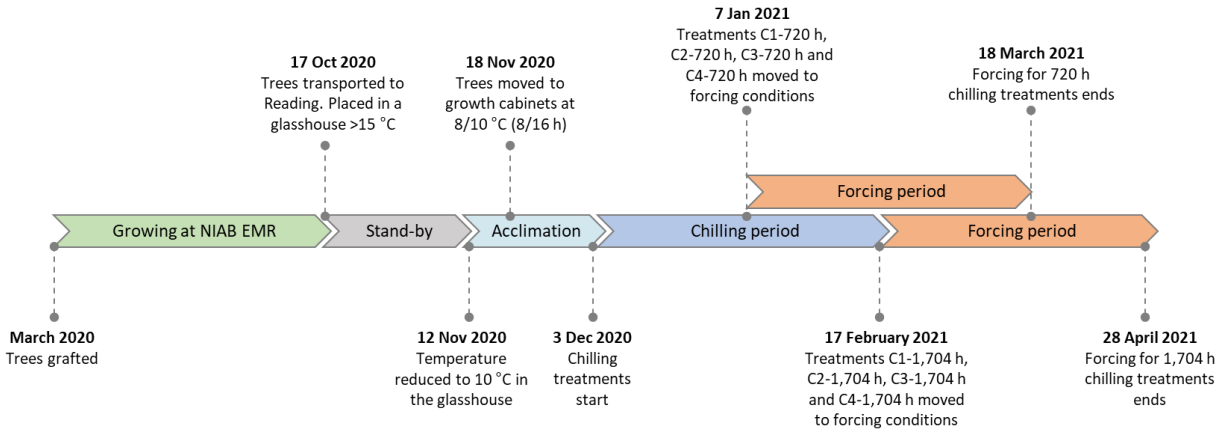


Figure 5.3 - Timeline of the experiment.

5.2.2 Treatments

All treatments were imposed in controlled environment growth cabinets (Fitotron 3 Wiss Technik UK Ltd.) at the Crops and Environment Laboratory (CEL) at the University of Reading. Trees were randomly distributed inside the growth cabinets throughout the experiment (Figure 5.4). Four chilling treatments, three forcing treatments and two chilling durations were used in a 4 x 3 x 2 completely randomised multi-factorial design.

Due to limited space in the growth cabinets, main stems were pruned to 140 cm before starting the treatments, but at least one terminal bud was left intact on each tree and at least one branch was cut in all trees (Naor et al., 2003). All branches growing below 70 cm and longer than 15 cm were removed. Trees were also defoliated before being moved into the growth cabinets but leaves growing immediately next to terminal buds were left intact to avoid possible bud damage (Figure 5.5).

Trees were moved into the growth cabinets on 18 November 2020. To encourage growth cessation and ensure acclimation to cold temperatures, all cabinets were set to 10/8 °C in a 8/16h (day/night) photoperiod for two weeks. On 3 December 2020, chilling treatments were imposed.

Chilling treatments

All chilling treatments consisted of an 8/16h (day/night) photoperiod using 4ft warm-white T5 fluorescent tubes (TL5 39W Master, Philips, UK) providing a radiation intensity of approximately 100 µmol/m^2 at 60 cm height. Relative humidity was adjusted to achieve 0.6kPa VPD (Grange and Hand, 1987). Four different temperature and relative humidity treatments were imposed:

- C1: 2/-4 °C temperature (mean daily temperature of -2 °C) and 16/0% relative humidity.
- C2: 5.5/-0.5 °C temperature (mean daily temperature of 1.5 °C) and 34/0% relative humidity.
- C3: 9/3 °C temperature (mean daily temperature of 5 °C) and 48/22% relative humidity.
- C4: 13/7 °C temperature (mean daily temperature of 9 °C) and 60/41% relative humidity.

A two-and-a-half-hour ramp was set for the day/night temperature and humidity change whilst light intensity was adjusted in 30 minutes, simulating natural conditions.

Four trees from each variety and temperature were moved into growth cabinets to force bud break at two time points (durations of chilling):

- 720 hours of chilling accumulation
- 1,704 hours of chilling accumulation

Temperature in each treatment was maintained within the desired range ± 1 °C throughout the chilling period (Figures A3 and A4 - Appendix A). Due to technical problems with one of the growth cabinets, temperature increased above 4 °C on three occasions during the C1–1,704 h treatment; in one of these events, temperature remained above 4 °C for five consecutive days, whilst in the other two occasions the temperature increase lasted less than 24 h.

Figure 5.4 – Trees inside a growth cabinet at the start of chilling accumulation. Photo taken on 5/12/2020.

Figure 5.5 – Trees before and after being defoliated. Photo taken on 18/11/2020.

Forcing treatments

All forcing treatments consisted of an 18/6h (day/night) photoperiod at an average light intensity of 300 µmol/m^2 (4ft warm-white T5 fluorescent tubes, TL5 39W Master, Philips, UK) measured at 60 cm height. Temperature and relative humidity remained constant throughout the day. Different relative humidity values were selected at each temperature to achieve 1 kPa VPD (Grange and Hand, 1987). The three forcing treatments were:

- F1: 14 °C and 14% relative humidity
- F2: 18 °C and 52% relative humidity
- F3: 22 °C and 62% relative humidity

A two-and-a-half-hour ramp was set for the day/night temperature, relative humidity, and light intensity change. Hourly temperature remained within ± 1 °C of the desired temperature treatment throughout most of the forcing period. Deviations of ± 2 °C from the temperature treatment occurred in three occasions for less than two hours (Figures A5 and A6 – Appendix A). Due to battery failure in the sensor, no data was recorded during 10 days in treatment F2 after 1,704 h of chilling. All forcing treatments were imposed for 70 days.

Treatment abbreviations

Throughout this chapter, treatment combinations are abbreviated such as: Chilling treatment – Chilling Duration - Forcing treatment. For example: C1-1704 F2 indicates a chilling treatment of 2/-4 °C; a chilling duration of 1,704 hours, and a forcing at 18 °C.

5.2.3 Data collection

The total number of buds per tree was counted at the beginning of the experiment. During forcing (Figure 5.6), bud break was assessed twice a week in all trees and open buds (buds at the green tip stage 3 of development, as defined by Chapman and Catlin (1976), Figure 3.3) were recorded.

Figure 5.6 – Trees inside a growth cabinet during a forcing treatment. Photo taken on 5/03/2021.

Soil moisture content was monitored every 2 weeks during chilling treatments and weekly during forcing; a WET Sensor connected to an HH2 Moisture Meter (Delta-T Devices, UK) was used to ensure that that soil water availability was uniform across all treatments. Plants were hand watered when moisture content fell below 0.350m^3/m^3. In all growth cabinets, air temperature and humidity were recorded hourly with dataloggers (Tempo Disc™ 3-in-1 Bluetooth Sensor Logger from Blue Maestro, UK) to provide an independent record of environmental conditions within each growth cabinet.

5.2.4 Data analyses

Data analyses were carried out in different phases: first, the effect of mean daily temperature during chilling, forcing temperature, and chilling duration, on rate of progress to bud break and percentage of bud break were investigated. Secondly, results from these first analyses and from previous experiments (Chapter 4) were used to inform the development of a new chilling accumulation model, which converted hourly chilling temperatures and chilling duration into chilling accumulation. Chilling accumulated was combined with forcing temperature to calculate the base temperature for heat accumulation as in a thermal time model (Arnold, 1959). Finally, heat and

chilling requirements to reach 20% bud break were calculated to explore the possibility of compensating insufficient chilling with higher heat accumulation.

All analyses and graphs were performed using R statistical software (R Core Team, 2021). A significance level of 0.05 was used.

5.2.4.1 Analyses of untreated data

Cultivar-specific Generalised linear models (Thomas et al., 2017) were performed to explore the effect of mean daily chilling temperature, chilling duration, and forcing temperature on rate of progress to bud break ($days^{-1}$) and Maximum percentage of bud break (%). Rate of progress to bud break was calculated as the mean of the reciprocal of the average number of days to bud break within a tree. Maximum percentage of bud break is the total percentage of bud break observed by the end of the forcing period.

The total number of buds per tree was included in all models to account for the initial natural variability between trees. Manual backwards-stepwise model refinement based on p-values was used to delete non-significant terms (Thomas et al., 2017), and model assumptions were visually assessed.

For each cultivar, the two chilling durations (720 and 1,704 h) were initially analysed separately, and only the effect of chilling and forcing temperatures were considered, such as:

(i) Rate of progress to bud break = a + bChilling temperature + cForcing temperature

(ii) Max % bud break = a + bChilling temperature + cForcing temperature

Where *a, b* and *c* are cultivar-specific parameters. *Chilling temperature* is the mean daily temperature for each chilling treatment, i.e. C1 = -2 °C, C2 = 1.5 °C, C3 = 5 °C, and C4 = 9 °C. *Forcing temperature* as in F1, F2 and F3 treatments.

A second set of models were generated for each cultivar considering both chilling durations together:

(iii) Rate of progress to bud break = a + bChilling temperature + cForcing temperature + dChilling duration

(iv) Max % bud break = a + bChilling temperature + cForcing temperature + dChilling duration

Where *Chilling duration* is the number of hours of chilling (720 or 1,704 h).

5.2.4.2 Combining chilling accumulation and forcing temperature

Analyses described below were carried out for the cultivars “Galaxy Gala” and “Braeburn Lochbuie”, whilst no further analyses were performed in “Dabinett” due to a reduced temperature response in this cultivar (see section *5.3 Results*).

Cultivar-specific models on rate of progress to bud break were created, including two predictive variables, warm temperature during forcing and chilling accumulation, calculated as described below. For modelling purposes, a value of 70 days (maximum number of days bud break was assessed for) was given to trees where no bud break was observed, as done previously in similar studies (Guak and Neilsen, 2013).

Chilling temperature and chilling duration were combined to create a new chilling accumulation model (hereafter *Malus model*). As in existing chilling models developed with other fruit trees (Richardson et al., 1974; Fishman et al., 1987), the *Malus model* is based on a weighting system to account for different temperature effectiveness in contributing to chilling accumulation. An optimum temperature for chilling accumulation of -2 °C was established so that 1 hour at -2 °C accounted for 1 Chill Fraction (CF). Temperatures between -10 and 13 °C contributed to chilling as a fraction of a CF (Table B4 – Appendix B), assuming a linear relationship between the effectiveness of the optimum chilling temperature and the upper and lower chilling temperature thresholds (Figure 5.7). Model parameters were selected based on results obtained here and in Chapter 4 (see section *5.4 Discussion*).

Chilling accumulation was calculated for each chilling temperature-chilling duration combination based on the *Malus model*. Hourly temperatures, as recorded with dataloggers in the growth cabinets (Figures A5 and A6 – Appendix A), were used for chilling calculations. For comparison, chilling accumulation was also calculated according to the Utah (Richardson et al., 1974) and Dynamic models (Fishman et al., 1987). All calculations were performed using the *chillR* package (Luedeling, 2021), with a new function created to calculate CF.

After calculating CF for each chilling temperature and duration combination, a Generalised linear model including chilling accumulation (Chill Fractions) and forcing temperature was built for each cultivar (Thomas et al., 2017):

(v) Rate of progress to bud break = a + b Forcing temperature + c Chilling accumulation

Equation (v) was used to calculate the *Tb* for heat accumulation using a similar approach to the development rate method (Arnold, 1959), but accounting for chilling accumulation. In simple thermal time models (*Rate = a + bTemperature*), *Tb* is calculated as the temperature when no development occurs (*Rate = 0, therefore Tb = -a/b*) (Arnold, 1959). Here, the same approach is followed, but *Tb* also depends on previous chilling accumulated:

(vi) $Tb = \frac{-a - c\ Chilling\ accumulation}{b}$

Tb was calculated for a range of chilling accumulations (800 to 2,000; at 100 CF intervals) for "Braeburn Lochbuie" and "Galaxy Gala".

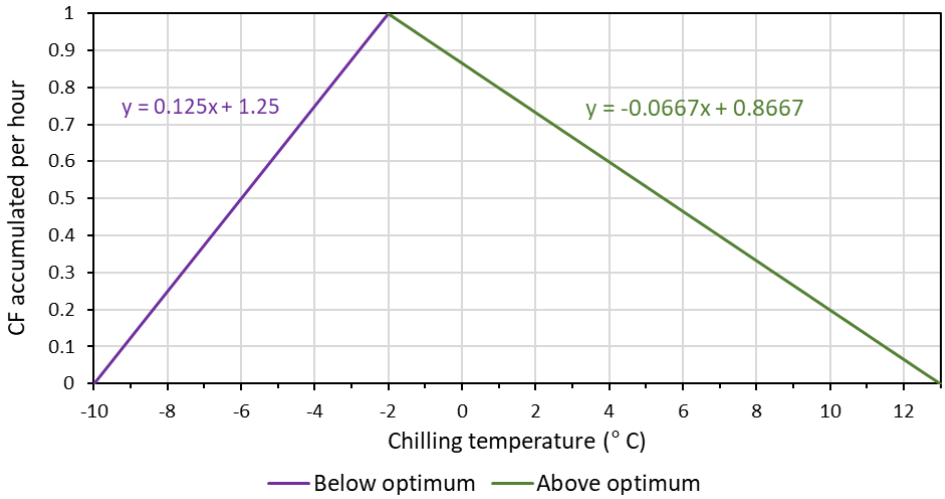


Figure 5.7 – Graphical representation of the temperature contributions in the *Malus model.* One hour at -2 °C is equivalent to 1 Chill Fraction (CF). Chilling temperatures between -10 and 13 °C contribute to chilling with a proportion of a CF (y-axis), as calculated by solving the linear regressions represented in the graph.

5.2.4.3 Combining chilling and heat accumulation

Based on the methodology described above, the amount of chilling (CF) and heat accumulated to reach 20% bud break was calculated for each treatment. Heat accumulation was calculated according to the Growing Degree Hour model (Anderson et al., 1986), with modifications. The GDH function for heat accumulation is based in two cosine equations. For temperatures below the optimum, hourly heat accumulation is calculated as:

(vii) $GDH = (FA/2) * (1 + \cos(\pi + \pi (TH - TB) / (TU - TB)))$

Where: *TH = hourly temperature*

TB = base temperature (4 °C)

TU = optimum temperature (25 °C)

F = a stress factor, considered 1 when plants are not growing under stress

A = TU – TB

The optimum temperatures (25 °C) for heat accumulation from the GDH model (Anderson et al., 1986) was maintained, whilst the base temperature was calculated as in equation *(vi)* and adjusted for each calculation according to the amount of chilling previously accumulated.

Finally, the ratio of heat required to reach 20% bud break (GDH) in relation to previous chilling accumulated (CF) was calculated:

(viii) $$Ratio = \frac{Heat\ accumulated\ to\ reach\ 20\%\ bud\ break\ (GDH)}{Chilling\ accumulated\ (CF)}$$

5.3 Results

Effect of chilling, forcing temperature and duration of chilling on rate of progress to bud break

The effect of chilling and forcing temperature were first investigated independently for each duration of chilling (Figure 5.8), and then in combination with chilling duration (Table 5.1). The interaction between chilling and forcing temperature was not significant.

After 720 h of chilling, a higher chilling temperature significantly reduced the rate of progress to bud break in all cultivars, whilst the opposite was observed with higher forcing temperatures ($p<0.05$, Figure 5.8). More than 60% ($R^2 = 0.6271$) of the variability in rate of progress to bud break of "Braeburn Lochbuie" (Figure 5.8A) was explained by chilling and forcing temperature, but less than 50% ($R^2 < 0.5$) of the variability was explained in "Dabinett" (Figure 5.8C) and "Galaxy Gala" (Figure 5.8E). In "Braeburn Lochbuie" and "Galaxy Gala", a higher R^2 was observed after 1,704 h of chilling, with R^2 values of 0.7277 (Figure 5.8B) and 0.605 (Figure 5.8F), respectively.

Overall, rate of progress to bud break was highest in "Braeburn Lochbuie", and lowest in "Dabinett". In "Braeburn Lochbuie" and "Galaxy Gala", maximum rate was observed after 1,704 h of chilling, in trees chilled at -2 °C and forced at 22 °C (Figures 5.8B and 5.8F). The same temperature treatments appeared most effective at increasing rate of progress to bud break in "Dabinett", but here it was highest after 720 h of chilling (Figure 5.8C). In this cultivar, differences between chilling and forcing temperatures were significantly reduced after 1,704 h, when chilling temperature did not appear to significantly affect rate of progress to bud break (Figure 5.8D).

When data from both chilling durations were combined (Table 5.1), colder chilling temperature, warmer forcing temperature, and longer chilling duration significantly increased rate of progress to bud break in "Braeburn Lochbuie" and "Galaxy Gala", explaining 73% and 55% of the observed variability, respectively ($p<0.01$, Table 5.1). The significance of each parameter on rate of progress to bud break varied between cultivars, whilst in "Braeburn Lochbuie", forcing temperature had the greatest effect on rate of progress to bud break, chilling temperature appeared more effective in "Galaxy Gala" ($p<0.01$, Table 5.1). A significant interaction between chilling temperature and duration was observed in "Dabinett" ($p<0.05$, Table 5.1), as well as a negative effect of duration of chilling on rate of progress to bud break. However, in this cultivar the variables studied only explained 36% of the observed variability on rate of progress to bud break (Table 5.1).

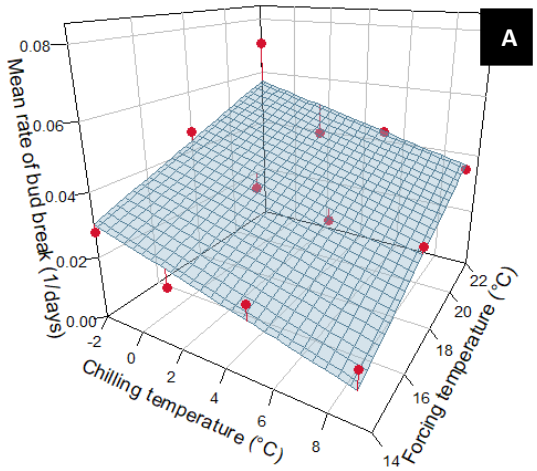
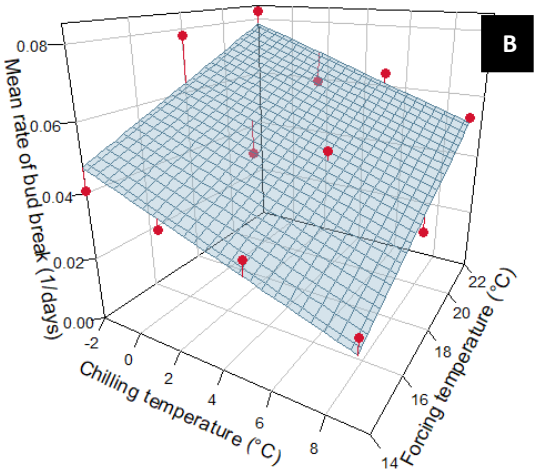
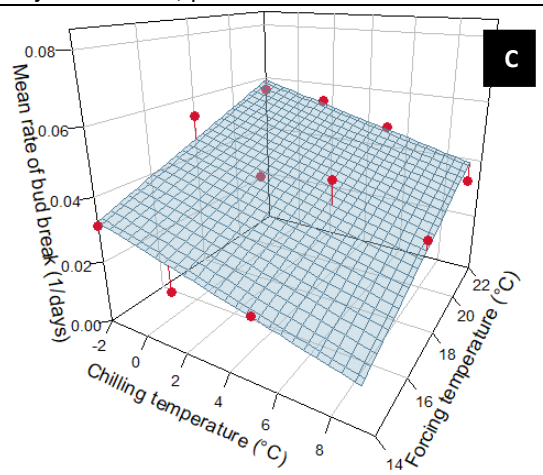
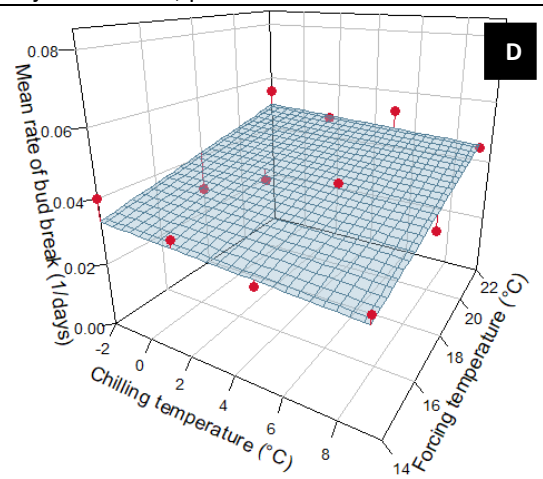
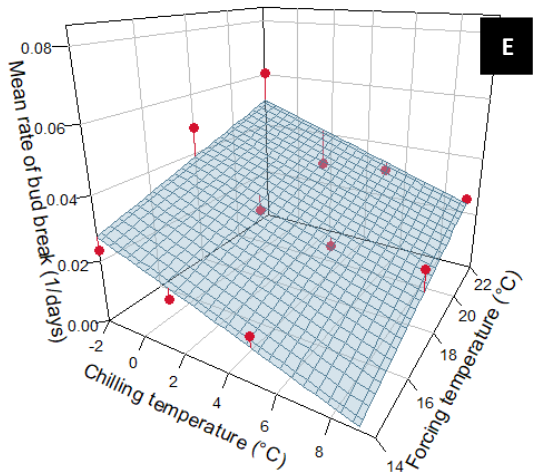
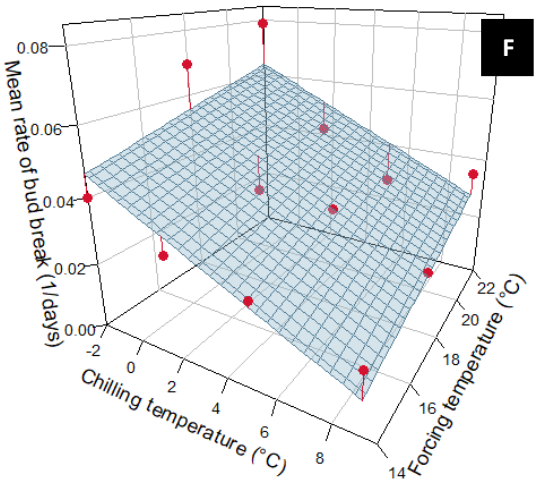
	720 h	1704 h
Braeburn Lochbuie	 y = -0.0190784 -0.0020656C + 0.0033072F Adj. R^2 = 0.6271, p-value: 3.795e-10	 y = -0.0105337 -0.0023651C + 0.0037919F Adj. R^2 = 0.7277, p-value: 2.696e-13
Dabinett	 y = -0.0142124 -0.0021844C + 0.0033400F Adj. R^2 = 0.4703, p-value: 7.694e-06	 y = 0.0026817 -0.0005275C + 0.0022421F Adj. R^2 = 0.2639, p-value: 0.000519
Galaxy Gala	 y = -0.0189377 -0.0025872C + 0.0030642F Adj. R^2 = 0.4418, p-value: 6.041e-05	 y = 0.0042206 -0.0037687C + 0.0025720F Adj. R^2 = 0.605, p-value: 1.273e-09

Figure 5.8 – Cultivar-specific 3D graphs representing the observed (●) and modelled (blue surface) rate of progress to bud break (days^{-1}) as a response to mean chilling and forcing temperatures. From top to bottom: "Braeburn Lochbuie", "Dabinett", "Galaxy Gala", results shown for trees chilled during 720 h (left column) and 1,704 hours (right). Inside each panel: equation, adjusted R^2 and p-value from the model.

Table 5.1. – Cultivar-specific generalised linear models on rate of progress to bud break, including chilling temperature, forcing temperature, and chilling duration (hours). Results presented: variable estimates, standard error, t-value, p-value indicating significance level of each variable and overall model parameters including the adjusted R^2 and overall model significance. F value and degrees of freedom of numerator, denominator.

Cultivar	Variable	Estimate	Std. Error	t value	P-value	Model parameters
Braeburn Lochbuie	Intercept	-3.54e-02	6.16e-03	-5.748	1.31e-07***	Adj. R^2: 0.7327 p = <2.2e-16 F (3, 87) = 83.24
	Chilling temp	-2.24e-03	2.48e-04	-9.008	2.16e-10 ***	
	Forcing temp	3.57e-03	3.05e-04	11.725	< 2e-16***	
	Chilling duration	1.66e-05	2.02e-06	8.232	1.67e-12***	
Dabinett	Intercept	1.02e-04	9.20e-03	0.011	0.991161	Adj. R^2: 0.3674 p = 4.115e-08 F (4, 78) = 12.91
	Chilling temp	-3.22e-03	9.34e-04	-3.449	0.002346 **	
	Forcing temp	2.69e-03	4.32e-04	6.221	5.19e-08 ***	
	Chilling duration	-3.22e-06	3.37e-06	-0.956	0.730764	
	Chilling temp * Chilling duration	1.58e-06	6.88e-07	2.298	0.024215*	
Galaxy Gala	Intercept	-2.12e-02	1.02e-02	-2.089	0.040156*	Adj. R^2: 0.5507 p = 1.684e-13 F (3, 74) = 32.46
	Chilling temp	-3.20e-03	3.77e-04	-8.472	2.36e-10 ***	
	Forcing temp	2.82e-03	4.78e-04	5.904	9.99e-08 ***	
	Chilling duration	1.14e-05	3.04e-06	3.739	0.004086 **	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

Effect of chilling, forcing temperature and duration of chilling on percentage of bud break

In all cultivars, percentage of bud break increased with longer chilling duration, lower chilling temperature and warmer forcing temperature (Figure 5.9). When the two durations of chilling were considered independently, chilling and forcing temperature explained less of the variability in percentage of bud break (Figure 5.9), compared to rate of progress to bud break (Figure 5.8).

Percentage bud break approximately doubled when chilling time was increased from 720 h to 1,704 h in the three studied cultivars, and maximum bud break always occurred in trees chilled at -2 °C and forced at 22 °C (Figure 5.9). Percentage of bud break was lowest in "Dabinett" (Figure 5.9C and D), whilst in "Galaxy Gala" (Figure 5.9F) and "Braeburn Lochbuie" (Figure 5.9B) approximately 80% of buds broke under some treatments. In "Galaxy Gala", chilling and forcing temperature did not significantly affect percentage of bud break after 720 h of chilling (Figure 5.9E).

More variability in the overall percentage of bud break was explained for all cultivars when both durations of chilling were considered together (Table 5.2), increasing to over 80% in "Braeburn Lochbuie" (R^2 = 0.8147) and 62% in "Galaxy Gala" (R^2 = 0.629). In these two cultivars, a significant interaction was observed between chilling duration and chilling temperature, such that the effect of lower chilling temperature on percentage of bud break decreased with longer chilling duration ($p<0.01$, Table 5.2). Chilling duration had a greater promotive effect on percentage bud break in "Braeburn Lochbuie" and "Galaxy Gala", with an increment of one hour of chilling increasing percentage bud break by more than 0.04%, this effect declined to 0.01% in "Dabinett" (Table 5.2). As with rate of progress to bud break, the fitted relationship explained less of the variability in percentage of bud break in "Dabinett" (R^2 = 0.4819), compared to the other cultivars.

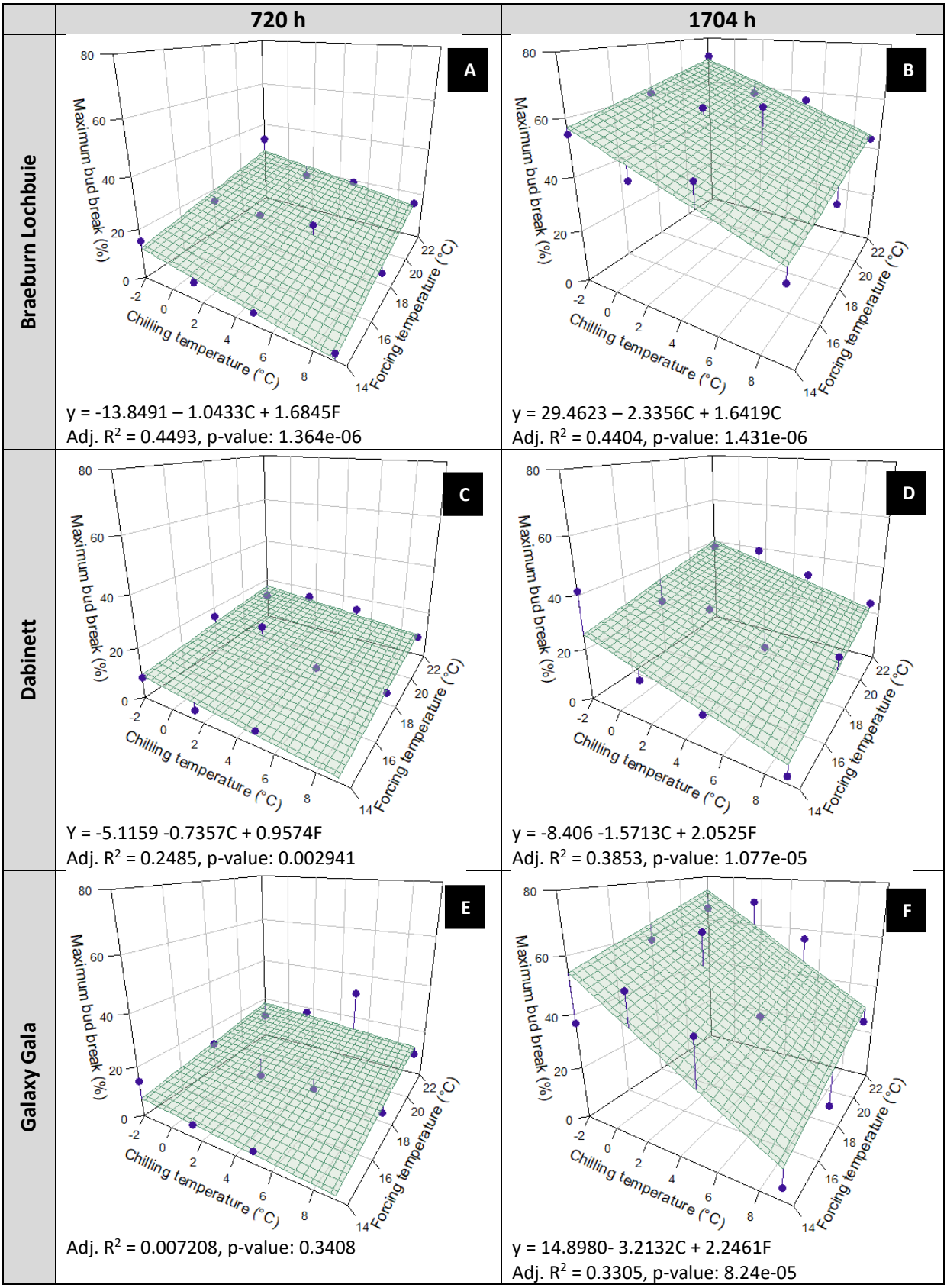


Figure 5.9 - Cultivar-specific 3D graphs representing the observed (●) and modelled (green surface) maximum percentage of bud break (%) as a response to mean chilling and forcing temperatures. From top to bottom: "Braeburn Lochbuie", "Dabinett", "Galaxy Gala", results shown for trees chilled during 720 h (left column) and 1,704 hours (right). Inside each panel: equation, adjusted R^2 and p-value from the model.

Table 5.2. – Cultivar-specific generalised linear models on percentage of bud break, including chilling temperature, forcing temperature, and chilling duration (hours). Results presented: variable estimates, standard error, t-value, p-value indicating significance level of each variable and overall model parameters including the adjusted R^2 and overall model significance. F value and degrees of freedom of numerator, denominator.

Cultivar	Variable	Estimate	Std. Error	t value	P-value	Model parameters
Braeburn Lochbuie	Intercept	-44.58	6.53	-6.83	1.14e-09***	Adj. R^2: 0.8147 p = <2.2e-16 F (4, 86) = 99.95
	Chilling temp	-9.22e-02	6.86e-01	-0.134	3.089e-05 ***	
	Forcing temp	1.662	3.14e-01	5.298	3.980e-07 ***	
	Chilling duration	4.32e-02	2.69e-03	16.052	< 2.2e-16 ***	
	Chilling temp * Chilling duration	-1.32e-03	5.17e-04	-2.546	0.01268 *	
Dabinett	Intercept	-27.626	7.00594	-3.943	0.000173***	Adj. R^2: 0.4819 p = 6.181e-12 F (3, 79) = 26.42
	Chilling temp	-1.24419	0.259809	-4.789	0.0004134 ***	
	Forcing temp	1.651339	0.332459	4.967	0.0001074 ***	
	Chilling duration	0.014884	0.002126	7.002	7.417e-10 ***	
Galaxy Gala	Intercept	-54.85	12.64	-4.339	4.53e-05 ***	Adj. R^2: 0.629 p = 6.712e-16 F (4, 73) = 33.64
	Chilling temp	1.187	1.255	0.946	0.003046 **	
	Forcing temp	1.768	5.92e-01	2.988	0.001845 **	
	Chilling duration	4.60e-02	4.32e-03	10.656	5.638e-16 ***	
	Chilling temp * Chilling duration	-2.56e-03	9.35e-04	-2.738	0.007769 **	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

Chilling accumulation in the Malus chilling model

The amount of chilling accumulated for each chilling temperature-duration combination was calculated as Chill Fractions (CF), and also as Chill Units (CU, Richardson et al., 1974) and Chill Portions (CP, Fishman et al., 1987) for comparison (Table 5.3).

Table 5.3 – Chilling accumulated per treatment, calculated as Chill Fractions (CF) (Malus model), Chill Units (CU) (Utah model, (Richardson et al., 1974)), and Chill Portions (CP) (Dynamic model, (Fishman et al., 1987)).

Treatment	Day/night chilling temperature (°C)	Chilling duration (hours)	CF accumulated	CU accumulated	CP accumulated
C1-720	2/-4	720	756.7	437.5	23.7
C2-720	5.5/-0.5	720	652.5	669.5	35.8
C3-720	9/3	720	495.2	1080	39.2
C4-720	13/7	720	299.8	918.5	51.5
C1-1704	2/-4	1704	1461.1	742.5	51.5
C2-1704	5.5/-0.5	1704	1354.1	1032.0	64.5
C3-1704	9/3	1704	995.0	1958.5	68.3
C4-1704	13/7	1704	546.2	1611.5	70.4

Rate of progress to bud break in both cultivars increased with CF accumulated whilst a pattern was not evident between CU or CP accumulated and rate of progress to bud break (Figure 5.10). Percentage of bud break also increased with CF accumulated (Figure 5.11), but in "Braeburn Lochbuie" percentage of bud break after 546.2 CF and 995.0 CF was higher than in other treatments where more CF had accumulated (Figure 5.11). No pattern was observed between CU accumulated

and percentage of bud break. When chilling was calculated with the Dynamic model (Fishman et al., 1987), percentage of bud break was low until 40 CP had accumulated, and increased sharply afterwards (Figure 5.11).

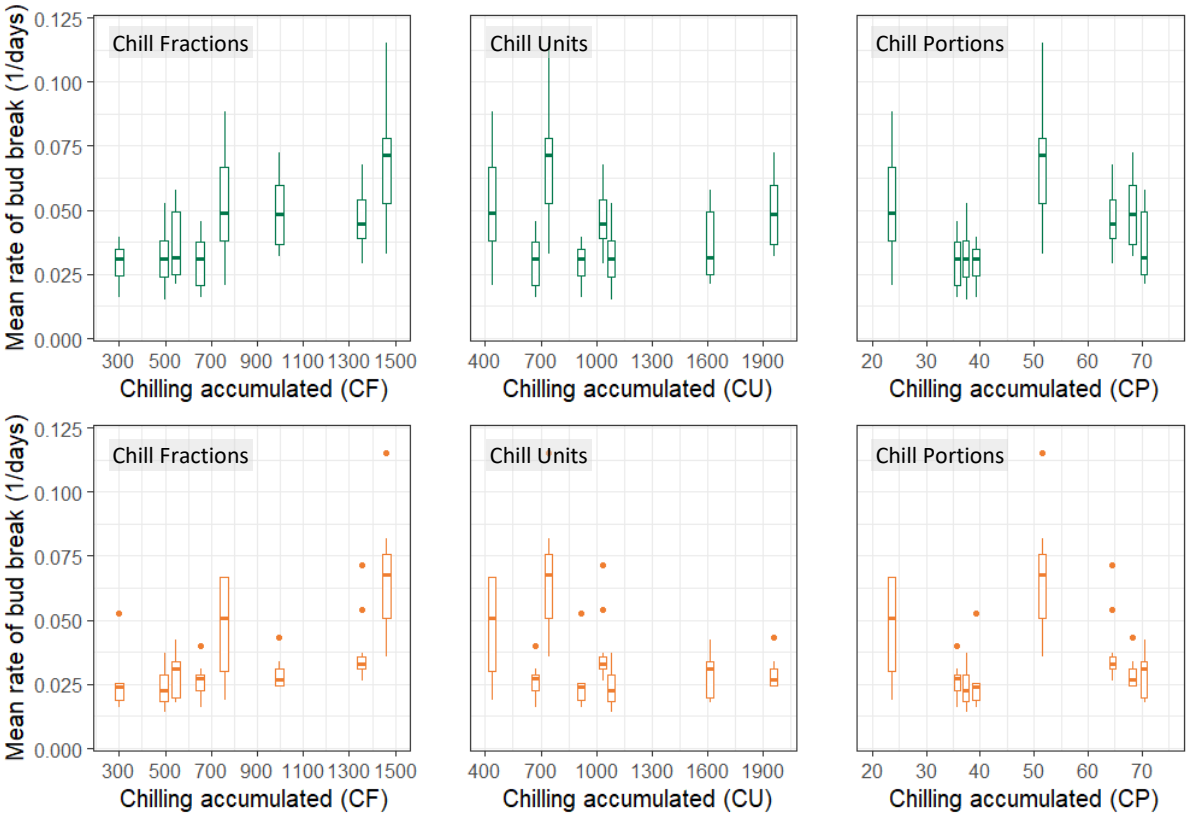


Figure 5.10 - Mean rate of progress to bud break (days^{-1}) in "Braeburn Lochbuie" (green) and "Galaxy Gala" (orange) as a response to Chilling accumulated in Chill Fractions (CF, Malus model, left), Chill Units (CU) (Utah model, centre, (Richardson et al., 1974)), and Chill Portions (CP) (Dynamic model, right, (Fishman et al., 1987)). Boxplots show median, interquartile range, whiskers expand from maximum to minimum values and outliers are represented as single dots.

The highest chilling accumulation in the *Malus model* was 1461.1 CF, recorded after the C1-1704 treatment (Table 5.3) which corresponded to the highest rate (Figure 5.10) and percentage of bud break (Figure 5.11). In the Utah model the highest chilling accumulation (1958.5 CU) occurred after the C3-1704 treatment (Table 5.3). Whilst a high percentage of bud break was observed after this chilling accumulation in both cultivars (Figure 5.11), rate of progress to bud break was low, particularly in "Galaxy Gala". Regarding the Dynamic model, maximum chilling accumulation (70.4 CP) occurred after the C4-1704 treatment (Table 5.3), where a lower rate and percentage of bud break was observed compared to other treatments (Figures 5.10 and 5.11).

The largest difference between models was observed in the C4 treatments, corresponding to the 13/7 °C chilling temperature (Table 5.3). Whilst these treatments accumulated significant amounts of CU and CP, chilling accumulation in C4 treatments was low with the *Malus model.*

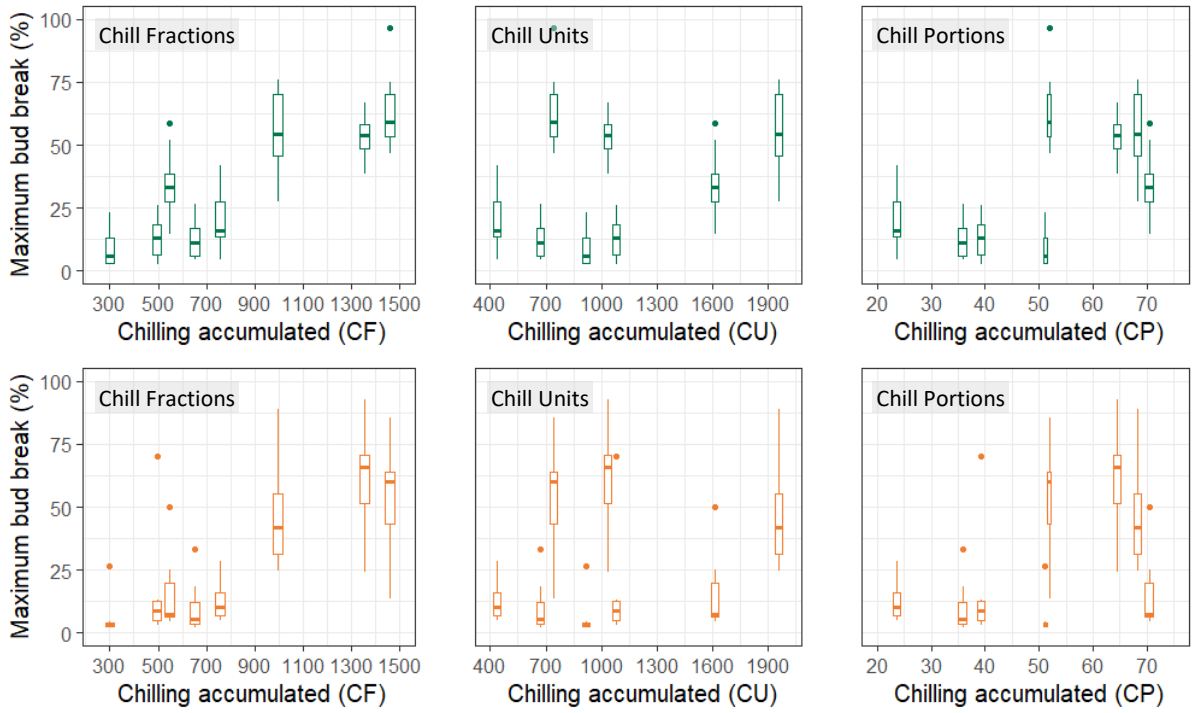


Figure 5.11 – Maximum percentage of bud break (%) in "Braeburn Lochbuie" (green) and "Galaxy Gala" (orange) as a response to Chilling accumulated in Chill Fractions (CF, *Malus model*, left), Chill Units (CU) (Utah model, centre, (Richardson et al., 1974)), and Chill Portions (CP) (Dynamic model, right, (Fishman et al., 1987)). Boxplots show median, interquartile range, whiskers expand from maximum to minimum values and outliers are represented as single dots.

Base temperature for heat accumulation after different chilling accumulations

Rate of progress to bud break increased with CF accumulation and warmer forcing temperature in both cultivars (Figure 5.12), with an observed maximum rate of progress to bud break of approximately 0.08 day^{-1}, after 1,400 CF and 22 °C. Greater differences between forcing temperatures were observed in "Braeburn Lochbuie" at all chilling accumulations, compared to "Galaxy Gala" (Figure 5.12). After 1,400 CF, the difference between the rate of bud break at 22 °C and 14 °C was 0.08 day^{-1} in "Braeburn Lochbuie", whilst it was only 0.03 day^{-1} in "Galaxy Gala".

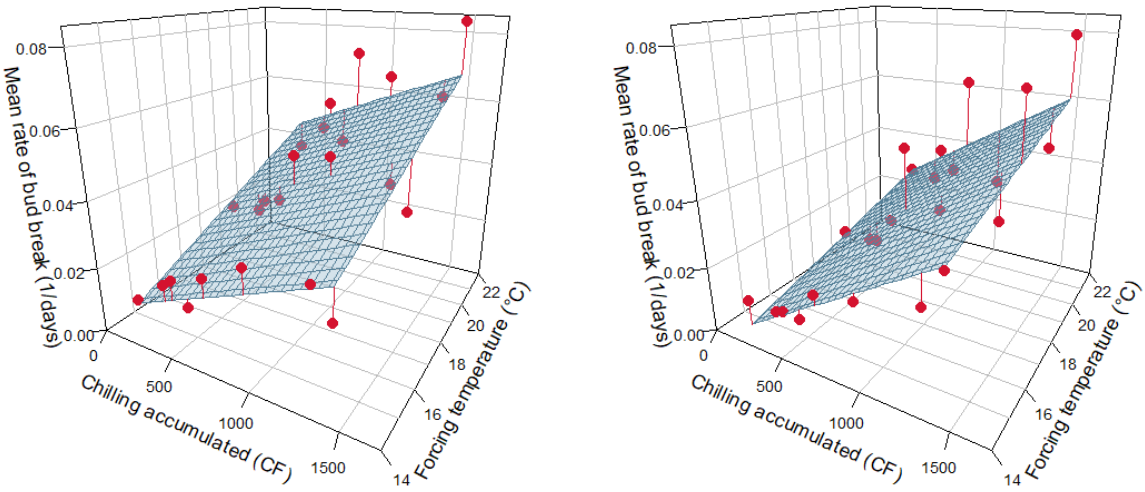


Figure 5.12 – Cultivar-specific 3D graphs (left: "Braeburn Lochbuie", right: "Galaxy Gala"), representing the observed (●) and modelled (blue surface) rate of progress to bud break (days^{-1}) as a response to chilling accumulated (CF, *Malus model*) and forcing temperature (°C).

Cultivar-specific generalised linear models showed that higher chilling accumulation and warmer forcing temperatures significantly increased rate of progress to bud break ($p<0.001$, Table 5.4). The interaction between both variables was not significant. The models developed explained approximately 62% ($R^2 = 0.6232$) of the variability in rate of progress to bud break in "Braeburn Lochbuie", and 53% in "Galaxy Gala" ($R^2 = 0.5201$). In "Braeburn Lochbuie", a 1 °C increase in forcing temperature accelerated rate of progress to bud break by 0.0036 day^{-1}, whilst this effect was reduced to 0.0025 day^{-1} in "Galaxy Gala". In contrast, the effect of chilling accumulation was slightly greater in "Galaxy Gala" than in "Braeburn Lochbuie" (Table 5.4).

Table 5.4 – Cultivar-specific generalised linear models on rate of progress to bud break, including chilling accumulated (CF) and forcing temperature. Results presented: variable estimates, standard error, t-value, p-value indicating significance level of each variable and overall model parameters including the adjusted R^2 and overall model significance. F value and degrees of freedom of numerator, denominator.

Cultivar	Variable	Estimate	Std. Error	t value	P-value	Model parameters
Braeburn Lochbuie	Intercept	-4.426e-02	7.126e-03	-6.211	1.46e-08 ***	Adj. R^2: 0.6232 p = <2.2e-16 F (2, 93) = 79.57
	Forcing temp	3.602e-03	3.592e-04	10.027	< 2e-16 ***	
	Chilling accumulated	2.480e-05	3.048e-06	8.136	1.76e-12 ***	
Galaxy Gala	Intercept	-3.621e-02	8.270e-03	-4.378	3.08e-05 ***	Adj. R^2: 0.5201 p = <2.2e-16 F (2, 95) = 53.56
	Forcing temp	2.498e-03	4.273e-04	5.847	7.03e-08 ***	
	Chilling accumulated	2.970e-05	3.545e-06	8.378	4.77e-13 ***	

Based on the methodology described in section 5.2.4.2 (*Combining chilling accumulation and forcing temperature*), the *Tb* for heat accumulation was calculated for both cultivars, for chilling accumulations from 800 to 2,000; at 100 CF intervals (Figure 5.13). As more chilling accumulated, the *Tb* for heat accumulation decreased in both varieties. After 800 CF, "Braeburn Lochbuie's" base temperature was 6.78 °C, and 4.98 °C in "Galaxy Gala". The base temperature decreased at a faster rate in "Galaxy Gala", reaching -9.28 °C after 2,000 CF, compared to -4.93 °C in "Braeburn Lochbuie" (Figure 5.13).

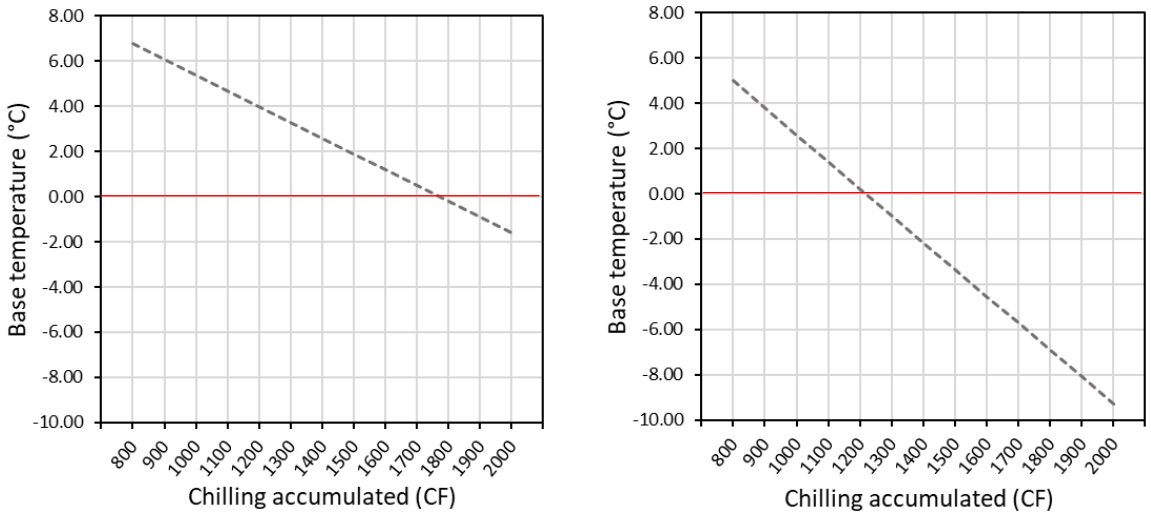


Figure 5.13 - Calculated base temperature (°C) for "Braeburn Lochbuie" (left) and "Galaxy Gala" (right), at different chilling accumulations (CF). Horizontal red line indicates 0 °C.

Minimum chilling and heat requirements for dormancy release

Only trees displaying at least 20% bud break by the end of the forcing period were used to explore the interaction between chilling and heat accumulation. When less than 20% bud break occurred, it was assumed that dormancy had not been fully released (see section *5.4 Discussion*). In "Braeburn Lochbuie", heat accumulation did not induce 20% bud break in trees that received less than 495 CF (or 1,080 CU and 39.2 CP), and less than 546 CF (1,611.5 CU and 70.4 CP) in "Galaxy Gala".

Although not very strong, a significant negative correlation between GDH to 20% bud break and CF accumulated was observed in both cultivars ($p<0.05$, Figure 5.14). A slightly higher correlation was noted in "Galaxy Gala" ($r = -0.4198$) compared to "Braeburn Lochbuie" ($r = -0.2882$). Therefore, in both cultivars, less heat was required to reach 20% bud break in trees chilled for 1,400 CF compared to those that only received 500 CF. However, some variability was observed in the data, particularly in "Galaxy Gala" (Figure 5.14).

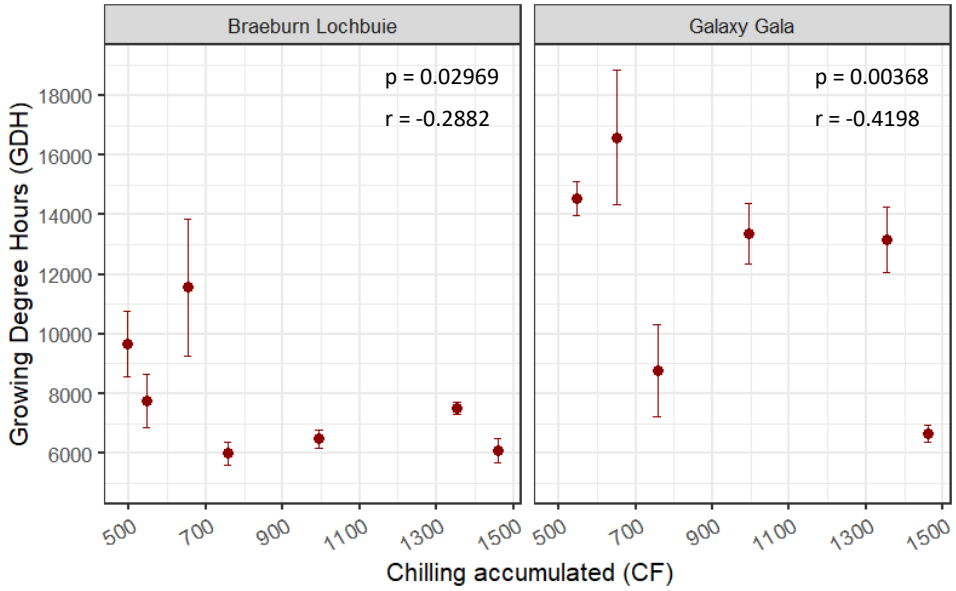


Figure 5.14 – Correlation between chilling accumulated (CF) and Growing Degree Hours (modified GDH model (Anderson et al., 1986)) to 20% bud break. Left: "Braeburn Lochbuie", right: "Galaxy Gala". Error bars represent ± 1 SE. Inside each panel: p-value and Pearson's correlation coefficient (r) for the cultivar-specific correlation.

Overall, "Braeburn Lochbuie" required less GDH to reach 20% bud break than "Galaxy Gala" throughout dormancy, with a difference of 1,000 GDH between cultivars after 1,400 CF. Cultivar differences were greater at the beginning of dormancy, when "Braeburn Lochbuie" required 9,000 GDH to reach 20% bud break compared to 14,500 GDH by "Galaxy Gala" (Figure 5.14).

The ratio of heating-to-chilling was calculated as described in equation (viii) (see section *5.2.4.3 Combining chilling and heat accumulation*). In "Braeburn Lochbuie", 20 times more heating than chilling could induce 20% bud break after less than 500 CF, whilst the ratio was more than 25 in "Galaxy Gala" (Figure 5.15). A sharp decrease was observed in both cultivars after 700 CF, with

"Braeburn Lochbuie" requiring seven times more heating than chilling, compared to more than 10 times in "Galaxy Gala". A ratio of less than 5:1 was observed in both cultivars after more than 1,400 CF (Figure 5.15).

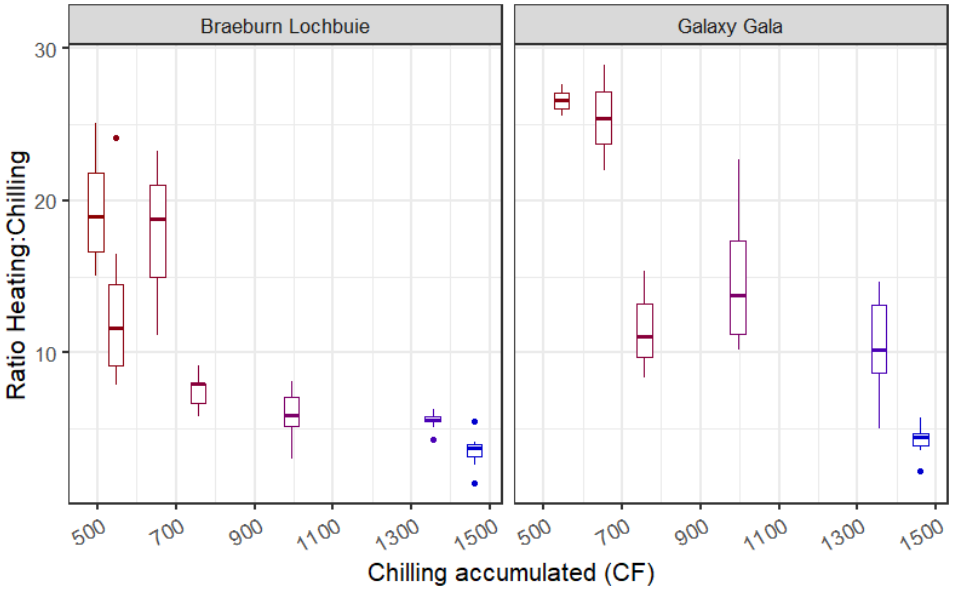


Figure 5.15 – Ratio of heat accumulated (modified GDH model (Anderson et al., 1986)) to 20% bud break by chilling accumulated (CF, *Malus model*), in relation to chilling accumulated (CF). Colour scale changes from red (low CF) to blue (high CF). Boxplots show median, interquartile range, whiskers expand from maximum to minimum values and outliers are represented as single dots.

5.4 Discussion

A different temperature response between cultivars

All existing bud break models use air temperature as the dependent variable to predict time of bud break, since it is the main factor affecting dormancy release and flower development. Photoperiod has a strong effect in some species (Li et al., 2003b; Fløistad and Granhus, 2010), but not in apple (Heide and Prestrud, 2005). As expected then, "Braeburn Lochbuie" and "Galaxy Gala" showed a strong response to temperature, with colder chilling and warmer forcing temperature increasing the rate and percentage of bud break in both cultivars. Temperature alone explained a large amount of the bud break variability in these varieties; however, in "Dabinett", the rate of progress to bud break was barely affected by temperature and only a limited effect was observed in percentage bud break.

It is possible that the reduced response in "Dabinett" is a consequence of an unusually high chilling requirement in this cultivar. Uneven flowering has been observed in "Dabinett" in the UK during recent years (Loraine Boddington, NACM, pers. comm.), which could be because chilling requirements have not been met. However, considering the chilling requirements of other apple cultivars (Hauagge and Cummins, 1991a; Parkes et al., 2020), it seems unlikely that none of the imposed temperature treatments provided sufficient chilling. Furthermore, "Braeburn Lochbuie" and "Galaxy Gala" showed a forcing temperature response even in treatments with insufficient

chilling. Other experiments with "Dabinett" (Chapter 3) also showed a weak response to forcing temperature, and a significantly different bud break pattern compared to other cultivars. Experiments with longer chilling and forcing periods would help to determine if the results observed here are a consequence of insufficient chilling, and if there is indeed a temperature response in this variety.

It is more likely that the reduced temperature response observed in "Dabinett" is a consequence of intrinsic physiological differences between cultivars. In an experiment investigating the carbohydrate content in buds of different apple cultivars (Chapter 6), "Dabinett" showed a higher sorbitol level, compared to other varieties tested. A high sorbitol to sucrose ratio in leaves has been previously correlated to late flowering (Escobar-Gutierrez and Gaudillere, 1994). Here, trees were pruned and defoliated at the beginning of the study, which could have affected their carbohydrate reserves (Breen et al., 2020). However, a study with "Royal Gala" trees showed that although early artificial defoliation reduced starch concentration in spurs during spring, the reduction did not affect flowering abundance or fruit set (Breen et al., 2020); and the immature trees used here had not cropped before the experiment, so it is unlikely that stored carbohydrate was a limiting factor. However, it is possible that pruning and defoliating had a greater effect on "Dabinett's" ability to bud burst; future experiments could explore this by comparing bud break in defoliated versus non-defoliated potted trees undergoing dormancy under natural conditions.

Until the molecular, biochemical and physiological mechanisms regulating bud break are fully elucidated and, most importantly, linked to observed bud break patterns in the field, it will be difficult to develop accurate models for cultivars exhibiting abnormal behaviours. This knowledge is also relevant to identify factors responsible for the remaining unexplained variability in bud break models of the other studied cultivars. Differences in how cultivars respond to chilling and heat accumulation are expected (Ruiz et al., 2007; Parkes et al., 2020), but such a weak response has not been reported before. It is difficult to hypothesise about the consequences of a reduced temperature response without knowing the cause; although a weaker temperature response could be advantageous under a climate change context, it might also be a sign that a much longer chilling period is needed, putting future fruit production at risk.

The Malus model is a better approximation of chilling temperature contributions

Results obtained here and in previous experiments (Chapter 4) were used to establish the parameters of the new *Malus* chilling model, which consisted of a chilling temperature range from -10 to 13 °C and an optimum temperature for chilling accumulation of -2 °C.

As discussed extensively in Chapter 4, previous dormancy studies on apple have reported a range of optimum temperatures for chilling accumulation (Thompson et al., 1975; Shaltout and Unrath, 1983; Naor et al., 2003; Guak and Neilsen, 2013). Some reports have highlighted the potential contribution

of below-zero temperatures (Naor et al., 2003; Harrington et al., 2010; Guak and Neilsen, 2013) but it has never been suggested as the optimum. Technical challenges associated with exposing trees to freezing temperatures have probably hindered this research, but results obtained here, and in Chapter 4, demonstrate that freezing temperatures are highly effective for chilling accumulation and should therefore be considered in chilling models.

Rate of progress to and percentage of bud break in all cultivars was highest in the -4/2 °C temperature treatment; whilst in Chapter 4, the optimum chilling temperature observed for "Braeburn Lochbuie" was 0.8 °C. In this previous experiment, 1,080 h of chilling had accumulated when the optimum temperature was determined, which increased as further chilling accumulated. Here, the rate of progress to bud break after 720 h in the -4/2 °C treatment was as high as in trees chilled at 1.75 °C for 1,080 h (Chapter 4), suggesting that the real optimum for "Braeburn Lochbuie" could be lower than previously suggested. Another explanation for the different results compared to Chapter 4 is that alternate day/night temperatures were used here, which could have had a promoting effect on bud break (Hänninen, 1990). No differences were observed in Chapter 4 between a constant chilling treatment at 4.5 °C and alternating temperature of 7/2 °C; however, -4 and 2 °C fluctuate around the optimum temperature, potentially having a stronger bud break inducing effect.

A study with excised shoots of "Gala" suggested an optimum temperature of 3.5 °C for this cultivar, with temperatures between -2 and 5.5 °C being the most effective for chilling accumulation (Guak and Neilsen, 2013). Long-term phenology data from NIAB EMR shows that in the UK, "Gala" flowers at a similar time to "Discovery", which has an optimum chilling temperature of -2.5 °C (Chapter 4). Whilst it is not known if there is a link between flowering time and optimum chilling temperature, this information together with the observed greater bud break response after the -4/2 °C treatment suggests a low optimum for this cultivar, thus -2 °C was also selected.

The upper and lower temperature thresholds of the *Malus model* were selected based on previous results obtained with "Braeburn Lochbuie" (Chapter 4), where it was noted that rate of progress to bud break at -4 ° was significantly higher than 10 ° C, the extremes of the temperature range imposed. This observation suggested that the low temperature threshold was lower than -4 °C. Previous studies have shown a greater accuracy of blooming predictions when negative temperatures down to -5 °C were included in chilling models (Harrington et al., 2010; Kaufmann and Blanke, 2019). Rate of progress to bud break in trees chilled at 10 °C was not significantly higher than in trees that did not receive any chilling, and percentage of bud break was very low (Chapter 4). Here, percentage of bud break in trees chilled at 7/13 °C was below 10%, even after 1,776 h of chilling. Whilst existing models suggest a higher upper-temperature threshold for chilling accumulation (14 °C in the Dynamic model (Fishman et al., 1987), 15 °C in the Utah model

(Richardson et al., 1974)), based on the results described here, 13 °C was chosen as the upper-temperature threshold.

The highest chilling accumulation calculated with the Utah (Richardson et al., 1974) and Dynamic (Fishman et al., 1987) models were not achieved in those treatments with a higher rate and percentage of bud break observed, suggesting that chilling temperature contributions with these models are inaccurate. Furthermore, the highest CP and CU accumulated with the Dynamic and Utah models, respectively, should be sufficient to release endodormancy in most existing apple cultivars (Hauagge and Cummins, 1991a; Parkes et al., 2020); however, a lower rate and percentage of bud break were observed here compared to other treatments.

It is important to highlight that the *Malus model* needs to be validated with an external dataset to assess its accuracy; and that cultivar-specific calibration is required, as a better fit was obtained in "Braeburn Lochbuie", results from which were used to establish chilling thresholds in the *Malus model*, compared to "Galaxy Gala". However, the results indicate that the proposed model is a better representation of the temperatures contribution to chilling accumulation than those suggested by the Utah (Richardson et al., 1974) or the Dynamic (Fishman et al., 1987) models.

A new methodology to define the interaction between chilling and heat accumulation

In the proposed method to predict time of bud break, the rate of progress to bud break was modelled by combining the *Malus chilling model* with a thermal time approach (Arnold, 1959) used to calculate *Tb* for heat accumulation. To our knowledge, this is the first attempt to simultaneously investigate the effect of a range of chilling and heat accumulation temperatures, to try to overcome the limitation inherent in parallel models that do not account for different temperature contributions towards heat and chilling accumulation (Landsberg, 1974; Cannell and Smith, 1983). Whilst growing evidence indicates that chilling and heat might accumulate simultaneously (Guo et al., 2014, 2015a; Pope et al., 2014; Darbyshire et al., 2017), it is challenging to explore this in controlled environments as there can only be one temperature at any given time. However, the combined effect of chilling and heat, occurring successively, was investigated.

Bud break models are usually developed by assessing the effect of different temperatures on time of bud break or growth, albeit measured in different ways (Dennis, 2003); but no studies have considered the rate of progress to bud break in apple (but see Hadley et al., 1983). The modelling approach proposed here is based on the x-intercept method, which assumes a linear relationship between temperature and rate of development (Arnold, 1959), as observed here. This linear relationship was used to calculate the base temperature for heat accumulation, after different amounts of chilling. Whilst the x-intercept method (Arnold, 1959) has been widely used in crop growth models (i.e. Ellis et al., 1986; Steinmaus et al., 2000; Hadley et al., 1983), some have argued that it is inappropriate as it is based on extrapolating the results of a regression model (Yang et al.,

1995). However, this method avoids the need to pre-define base temperatures as is done in other methods (Yang et al., 1995; Snyder et al., 1999; Zapata et al., 2015). Estimating *Tb* correctly is important as heat accumulation will vary with time depending on this parameter. Here, *Tb* decreased as further chilling accumulated, creating a model where heat requirements are met sooner if there is a longer chilling period, as previously observed (Ruiz et al., 2007; Darbyshire et al., 2013; Guo et al., 2014).

In physiological terms, the base temperature of a thermal time system is usually defined as the temperature below which no development can be detected. Here, *Tb* of both cultivars declined as further chilling accumulated, and negative values were observed after 1,800 CF in "Braeburn Lochbuie" and 1,200 CF in "Galaxy Gala". Below zero values are difficult to interpret physiologically as it is generally accepted that development in plants does not occur at these temperatures (Ritchie and Nesmith, 1991); but other studies using long-term datasets to estimate base temperatures have reported negative values (Zhang et al., 2015). Furthermore, it was observed in Chapter 4 that temperatures below zero significantly increased rate of progress to bud break, even after fulfilling chilling requirements. As first argued by Arnold (1959), discussions over the physiological relevance of *Tb* may neither be necessary or fruitful, and instead, it should perhaps be considered as a parameter that gives the least variability in heat accumulation.

Whilst the forcing temperatures used to develop this thermal time relationship are higher than current mean spring temperatures in the UK, they are appropriate in a climate change context as temperatures are predicted to increase in the coming decades (Murphy et al., 2018). An assumption of linearity has been made outside of the temperature range considered here, although it has been suggested that the relationship between temperature and rate of development might not be linear at temperature extremes (Ritchie and Nesmith, 1991). In future work, lower forcing temperatures should be investigated to ensure this assumption is accurate; a longer forcing period would be required to explore this effect, which could not be achieved in the current PhD programme because of time restrictions.

Some accountability of non-linearity has been considered in the approach presented here as a modified GDH model (Anderson et al., 1986) has been used to quantify heat accumulation to 20% bud break, using the base temperatures obtained at each chilling accumulation. Developed with peach trees, it is unclear how parameters of the GDH model were selected as no experiments comparing different temperatures were reported (Richardson et al., 1975; Anderson et al., 1986). Different studies have achieved more accurate results by changing these parameters (Parker et al., 2011; Zhang et al., 2015). In a long-term phenology study with pome fruit in Australia, Darbyshire et al., (2013) tested base temperatures for heat accumulation within the 4-12 °C range, and found the best model fit occurred between 4-8 °C, depending on the cultivar. They reported a better model fit predicting blooming dates, compared to green-tip, and suggested a potential non-linear response of

green tip to temperature as an explanation. I hypothesise that the lower fit observed by Darbyshire et al., (2013) in the green-tip stage is possibly the result of inadequate model parameters in either the chilling or GDH models used. Here, the *Tb* was adjusted depending on the previous chilling accumulated, whilst the optimum temperature from the GDH model (Anderson et al., 1986) was not modified. Although this value should also be calibrated at a cultivar level, calculations of heat accumulation were only carried out for temperatures below the optimum, and therefore any inaccuracies with this parameter would have no impact on the results obtained.

Another limitation of existing bud break models is that they require pre-defined dates for the start and/or end of chilling accumulation (see section *2.4.4 Selecting starting dates for chilling and heat accumulation*). These are often selected based on arbitrary decisions such as the date of first frost day, leaf fall or when a sudden decrease in temperatures occurs (Richardson et al., 1974; Cannell and Smith, 1983; Fishman et al., 1987). Most modern approaches include Partial Least Squares (PLS) analyses to determine chilling and heat phases from long-term phenology and temperature records (Luedeling et al., 2013; Guo et al., 2015a), but these require many years of data and rely on existing chilling and heat models. Whilst a decision on the starting date for chilling accumulation is still required with the model developed here, an end date for chilling accumulation is not needed as the model can determine the rate of progress to bud break according to a range of chilling accumulations.

Results obtained here should not be extrapolated to different temperature regimes, cultivars, or growing conditions; and it is crucial to test this model with external datasets including climate and apple phenology data from orchard-grown trees, to assess its validity and effectiveness. However, considering these limitations, the approach described here constitutes the first attempt to incorporate the interaction between chilling and heat accumulation in a single model in apple, by adapting *Tb* for heat accumulation according to previous chilling accumulated.

Chilling and heat accumulation safe thresholds

Chilling and heat requirements to reach 20% bud break were calculated to investigate if reduced chilling can be totally or partly substituted with increased heat accumulation, as previously suggested in sweet cherry (Kaufmann and Blanke, 2019). It was assumed that trees exhibiting less than 20% bud break at the end of the forcing period had not received sufficient chilling. Whilst 20% might seem a low threshold, low percentages of bud break in dormancy studies with potted trees are common (Cook and Jacobs, 2000; Naor et al., 2003), and possible reasons, such as the effect of apical dominance (Faust et al., 1995; Cook and Jacobs, 1999) were discussed in Chapter 3. Previous studies have chosen similar thresholds as a measure of dormancy break (Cook and Jacobs, 2000; Naor et al., 2003; Ruiz et al., 2007; Campoy et al., 2012).

Surprisingly, whilst a significant negative correlation between chilling and heat was observed, it was not as strong as in previous studies (Cannell and Smith, 1983; Ruiz et al., 2007). A possible explanation is that there was not enough variability in the data to clearly identify this relationship, as only eight levels of chilling accumulation were available and only three forcing temperatures were used. Whilst these provided different amounts of heat accumulation, the forcing period was limited to 70 days, potentially masking the effect of longer heat accumulation.

Nevertheless, results showed that lower chilling levels were compensated by higher heat accumulations, but a minimum amount of chilling was required in both varieties, as previously observed in sweet cherry (Kaufmann and Blanke, 2019). Here, a minimum of 495.2 CF was required in “Braeburn Lochbuie” and 546.2 CF in “Galaxy Gala”, reflecting the lower chilling requirements previously suggested in “Braeburn” (Jackson, 2003), compared to “Galaxy Gala” (Chapter 3 and Parkes et al., 2020). Heat-to-chill ratios presented provide information on safe chilling thresholds, which could be valuable under future climate change scenarios, assuming heat accumulation will not be a constraint. After 1,400 CF, a ratio of 5:1 released dormancy and induced bud break in both cultivars, but any chilling above 500 CF could be compensated by longer and/or warmer forcing periods.

Whilst it appears that after limited chill accumulation, longer and warmer forcing periods can release dormancy and promote the same percentage of bud break; the impacts of lower chilling levels on flowering and fruit quality (Petri and Leite, 2004) were not considered here. Further experiments should investigate the effect of heat-to-chilling ratios on flowering, fruit yield and quality.

Chapter 6

Winter carbohydrate dynamics in buds and xylem sap of different apple cultivars

6.1 Introduction

Temperate woody perennials like apple, accumulate carbohydrates before winter, often in the form of starch. To support resumption of bud growth in spring, starch is degraded into soluble sugars and transported to the growing buds (Bonhomme et al., 2010; Tixier et al., 2017) until leaves become source organs (Loescher et al., 1982; Naschitz et al., 2010).

Carbohydrate storage is linked to frost tolerance and key for surviving winter months (Sauter et al., 1996). Seasonal changes in carbohydrate concentrations have been observed in several species (Yoshioka et al., 1988; Rinne et al., 1994b; Sivaci, 2006), and many studies have suggested a close link between carbohydrate dynamics and dormancy progression (Ito et al., 2012; Kaufmann and Blanke, 2017; Fernandez et al., 2019). Furthermore, lack of chilling can alter carbohydrate dynamics in buds (Bonhomme et al., 2005) and reduce sugar levels in xylem sap of fruit trees (Ito et al., 2013).

Although buds remain isolated during dormancy, plasmodesmata communication is restored when chilling requirements are fulfilled (Rinne et al., 2001). After that, plants import sugars into the buds from other areas of the plant, required for bud burst and development (Tixier et al., 2017). As phloem activity becomes highly reduced during winter, xylem sugar transport plays a major role to ensure bud growth in spring (Loescher et al., 1990; Decourteix et al., 2008; Ito et al., 2012). The type and amount of carbohydrates detected during winter varies between species and plant part considered, with some studies highlighting the role of hexose (Fernandez et al., 2019), and others focusing on sorbitol (Ito et al., 2012).

Because buds remain isolated during dormancy (Rinne et al., 2001), many studies have focused on investigating carbohydrate dynamics inside the buds. Overall, levels of soluble carbohydrates in buds and floral structures appear to be highest during endodormancy, and then decline before bud break; this has been observed in pear (Ito et al., 2012), sweet cherry (Kaufmann and Blanke, 2017; Fernandez et al., 2019), walnut (Bonhomme et al., 2010) and peach (Bonhomme et al., 2005), amongst others. Starch levels remain low during dormancy (Bonhomme et al., 2010; Ito et al., 2012; Kaufmann and Blanke, 2017); some studies detected a decrease during winter (Fernandez et al.,

2019) and others identified an increase before bud break (Bonhomme et al., 2010). In peach stems, a delay in starch hydrolysis due to lack of chilling was observed (Bonhomme et al., 2005).

Xylem transport plays a key role during winter (Loescher et al., 1990; Decourteix et al., 2008; Ito et al., 2012) and carbohydrates in xylem sap have been linked to freezing tolerance and embolism restoration (Améglio et al., 2000, 2004). Whilst most higher plants transport carbohydrates primarily in the form of sucrose, in plants from the *Rosacea* family, such as apple, sorbitol is the main photosynthetic product and their main translocatable carbohydrate (Webb and Burley, 1962; Bieleski, 1969). Sorbitol is a sugar alcohol, also known as polyols (Kanayama, 2009), and can be synthesised from hexoses (Moing, 2000 and references within) or starch (Loescher et al., 1990). Sorbitol levels in xylem sap peak during endodormancy and lower sorbitol levels in pear have been linked to insufficient chilling (Ito et al., 2013). In apple, sorbitol concentration increased in shoots exposed at lower temperatures (Raese et al., 1977).

Whist a correlation between sugar levels and dormancy progression has been observed in various fruit tree species (Bonhomme et al., 2010; Ito et al., 2012; Fadón et al., 2018; Fernandez et al., 2019), winter carbohydrate dynamics have been proposed as a mechanism to distinguish dormancy phases only in sweet cherry buds (Kaufmann and Blanke, 2017). However, this study did not explore carbohydrate concentrations in other parts of the plant with a central role during growth resumption, such as xylem sap (Loescher et al., 1990; Decourteix et al., 2008; Ito et al., 2012).

In this Chapter, winter carbohydrate dynamics in floral buds (eight cultivars) and xylem sap (three cultivars) were investigated to study the possibility of using changes in carbohydrate concentrations to distinguish dormancy phases in apple and, ultimately, to differentiate varieties with different chilling requirements. Cultivars were selected based on commercial importance and to include a range of chilling requirements, particularly “Anna”, known for being a very low-chill variety (Hauagge and Cummins, 1991a).

6.2 Materials and methods

Two experiments were carried out between October 2020 and March 2021. In the first experiment, the dynamics of carbohydrate concentrations and Relative Water Content (RWC) in floral buds were determined (section 6.2.1) and in the second, carbohydrate concentrations in apple bark and xylem sap were quantified (section 6.2.2). A preliminary experiment in which the sampling methodology was developed and refined was carried out in 2019/20; outputs from this work are presented in Figure A7 - Appendix A.

6.2.1 Carbohydrate concentrations and RWC in floral buds

6.2.1.1 Plant material, floral buds' collection and preparation

Buds on spurs of nine apple cultivars were sampled in this experiment: "Anna", "Bramley", "Braeburn Mariri Red", "La Vera Cox", "Fuji Aztec", "Galaxy Gala", "Jonagold Robijn", and two cider varieties, "Dabinett" and "Kingston Black". All the trees that were sampled, with the exception of "Anna", were the same trees used for work described in Chapter 3. Trees from all cultivars except "Anna" were sampled from an orchard at NIAB EMR, East Malling, south-east England (51.287089, 0.445985); and were grafted on "M9" rootstock and planted out in 2014. "Anna" was grafted on "M27" rootstock and planted out in 1995, in the NIAB EMR gene bank collection approximately 100 m away from the other varieties.

Due to limited availability of some cultivars, a different number of trees per variety was used: one "Anna" tree, two trees per cider variety, and ten trees from all other cultivars. All trees were labelled at the beginning of October 2020. In dessert cultivars where multiple trees were available, ten trees of similar size and number of growing spurs were selected, and trees showing signs of diseases and on edge rows were avoided.

Table 6.1 - Dates of bud, bark and sap collections, and accumulated chilling and heat on each sampling date. Chilling was calculated in Chill Units (CU) (Utah model (Richardson et al., 1974)) and Chill Portions (CP) (Dynamic model (Fishman et al., 1987)). Heat accumulated was calculated in Growing Degree Hours (GDH) (Anderson et al., 1986).

Experiment number	Samples collected	Collection number	Collection date	CU accumulated	CP accumulated	GDH accumulated
1	Buds	1	22/10/2020	0	28.56	0
1	Buds	2	12/11/2020	110.5	38.55	0
1	Buds	3	30/11/2020	385.5	47.75	0
1	Buds	4	14/12/2020	646.5	54.08	0
1	Buds	5	08/01/2021	992.5	76.87	0.81
1	Buds	6	21/01/2021	1188	86.11	278.50
1	Buds	7	06/02/2021	1470.5	99.87	765.05
2	Sap	1	01/12/2020	409.5	50.75	0
2	Bark and sap	2	15/12/2020	667.5	63.29	0
2	Bark and sap	3	22/12/2020	771	69.32	0
2	Bark and sap	4	06/01/2021	987	76.34	0.81
2	Bark and sap	5	20/01/2021	1164	85.34	259.70
2	Bark and sap	6	03/02/2021	1403.5	96.77	669.65

Floral buds growing on spurs were collected on seven occasions between October 2020 and February 2021 (Table 6.1). The collection of samples was carried out between 9.00-11.00 h. At each time point, a total of 10-40 buds were collected per cultivar from the selected trees. Buds were sampled from branches between 1 and 1.8 m height, sealed in a plastic bag and transported to the

laboratory for processing. In the laboratory, bud scales were carefully removed (Figures 6.1 and 6.2). Buds were weighed, immersed in liquid Nitrogen, and stored at -80 °C until carbohydrate analyses. The time between sample collection and storage was approximately 1 h.

Air temperature in the experimental orchard was recorded hourly using an Adcon system with telemetry installed on site and data were acquired with addVANTAGE Pro 6.4 Software.

Figure 6.1 – Bramley floral buds after collection 7. Photo taken on 6/2/2021.

Figure 6.2 – Bramley floral buds after removing bud scales. Photo taken on 6/2/2021.

6.2.1.2 Carbohydrate analyses and RWC determination in buds

Bud samples were freeze-dried then immediately weighed, and RWC was calculated using the equation:

Relative water content (%) = $\frac{fresh\ weight - dry\ weight}{dry\ weight} * 100$

Flower buds were then ground using a pestle and mortar and prepared for the analysis of starch and soluble sugars according to Kaufmann and Blanke (2017), with some modifications.

Powdered samples were diluted in distilled water (0.5 g DM in 10 ml HPLC-grade H_2O or 0.3 g DM in 6 ml HPLC-grade H_2O) and the mixture was stirred at room temperature for 1 h, incubated in a heated water bath at 60 °C for 1 h, and then centrifuged at 4500 g for 30 min (Centrifuge Sigma 4-16 kg Henderson Biomedical, UK). The pellet was used for starch analyses and the supernatant was analysed for fructose, sorbitol, glucose and sucrose. Carbohydrate concentrations were quantified using a high-performance liquid chromatograph (HPLC) (Alliance e2695, Waters, UK) with a refractive index detector, an Amino Column (Luna 5µm NH2 100A, 250 x 4.6mm) set to 40 °C, and a mobile phase of 80:20 Acetonitrile (MeCN): water at a flow rate of 1.8 ml/min.

For starch analyses, the pellet was mixed with 5 ml H_2O, stirred for 10 min, and centrifuged for 15 min, after which the supernatant was discarded. The process was repeated a second time, but 7 ml of H_2O were added, and the sample was centrifuged for 25 min. After discarding the supernatant, pellets were rehydrated with 5 ml of HPLC-grade H_2O; each sample was manually shaken to ensure that pellets were fully dissolved in the water. Samples were incubated in a heated water bath at 100 °C for two hours. After cooling, 2 ml of acetate buffer (0.2 M pH 4.6) and 50 µL of a solution

containing 250 mg of amyloglucosidase in 10 ml of acetate buffer were added to each sample. Tubes were stirred manually and then placed in a water bath at 60 °C for 24 h. Finally, samples were centrifuged at 4500 g for 15 min. The supernatant was analysed for soluble sugars as described above. The quantification of carbohydrates was adjusted according to the initial sample weight and subsequent dilution.

6.2.2 Carbohydrates in apple bark and xylem sap

6.2.2.1 Plant material, xylem sap extraction and bark preparation

Bark and xylem sap samples were collected from apple cultivars “Galaxy Gala”, “Braeburn Hillwell” and “Bramley” grafted on “M9” rootstock at NIAB EMR (51.287089, 0.445985). Trees were planted in 2009, with 3.5 m between rows and 1 m within row spacing. Trees were irrigated with trickle irrigation with one 2 L/h dripper per tree; irrigation was scheduled by the NIAB EMR Farm Irrigation Manager.

In October 2020, ten trees per cultivar were selected based on similar canopy volume and extent of new growth, avoiding trees showing signs of diseases and those on edge row. Between December 2020 and February 2021, bark and xylem sap were collected fortnightly; a total of six sap and five bark collections were carried out (Table 6.1). At each collection day, a one-year-old branch growing between 1.20 and 2 m high was collected per tree. All branches were between 40-90 cm length and 0.7-1.5 cm thickness at the base. Branches were placed in a plastic bag to avoid desiccation and taken to the laboratory, 5 min away, for sample preparation. Only five branches were collected at a time to minimise the time between collection, sample processing and storage at -80 °C, which was between 30 min and 1 h.

Xylem sap was extracted using the method developed by Bollard (1953). For each branch, an 8 cm length of bark and phloem at the base of the cut branch was removed with a scalpel. The branch was pushed through a previously perforated rubber bung, ensuring 2 cm of the branch base extended below the bung to enable sap to be extruded (Figure 6.3). An air-tight fit between the branch and the bung was required in order to create a strong vacuum. The bung and the branch were inserted into a metal cylinder with a narrow side tube; and a screw cap plastic bottle for sap collection was inserted in the bottom hole (Figure 6.4). Using a plastic tube connecting the side tube of the cylinder to a vacuum pump (Figure 6.5), a vacuum was applied and then a *ca.* 4 cm piece of the distal part of the branch was cut using sharp secateurs to release xylem tension, which resulted in xylem sap dripping from the proximal cut surface. The 4 cm piece was discarded. Approximately 4-cm-sections of branch were cut at regular intervals (2-5 seconds) (Williams and Raese, 1974), which maintained a flow of xylem sap from the proximal end of the branch.

The 4 cm branch sections cut during sap extraction were collected for later analyses of soluble sugars in the bark. Only the internode sections of the branch were collected and a minimum distance of 0.5 cm was left from the nearest bud (Figure 6.6). Two pieces of internode bark were collected from each branch.

Xylem sap or bark from branches of the same cultivar were combined to ensure sufficient sample volumes for laboratory analyses. Samples were immersed in liquid Nitrogen immediately after collection and stored at -80 °C until carbohydrate analyses.

Figure 6.3 - Branch with phloem removed and inserted in a rubber bung for sap extraction. Photo taken on 18/03/2020.

Figure 6.4 - Metal cylinder with bottle for sap extraction. Photo taken on 18/03/2020.

Figure 6.5 - Set up for xylem sap extraction. Photo taken on 25/11/19.

Figure 6.6 – Example of piece used for bark carbohydrate analyses. Photo taken on 10/12/2020.

6.2.2.2 Carbohydrate analyses of bark and xylem sap

Soluble sugars in xylem sap were extracted according to Ito et al., (2012) and Kaufmann and Blanke (2017), with some modifications. Frozen sap samples were defrosted in a water bath at 25 °C for approximately 1 h, and after agitation, 1 ml aliquots of sap were combined with 0.01 g of polyvinylpolypyrrolidone (PVPP) to remove phenolic compounds. Sugars were then extracted using the procedure described for floral buds.

The same protocol described for floral buds was followed to quantify sugar and starch concentrations in bark samples, however, bark tissue was first ground using an electric grinder.

6.2.3 Data analyses

All data analyses and graphs were performed using R statistical software (R Core Team, 2021).

In apple buds, results from all cultivars, except “Anna”, were combined to investigate differences in the concentration of each carbohydrate between sampling dates. If an ANOVA showed significant differences ($p<0.05$), a post-hoc Duncan’s Multiple Range Test was applied to identify which sampling dates had significantly different sugar concentrations. “Anna” was not included in these analyses as less data was available for this cultivar, due to the limited number of trees.

For each collection date, chilling and heat accumulation were calculated using the R package *chillR* (Luedeling, 2021) (Table 6.1). To enable comparison with previous studies, chilling was calculated according to two existing models: Chill Units (CU) from the Utah Model (Richardson et al., 1974), and Chill Portions (CP) from the Dynamic Model (Fishman et al., 1987). The start date of chilling accumulation was selected based on the methodology established by each model (see section *2.4.4 Selecting starting dates for chilling and heat accumulation*), CU began to accumulate on 2 November 2020, and CP began on 1 September 2020.

Heat accumulation was calculated as Growing Degree Hours (GDH) (Anderson et al., 1986) and the starting date was set at the beginning of ecodormancy (Lang et al., 1987). The time of progression from endo to ecodormancy was established based on times of bud break recorded in previous experiments (see Chapter 3).

6.3 Results

6.3.1 Carbohydrates and relative water content dynamics in floral buds

Carbohydrate dynamics during dormancy in floral buds of nine apple cultivars

Sucrose was not detected in apple bud tissue throughout dormancy, but changes in concentrations of fructose and glucose (hereafter referred to as hexoses) followed a similar pattern across all cultivars during winter (Figure 6.7). Concentration of hexoses remained stable until the end of November in most cultivars (Figure 6.7), although a slight significant increase ($p<0.05$) was observed when all varieties where combined (Figure 6.8A and 6.8B). A significant increase was observed in all cultivars on 14 December ($p<0.05$), reaching a maximum of approximately 20-25 mg/g of fructose and 20 mg/g of glucose on 8 January 2021 (Figure 6.8A and 6.8B). A significant decrease in fructose ($p<0.05$) was detected between 8 and 21 January (Figure 6.8A), although this was more evident in some cultivars ("Anna", "Braeburn Mariri Red", "Bramley", "Galaxy Gala") than others (Figure 6.7). A reduction in concentration of glucose ($p<0.05$) was also observed at the end of January but this change was more gradual (Figure 6.8B).

The concentration of starch in floral buds followed the opposite pattern to hexoses in all cultivars investigated (Figure 6.7). During autumn, starch concentrations remained relatively constant (*ca.* 10 - 15 mg/g) but a significant decrease ($p<0.05$) was detected between 30 November and 14 December (Figure 6.8D). No starch was detected in the following collections in some cultivars; whilst starch levels remained above zero in other varieties (i.e. "Jonagold Robjin", "Dabinett") (Figure 6.7). An increase in starch was observed on 8 February 2021 in all cultivars except "Bramley", "Braeburn Mariri Red" and "Kingston Black" (Figure 6.7).

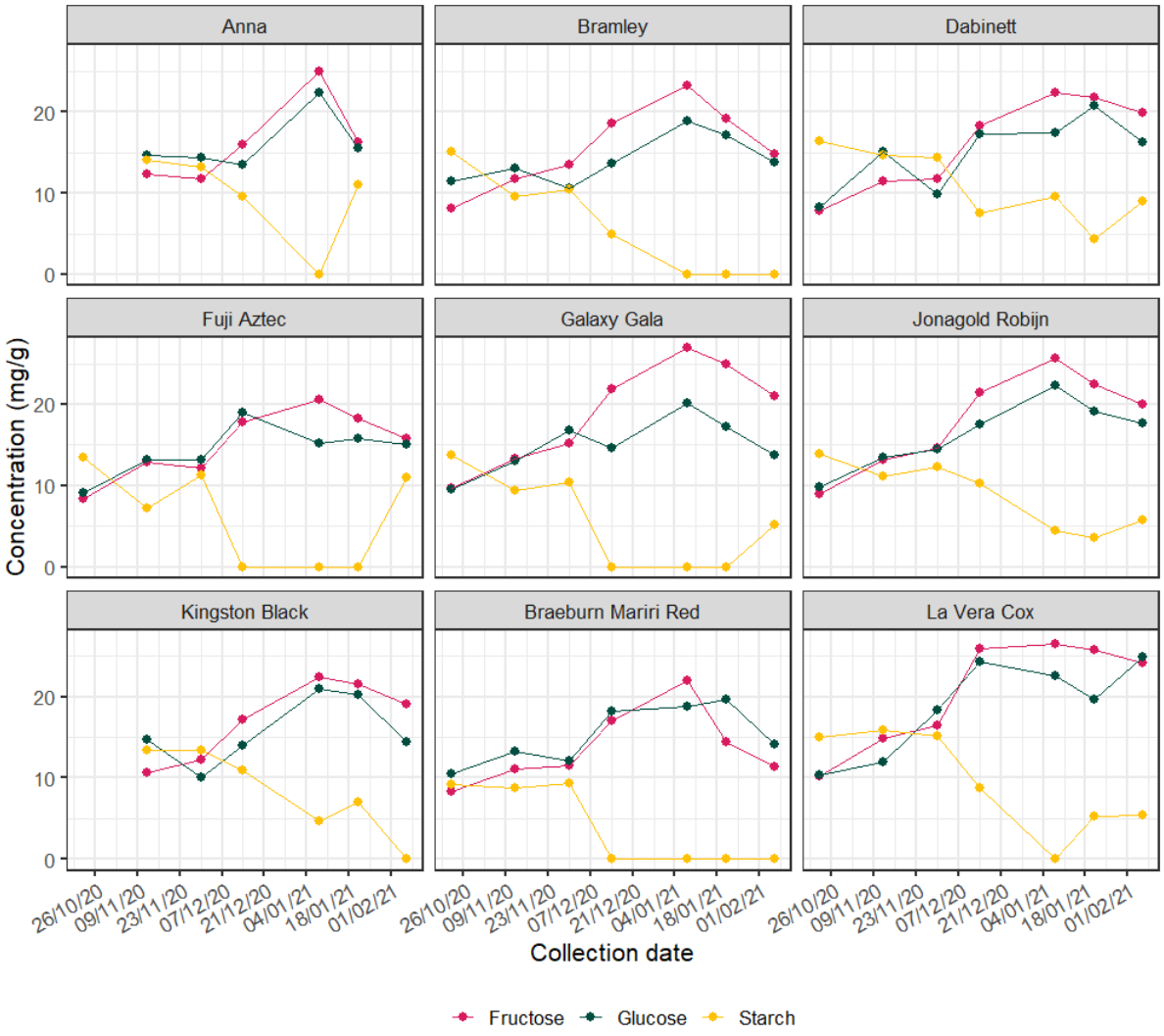


Figure 6.7 – Concentration of fructose, glucose and starch (mg of carbohydrate per gram of dry bud) in floral buds of nine apple cultivars during dormancy progression.

Sorbitol was the most abundant carbohydrate in flower buds of all cultivars and remained above 50mg/g throughout the time of study (Figure 6.9). In most cultivars, bud sorbitol concentrations remained stable throughout winter; the exception was “Fuji Aztec” where fluctuations were detected. Sorbitol concentration was highest in “Dabinett” throughout dormancy, compared to all other cultivars, peaking at almost 100 mg/g on 21 January 2021 (Figure 6.9). A gradual increase was detected in the last two collections in some cultivars (i.e., “Galaxy Gala” and “La Vera Cox”) (Figure 6.9).

When results from all cultivars were combined, no significant differences in sorbitol concentration were observed between collection dates (Figure 6.8C). In most cultivars, mean sorbitol concentration remained around 60 mg/g during winter and a slight not-significant decrease was only observed between 12 and 30 November 2020 (Figure 6.8C).

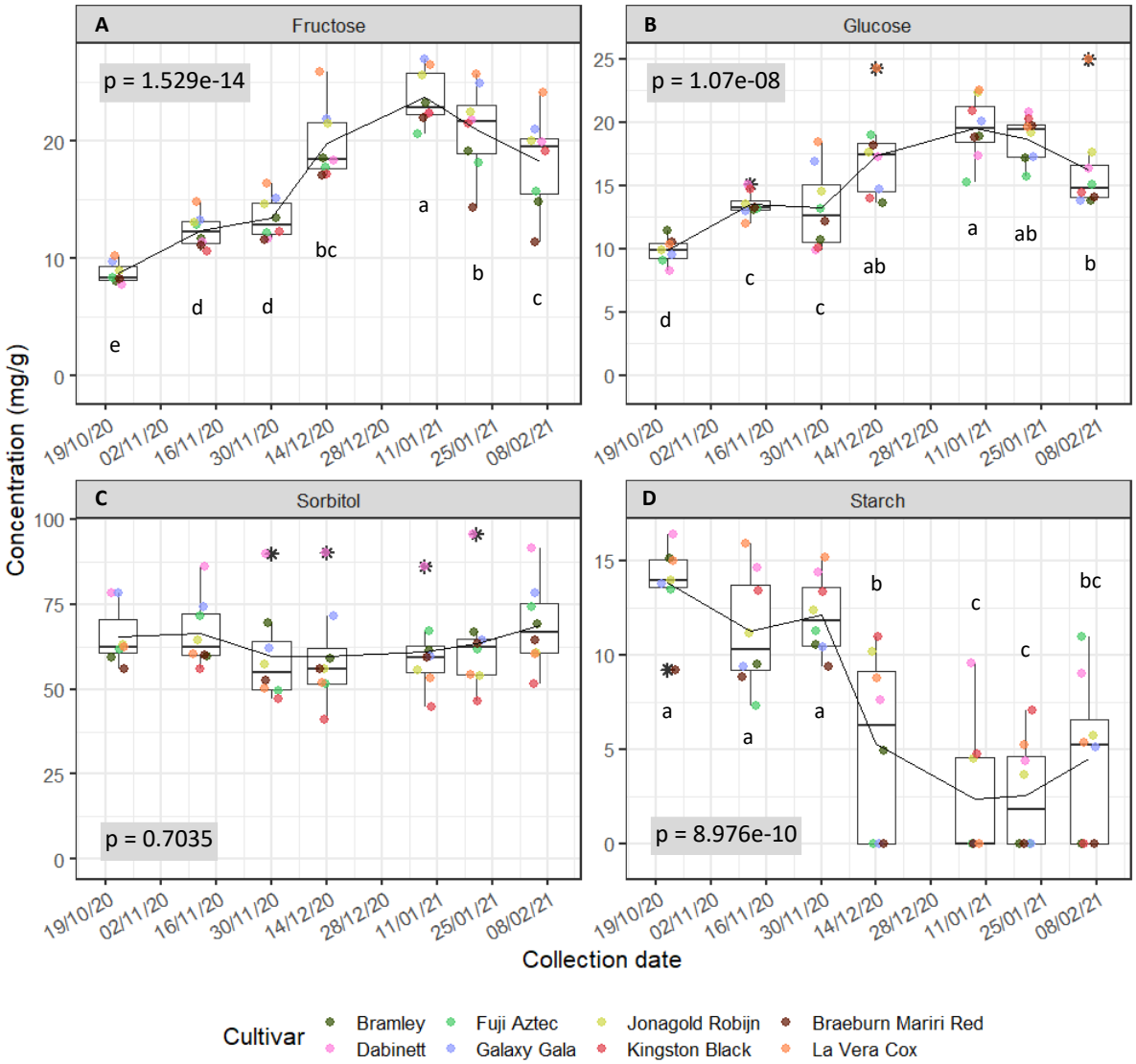


Figure 6.8 – Mean concentration (mg per gram of dry bud) of carbohydrates (A = Fructose, B = Glucose, C = Sorbitol, D = Starch) in buds of eight apple cultivars throughout dormancy. Boxplots show the median, first and third quartile; (*) are outliers and colour dots represent each cultivar. An ANOVA was used to determine differences between sampling dates for each carbohydrate type; significance is indicated with a p-value inside each box. Letters above or below boxplots indicate significant differences ($p<0.05$) between dates within each sugar type as determined with a post-hoc Duncan's multiple-range test.

Relative water content in floral buds during dormancy

Relative water content remained around 55% until February 2021 in all cultivars except "Anna" and "Kingston Black". In "Anna" buds, RWC was 60% until 14 December, after which it increased sharply reaching more than 65% on 21 January 2021 (Figure 6.10). In the field, buds of this cultivar were open on 6 February 2021 (Table B5 – Appendix B) so no samples were taken on the final collection date. A lower RWC was detected in "Kingston Black" throughout the study, which fell below 50% at the beginning of January (Figure 6.10).

An increase of 8% in RWC was observed in February in "Galaxy Gala", whilst <5% increase was measured in "Bramley", "Dabinett", "Fuji Aztec", "Jonagold Robjin", "Braeburn Mariri Red" and "La Vera Cox" (Figure 6.10).

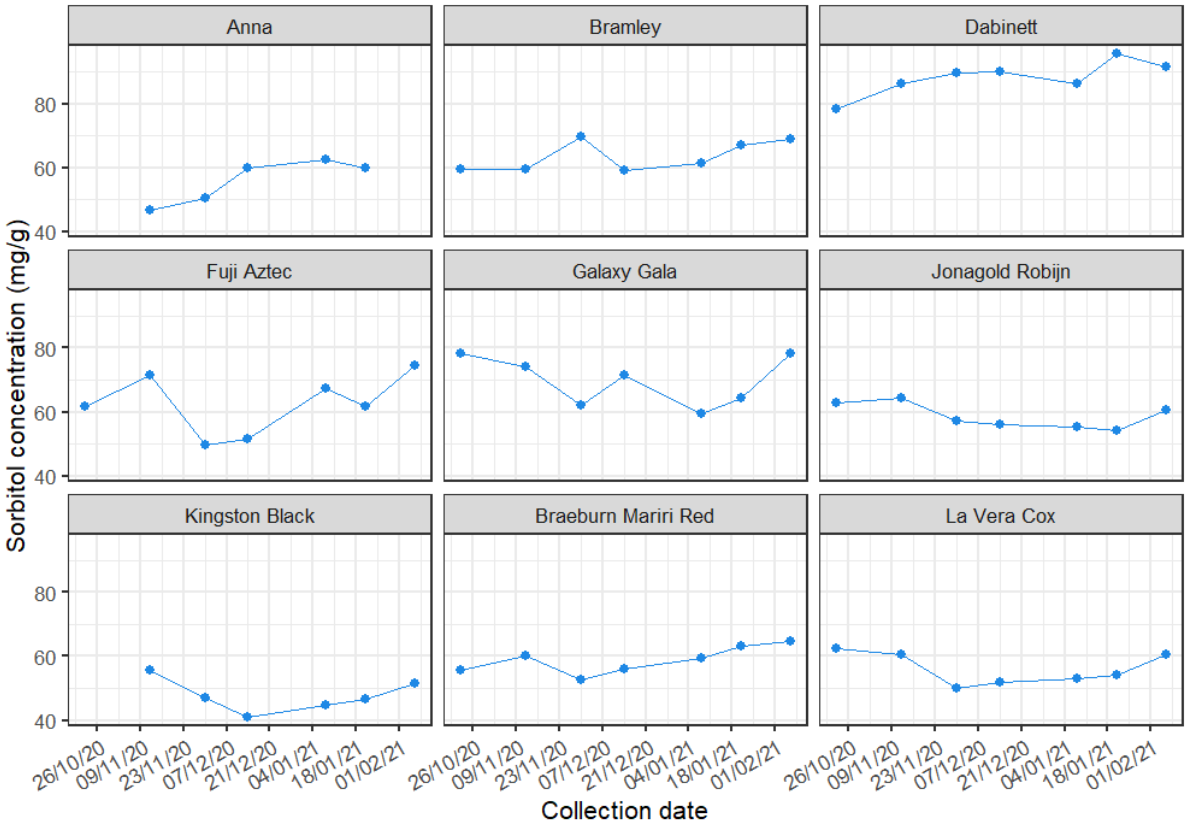


Figure 6.9 – Concentration of sorbitol (mg per gram of dry bud) in floral buds of nine apple cultivars during dormancy.

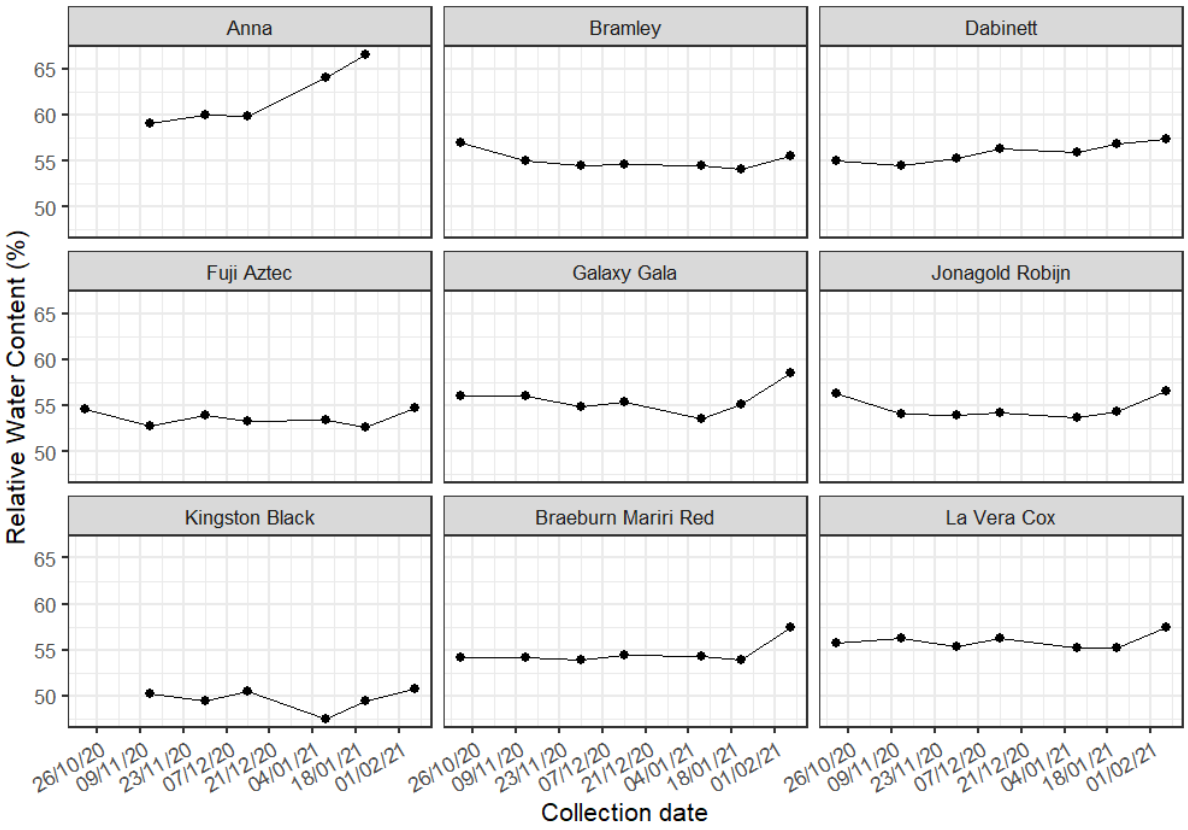


Figure 6.10 – Relative water content (%) of floral buds of nine apple cultivars during dormancy.

6.3.2 Carbohydrate dynamics in apple bark and xylem sap

Carbohydrate dynamics during dormancy in bark and xylem sap of three apple cultivars

Throughout the time of study, sorbitol was the most abundant sugar in bark and xylem sap (Figure 6.11). In all cultivars studied, xylem sap concentrations of hexose, starch and sucrose were at the limits of detection during winter, whilst sorbitol concentration remained stable until 22 December 2020 and then increased sharply, peaking at more than 6 mg/ml in "Braeburn Hillwell", 4mg/ml in "Bramley" and 3 mg/ml in "Galaxy Gala". Sorbitol concentrations fell in all cultivars after 20 January 2021, but were more marked in "Braeburn Hillwell" and "Galaxy Gala". In February 2021, xylem sap sorbitol concentration remained above 2 mg/ml in "Braeburn Hillwell" and "Bramley", but was less than 1 mg/ml in "Galaxy Gala" (Figure 6.11).

Bark concentrations of glucose and fructose followed the same pattern in all cultivars throughout winter, with only minor differences (Figure 6.11). In "Braeburn Hillwell" and "Galaxy Gala", hexose concentrations peaked on 6 January 2021 at approximately 5 mg/g; whilst the highest concentration in "Bramley" was noted in February. Starch concentrations followed a similar pattern in "Braeburn Hillwell" and "Galaxy Gala", being highest at the end of December and then decreasing to approximately 2.5 mg/g. Sucrose was not detected in "Bramley" throughout the study, whilst in "Braeburn Hillwell" and "Galaxy Gala" a peak was observed on 20 January 2021 (Figure 6.11).

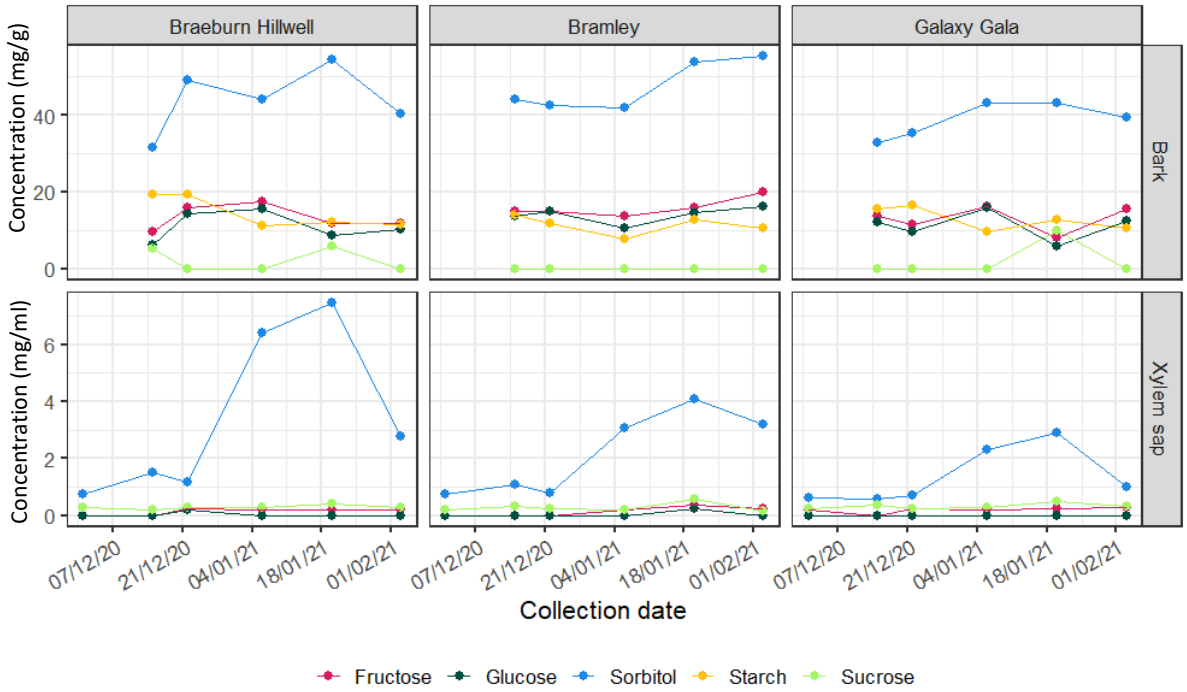


Figure 6.11 – Concentration of carbohydrates in bark (top, mg of carbohydrate in g of dry bud) and xylem sap (bottom, mg of carbohydrate in a ml of xylem sap) of three apple cultivars ("Braeburn Hillwell", "Bramley" and "Galaxy Gala") during dormancy progression.

6.4 Discussion

Carbohydrate dynamics in apple floral buds during dormancy progression

Sugars have a key role in bud growth (Bonhomme et al., 2010; Tixier et al., 2017), and sugar accumulation is linked to freezing tolerance (Sauter et al., 1996). Whilst a clear link to dormancy break has not been established, previous studies in other woody species have reported changes in carbohydrate concentrations in various parts of the plant during dormancy (Bonhomme et al., 2010; Ito et al., 2012; Fernandez et al., 2019). Here, concentration of sorbitol in floral buds was high throughout dormancy progression, whilst hexose and starch concentrations remained constant at the beginning of autumn and then diverged, with concentration of hexoses increasing and starch decreasing. These dynamics are similar to those reported for carbohydrate concentrations in sweet cherry buds (Kaufmann and Blanke, 2017), pear buds (Ito et al., 2012), walnut buds (Bonhomme et al., 2010), and floral primordia and other structures of peach buds (Bonhomme et al., 2005). The only study found in apple used stems instead of buds, but reported similar values of magnitude in starch and soluble sugar concentrations (Sivaci, 2006).

Perennial plants store carbohydrates during winter, often in the form of starch, to survive dormancy. To support the resumption of bud growth in spring, starch is degraded into soluble sugars and transported to the growing buds (Bonhomme et al., 2010; Tixier et al., 2017). The changes in concentration of hexoses and starch in apple buds at the beginning of dormancy reported here support this hypothesis, as the decrease of starch and increase in hexoses occurred simultaneously.

In the present study, a further decrease in hexoses and an increase in starch was observed at the beginning of January, when heat started accumulating (Figure 6.12A). A decrease in sucrose concentration at the end of dormancy was observed in pear (Ito et al., 2012) and walnut buds (Bonhomme et al., 2010) and a minor increase in starch was reported in sweet cherry (Kaufmann and Blanke, 2017). However, there are no reports of both changes occurring simultaneously. Kaufmann and Blanke (2017) suggested that changes in hexose and starch concentrations in buds could serve as markers to differentiate between dormancy stages in sweet cherry; other studies have linked concentrations of hexose and starch with chilling fulfilment (Bonhomme et al., 2005; Ito et al., 2012; Fadón et al., 2018; Fernandez et al., 2019). Since time of bud break under forcing conditions was not recorded in this experiment, it is not possible to establish a direct link between the change in starch and hexose concentrations and time of endodormancy release. However, data collected in 2019 and 2020 (see Chapter 3) indicate that the progression from endo to ecodormancy occurred when approximately 600-900 CU had accumulated, depending on the variety. Here, the second change in hexose and starch concentrations occurred between 14 December (646.50 CU) and 8 January (992.5 CU), coinciding with time of endodormancy break and the start of heat accumulation (see section *Carbohydrate dynamics to distinguish dormancy phases*).

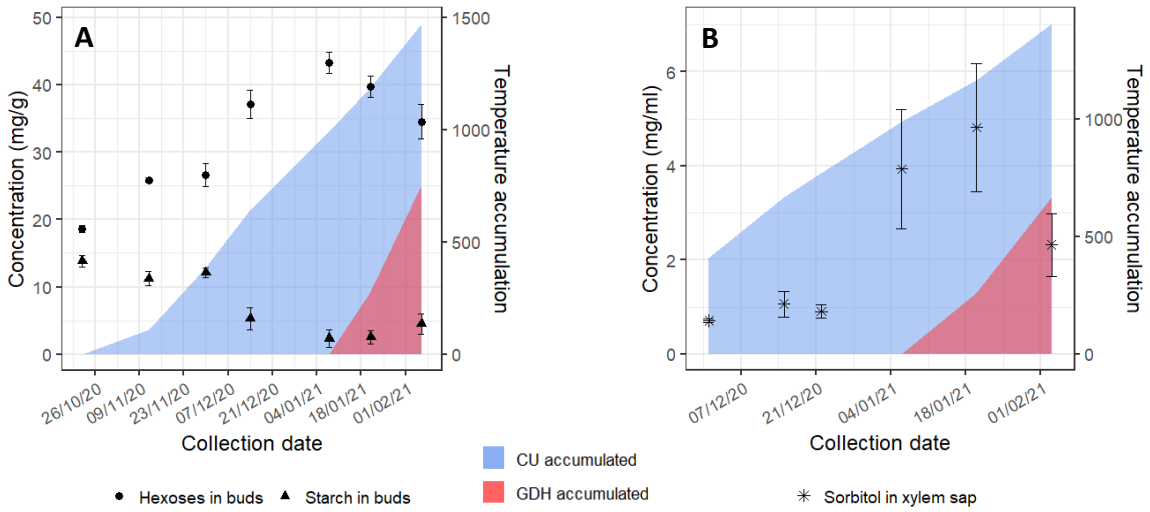


Figure 6.12 – Mean concentration of hexoses and starch (mg of carbohydrate in g of dry bud) in apple buds (A) and sorbitol in xylem sap (B) (mg of carbohydrate in a ml of xylem sap); in relation to chilling (CU accumulated, blue area) and heat (GDH accumulated, red area) accumulated. Each point represents the mean of all cultivars (eight cultivars in A, three cultivars in B), error bars show ±1 SE.

The observed changes in the levels of hexose and starch as heat accumulate can be explained by an increase in the rate of starch hydrolysation at warmer temperatures, which has been proposed as a mechanism to describe sugar fluxes in walnut xylem (Améglio et al., 2004). Whilst further studies are required to establish a causal link between endodormancy break and changes in hexose and starch levels, the current experiment indicates that in apple, a change in concentrations of these substances might occur at endodormancy release, which could potentially be used as a marker to identify chilling requirement fulfilment.

Changes in carbohydrate concentrations in apple buds during winter have not been reported previously, but information on stem cuttings and other woody parts exists (Raese et al., 1977; Yoshioka et al., 1988; Sivaci, 2006; Breen et al., 2020). These studies found sucrose in different concentrations, contrary to the findings presented here. Sucrose was also reported in pear (Ito et al., 2012), walnut (Bonhomme et al., 2010) and peach buds (Bonhomme et al., 2005), but was not found in buds of sweet cherry (Kaufmann and Blanke, 2017). It is possible that sucrose in apple buds is converted into hexoses and/or sorbitol quicker than in other *Rosacea* species and distributed in the xylem sap (see section *Carbohydrates in apple xylem sap*).

Sorbitol was the most abundant sugar in apple buds during winter, as previously reported in sweet cherry (Kaufmann and Blanke, 2017), pear (Ito et al., 2012) and peach buds (Bonhomme et al., 2010) (Bonhomme et al., 2005). No significant changes in the levels of sorbitol were detected throughout dormancy in the present study, whilst an increase around endodormancy break has been previously reported in pear and sweet cherry buds (Ito et al., 2012; Kaufmann and Blanke, 2017). In apple spur leaves, a link was also established between increased sorbitol concentration after bud break and the transition from carbohydrate sinks to sources in leaves, highlighting a major role of sorbitol in bud growth in apple trees (Loescher et al., 1982). Sorbitol is important in responses to cold temperatures

and it has been related to cold hardiness and resistance to abiotic stresses (Williams and Raese, 1974; Raese et al., 1977; Moing, 2000; Kanayama, 2009; Naschitz et al., 2010). Changes in sorbitol concentration in apple buds during winter were expected based on the literature (Williams and Raese, 1974; Ito et al., 2013; Kaufmann and Blanke, 2017), but these were not seen here; however, significant changes were detected in sorbitol xylem sap concentrations (see section *Carbohydrates in apple xylem sap*), which could be linked to chilling requirements.

Relative water content in dormant apple buds

Various authors have reported temperature dependent changes in the water content of different plant tissues during winter (Faust et al., 1991; Améglio et al., 2000). In apple buds, magnetic resonance imaging revealed conversion from bound to free water in apple buds, as chilling requirements were met (Faust et al., 1991). This change in water status occurred at different times depending on the chilling requirement of each cultivar, but it did not translate into changes in the overall RWC in the buds (Faust et al., 1991). Similarly, results obtained here showed RWC remained stable during winter and only increased at the end of dormancy, as previously observed (Rinne et al., 1994b; Götz et al., 2014; Malagi et al., 2015; Kaufmann and Blanke, 2017).

The increase in RWC at the end of winter was most significant in the cultivar "Anna", but less abrupt in other varieties. "Anna" is known for being a very low-chill cultivar, requiring less than 300 CU (Hauagge and Cummins, 1991a), which would explain the earlier sharp increase. It is reasonable to speculate that similar results would have been observed in subsequent bud collections in the other cultivars (Malagi et al., 2015). The RWC in "Anna" was higher throughout dormancy. Whilst this could be linked with low-chill requirements, cultivar-specific replication and earlier collections would be required to determine if the difference with other cultivars is significant and if a correlation with chilling requirements exists.

Kaufmann and Blanke, (2017) proposed that an increase in RWC could be used to identify the transition point from endo to ecodormancy (Lang et al., 1987), but no statistical analysis was reported to determine whether the observed increase (< 5% increase) was significant. Whilst an increase in RWC at the end of dormancy was also observed here, it is most likely that trees were ecodormant before this increase, as the transition from endo to ecodormancy in the studied cultivars occurred after approximately 900 CU (see Chapter 3). It is hypothesised that the increase in buds' water content is a consequence of heat accumulation, but not a sign of the transition from endo to ecodormancy (see section *Carbohydrate dynamics to distinguish dormancy phases*).

Differences in carbohydrates dynamics between cultivars

Similar changes in carbohydrate concentrations in floral buds were observed as dormancy progressed in all cultivars studied here. If there is a causal relationship between chilling fulfilment and changes in carbohydrate concentration (Bonhomme et al., 2005; Ito et al., 2012; Fadón et al.,

2018; Fernandez et al., 2019), significant differences between cultivars would be expected, and these should correlate with chilling requirements and timing of endodormancy break.

Previous studies have reported differences in carbohydrate concentration during winter between cultivars of other woody plants (Kaufmann and Blanke, 2017; Fadón et al., 2018; Farokhzad et al., 2018; Fernandez et al., 2019). Fernandez et al., (2019) used carbohydrate concentrations in sweet cherry stems of eight cultivars during winter to developed a model to estimate the probability of bud break based on concentration of hexoses and starch. They observed that including cultivar in the model provided better results than not including this parameter, suggesting meaningful differences between varieties (Fernandez et al., 2019). In apple stems, small differences in concentration of soluble sugars between three "Delicious" cultivars were reported at two time points in October and February, but no differences were detected in starch concentration (Sivaci, 2006). By quantifying the amount of starch in floral primordia using image analysis, Fadón et al., (2018) also detected differences in the dynamics of starch accumulation during winter between two cherry cultivars. In their study however, greater differences in starch accumulation were observed between cold and warm winters, suggesting that chilling accumulation could have a greater effect than cultivar on starch accumulation (Fadón et al., 2018).

In line with previous studies, larger differences in concentration of hexoses and starch between apple cultivars were anticipated here. Whilst some differences were noted, these could not be statistically examined due to the lack of replication because of limited plant material available. Adequate replication could reveal significant differences, but current results do not reflect differences in time of bud break between the cultivars studied particularly in "Anna", where bud break occurred at the beginning of February (Table B5 - Appendix B). A possible reason for this lack of variation between cultivars is that buds were collected every two-three weeks, compared to weekly collections in studies reporting significant differences (Fernandez et al., 2019). Future experiments should include replication for each variety and weekly bud collections, to investigate cultivar-specific dynamics.

Differences in concentration of sorbitol were observed between cultivars, although these changed throughout dormancy. A high sorbitol:sucrose ratio in mature peach leaves was negatively correlated with flowering date, suggesting a link between sorbitol and flowering time (Escobar-Gutierrez and Gaudillere, 1994). No sucrose was detected here, but "Dabinett" is one of the latest varieties to bud burst and had the highest sorbitol concentration throughout dormancy; whilst "Kingston Black", also a late variety (Table B5 - Appendix B), had lower sorbitol levels. These results are at variance with those of Escobar-Gutierrez and Gaudillere (1994) as there is no clear link between sorbitol levels and time of bud break. However, in their work, differences between cultivars in the sorbitol:sucrose ratio were mostly driven by changes in sucrose, suggesting differences in sorbitol between varieties were minimal (Escobar-Gutierrez and Gaudillere, 1994).

Greater differences in sorbitol between cultivars were detected at the start of dormancy. This parameter, in combination with initial starch levels, has been used to differentiate nine sweet cherry cultivars into two groups with different carbohydrate dynamics (Kaufmann and Blanke, 2017). However, no analyses comparing sorbitol concentrations between cultivars were presented (Kaufmann and Blanke, 2017). Nonetheless, sorbitol concentrations at the onset of dormancy were better correlated with time of bud break here; sorbitol concentrations in "Galaxy Gala" and "Dabinett" were initially high and bud break was later, whereas concentrations were low in "Anna" and this was the first cultivar to bud burst in the field (Table B5 - Appendix B).

Carbohydrates in apple xylem sap

As in buds, sorbitol was the most abundant sugar in xylem sap in all cultivars studied here, whilst other soluble sugars were detected in very small amounts. A clear peak of sorbitol was detected in all cultivars in January 2021. These observations are in agreement with previous studies in pear (Ito et al., 2012, 2013) and "Delicious" apple cultivars (Williams and Raese, 1974), which also showed higher sorbitol values in January and lower sorbitol levels before bud break.

In Williams and Raese's study (1974), observations of daily maximum and minimum temperatures indicated the increase in sorbitol in sap during autumn was triggered by low temperatures, although a correlation between lower temperature and sorbitol concentration was not confirmed statistically. Raese et al., (1977) also reported that sorbitol concentration in apple shoots increased after exposure to freezing temperatures, with a maximum concentration observed at -0.6 °C, and lower temperatures increasing concentrations only when the time of exposure was short. Concentrations of other soluble sugars (sucrose, fructose and glucose), also increased at lower temperatures (down to -3.9 °C) and with longer exposure (Raese et al., 1977). The results obtained in the present study also suggest a link between sorbitol concentration in xylem sap and cold temperatures (Williams and Raese, 1974), as the abrupt increase in sorbitol occurred after more than a week of cold temperatures at the end of January, which were maintained until mid-January (mean daily temperatures below 5 °C) (Figure A8 - Appendix A).

Here, the abrupt increase in sorbitol also occurred when approximately 900 CU had accumulated, which in these varieties coincides with the time of progression from endo to eco-dormancy (see Chapter 3). Further experiments are needed to understand whether the increase of sorbitol in xylem sap is just a response to colder winter temperatures (Williams and Raese, 1974; Améglio et al., 2000), or if it plays a role in the breaking of endodormancy.

Large differences were observed in the maximum sorbitol levels detected in each cultivar, with three times more sorbitol being detected in "Braeburn Hillwell" compared to "Galaxy Gala" in January. Differences have been reported between "Red Delicious" and "Golden Delicious" (Williams and Raese, 1974), but their magnitude was lower than results reported here. Whilst the increase in

sorbitol occurred at the same time in all cultivars and so could be linked to lower winter temperatures (Williams and Raese, 1974), differences in the maximum concentration of sorbitol detected in each cultivar could be related to cultivar-specific responses. “Braeburn Hillwell” is a “Braeburn” sport, a variety that cropped heavily in warm winters in Zimbabwe (Jackson, 2003) and one of the first cultivars to bud burst in the UK (Table B5 - Appendix B). On the other hand, “Bramley” and “Galaxy Gala” are later bud burst varieties, suggesting a possible link between sorbitol levels and chilling requirements.

In apple bark, sorbitol was also the most abundant sugar throughout dormancy; and hexoses and starch were found in higher amounts than in buds. Sugars in the bark could be translocated to buds via the xylem (Loescher et al., 1990; Decourteix et al., 2008). The small bark pieces used in this experiment included the phloem, which has been shown to play an important role during dormancy in walnut branches, as removing it reduced the concentration of carbohydrates and delayed time of bud break (Tixier et al., 2017).

Whilst the link between sorbitol dynamics in xylem sap and chilling temperature seem clear (Raese et al., 1977; Williams et al., 1979; Ito et al., 2012), results here also indicate that different concentrations could be linked to cultivar-specific chilling requirements. Further experiments including replication at a cultivar level and more frequent sap collections during the winter could provide more information.

Carbohydrate dynamics to distinguish dormancy phases

As described above, changes observed in carbohydrate concentrations in apple buds and xylem sap appear to correlate with chilling and heat accumulation (Figure 6.12), and to cold temperatures during winter (Figure A8 - Appendix A) (Raese et al., 1977; Améglio et al., 2004; Fadón et al., 2018). In sweet cherry, carbohydrate dynamics and RWC in buds during winter have been used to differentiate dormancy phases (Kaufmann and Blanke, 2017). Here, a similar approach was followed by combining the results obtained in buds and xylem sap, and a hypothesis to distinguish dormancy phases through carbohydrate dynamics is proposed.

At the beginning of winter, when hexose and starch concentrations in buds are stable, trees are entering dormancy, but are not fully endodormant at this stage. This hypothesis is consistent with results from previous experiments in this PhD programme (see Chapter 3), as time to bud break in excised shoots increased until more than 500 CU had accumulated. Here, the first change in the hexoses and starch curve was observed between 385.5 CU and 646.5 CU accumulated. During this first dormancy phase, trees do not require carbohydrates as growth has ceased, which would explain why starch and hexose concentrations remain constant. This suggested initial phase where trees are still not fully dormant also relates with the basis of the Dynamic Model (Fishman et al., 1987), which

assumes that at the beginning of chilling, chilling accumulation can be reversed by warmer temperatures.

After accumulating 385.5 CU, concentration of hexoses increased and starch decreased, which can be associated with the onset of the endodormancy phase (Lang et al., 1987). At this stage, trees are fully dormant, and buds become isolated (Rinne et al., 2001). To exit endodormancy, buds convert starch within the buds into soluble sugars, as observed in the change of the starch and hexose concentrations.

Between the fourth and fifth bud collection (646.5 – 992.5 CU), concentration of hexoses decreased again and a small increase in starch was observed. Simultaneously, sorbitol in xylem sap increased, and this coincided with the beginning of heat accumulation. Therefore, the change in the hexoses and starch curves, together with the increase of sorbitol in xylem sap, could mark the time of endodormancy break and the start of ecodormancy (Lang et al., 1987). The sorbitol detected in xylem sap is then transported into buds to support growth and development, as observed with the slight increase of sorbitol detected in buds of some cultivars at the end of winter.

Finally, when trees become ecodormant (Lang et al., 1987) and full xylem transport has been restored (Améglio et al., 2000), an increase of water content in the buds is observed, in preparation for bud break.

It is important to recognise the limitations of this hypothesis, as buds and xylem sap were collected from different trees, and there was no replication for each cultivar. However, these results provide some insight into the correlation between carbohydrate dynamics and dormancy progression in apple, which, with further experiments, could prove to be an effective tool to distinguish dormancy phases and differentiate between cultivars with different chilling requirements.

Chapter 7

General discussion

Introduction

UK apple production and value have increased steadily from the beginning of the 21st century, and whilst imports have declined during the last decade, they still represent more than 43% volume of the total production in the UK apple industry (DEFRA, 2021). Apples are imported mainly from Spain and South Africa (DEFRA, 2021), where the impacts of insufficient winter chilling are already becoming apparent (Cook and Jacobs, 1999; Campoy, 2009; Cook, 2010). Furthermore, the Covid-19 pandemic has accentuated the fragility of supply chains and has highlighted issues stemming from an over-reliance on long-distance food imports. These factors, together with an increasing consumer interest in locally grown food, create an ideal opportunity to expand and promote UK apple production.

Since the dormancy cycle in apple will be affected by climate change (Campoy et al., 2011a; Atkinson et al., 2013), establishing the severity of these impacts on the productivity of different cultivars is key if current growing and agronomic practices are to be adapted to help to mitigate the risks. Decision-makers should consider both present and future climate as apple trees can remain in production for more than 20 years (NACM, pers. comm.). However, to ensure future UK production remains economically profitable in our changing climate, short-, medium- and longer-term research that focusses on improving current chilling and heat accumulation models is essential to develop accurate predictions of annual marketable yields at a cultivar level. In this PhD programme of research, new fundamental knowledge needed to develop more accurate chilling and heat accumulation models was generated, and this will help to inform climate change adaptation strategies so that potential impacts on the UK apple sector can be managed and mitigated more effectively.

Approaches to study dormancy in apple

Many investigations into chilling requirements in fruit trees utilise experiments with excised shoots (Hauagge and Cummins, 1991c, 1991b; Cook and Jacobs, 2000; Guak and Neilsen, 2013; Anzanello et al., 2014), an inherent assumption being that these are a good model to study field-grown trees. In these studies, shoots were often collected during winter and placed at warm temperatures to induce bud break, and chilling requirements were assumed to have been met when a pre-determined percentage of bud break was reached within 10-15 days (Hauagge and Cummins, 1991b; Naor et al.,

2003; Ruiz et al., 2007; Campoy et al., 2012; Jones et al., 2013; Parkes et al., 2020). But this decision is arbitrary, and as discussed in Chapter 3, not all buds within a shoot have the same probability of opening. Furthermore, this methodology is not valid when studying dormancy release in all apple cultivars (Chapter 3), and so more reliable approaches are needed. Unless studies can establish that a specific percentage of bud break in a branch is equivalent to productive levels of bud break in orchard trees, using the percentage of bud burst as a measure of dormancy release should be avoided. Instead, winter progression curves of the time to bud break (Chapter 3) would be a more accurate approach in dormancy studies, as they illustrate how the depth of dormancy changes throughout winter.

Many chilling models were developed using excised shoots, with investigators placing shoots in different temperatures for varying lengths of time to study effects on bud break (Weinberger, 1950; Richardson et al., 1974; Shaltout and Unrath, 1983; Guak and Neilsen, 2013). In many of these studies, treatment effectiveness was assessed as a function of the time required to reach a certain percentage of bud break after moving shoots to a warmer temperature. Preliminary work carried out in this PhD programme used a similar approach (data not shown), but excised branches did not survive chilling treatments at freezing temperatures for extended periods. Existing models developed with excised shoots did not include temperatures below-zero (Weinberger, 1950; Shaltout and Unrath, 1983; Dennis, 1994), perhaps for this reason, the validity of using excised shoots as a model system in the study of chilling accumulation in fruit trees was not more widely questioned. Using potted trees is, perhaps, a more relevant way to study both chilling and heat accumulation (Chapters 4 and 5), as favoured in some previous studies (Fishman et al., 1987; Naor et al., 2003). This approach permits investigations into the effect of freezing temperatures, and whilst some damage occurred when constant temperatures were imposed (Chapter 4), no frost damage was observed in fluctuating temperature treatments (Chapter 5), which are a more realistic representation of true climatic conditions.

A clear role for rootstock-to-shoot signalling during dormancy has not been established, but an involvement seems logical as warmer temperatures in spring are linked to the resumption of osmotically-driven sap flow and changes in the composition of sap during winter were observed (Chapter 6). Intact xylem and phloem connections (Améglio et al., 2000; Tixier et al., 2017), and a functional polar auxin transport pathway between roots and shoots are crucial for plant growth and development (Friml and Palme, 2002), and so whilst the behaviour of excised shoots might superficially resemble that of an intact mature tree for a short period following excision, a greater impact as a consequence of excision is to be expected over time. Therefore, studies utilising excised shoots should be of short duration (< 2 months) and include numerous replicates (> 10) as variability between branches can be high (Chapter 3). Furthermore, unless and until there is unequivocal

evidence that excised shoots are indeed a good representation of intact field-grown trees, the approach should not be used in work to develop chilling or heat accumulation models.

Chilling accumulation in apple in the context of climate change

Key results presented in Chapters 4 and 5 demonstrate that chilling temperatures below zero are most effective for chilling accumulation. However, the most commonly used chilling models in fruit trees (Weinberger, 1950; Richardson et al., 1974; Fishman et al., 1987), do not incorporate freezing temperatures, although studies in blackcurrant (Jones et al., 2013) and forest trees (Harrington et al., 2010) have highlighted the effectiveness of sub-zero temperatures towards chilling accumulation.

Freezing temperatures occur frequently during winter in the UK and other important fruit growing regions, and a failure to account for these in existing models likely contributes to inaccurate predictions of bud break (Legave et al., 2008). Predictions of the extent of the decline in winter chilling as a consequence of climate change vary greatly depending on the model used to calculate chilling (Sunley et al., 2006; Luedeling et al., 2009a; Luedeling and Brown, 2011), but with freezing temperatures being optimal for chilling accumulation, greater reductions in winter chilling are predicted using the *Malus model* (Chapter 5) (see section *Implications for industry and adapting orchard management practices*).

As noted in Chapters 4 and 5, the lower threshold for chilling accumulation is much lower than that previously reported (Richardson et al., 1974; Fishman et al., 1987; Naor et al., 2003; Guak and Neilsen, 2013). As mean daily temperatures rise due to climate change, the frequency of extreme weather events and erratic weather fluctuations are also predicted to rise (Murphy et al., 2018). Under these changing climates, short duration freezing events could contribute towards fulfilling chilling requirements, so it is important to account for the potential impact of sub-zero temperatures. Studies with blackcurrant in the UK have suggested that long and warmer winters are not as effective at releasing dormancy as cold, shorter winters (Jones et al., 2013). Whilst this interaction was not directly investigated here, a similar percentage bud break was observed under warmer and longer chilling treatments when compared to shorter and colder treatments (Chapter 4), contrary to the observations by Jones et al., (2013). It is possible that in Jones et al., (2013), chilling requirements were not satisfied in the long, warmer treatments, and that extending the chilling period further could provide similar results to short, colder winters. Future research should focus on understanding the effectiveness of long, warmer chilling periods, compared to short, colder chilling periods, investigating not only on the effect on bud break but also on final fruit production.

An important element affecting chilling accumulation that has not been considered here is the potential negating effect of warm temperatures during chilling accumulation. Both the Utah (Richardson et al., 1974) and the Dynamic model (Fishman et al., 1987) include a mechanism by

which previously accumulated chilling can be “cancelled” if temperatures above the chilling range occur. The Dynamic model is the only model in fruit trees that was developed from controlled environment observations of bud break in potted trees after different temperature treatments (Erez et al., 1979; Couvillon and Erez, 1985; Erez and Couvillon, 1987); these authors reported that a diurnal cycle including 8 h at temperatures above 21 °C reduced previous chilling accumulated. Research to establish whether a possible negating mechanism exists in apple, with subsequent investigations to quantify any impact on chilling accumulation, would help to further improve the accuracy of predictions and should be included in future work programmes. However, whilst winter average temperatures are predicted to increase in the UK, it is unlikely that daily temperature fluctuations will reach these extreme values on a regular basis in the coming decades (Murphy et al., 2018).

The upper temperature threshold for chilling accumulation in the apple cultivars studied here is lower than that suggested in previous models (Richardson et al., 1974; Fishman et al., 1987; Harrington et al., 2010). This finding heightens existing concerns over important fruit growing areas such as California or Spain, where winter temperatures are already at the edge of the chilling temperature range (Luedeling and Brown, 2011). Current mean winter temperature in the UK is below 10 °C, fully within the chilling temperature range (Murphy et al., 2018). However, as temperatures rise, daily fluctuations are likely to reach maximums above the upper temperature threshold (Murphy et al., 2018), highlighting the need to investigate the high temperature chilling negating mechanism at cultivar, metabolic, and cellular levels.

Previous studies have shown that fluctuating daily temperatures during chilling, as opposed to constant temperatures, can have a positive effect on bud break (Hänninen, 1990). In experiments carried out during the development of the Dynamic model, the percentage of bud break increased in a low/moderate temperature cycle, compared to a constant low temperature treatment (Erez and Couvillon, 1987); however, the authors did not compare the low/moderate cycle with the corresponding mean temperature, and these experiments did not consider temperatures below zero. Results from Chapter 4 showed no differences between chilling at 2/7 °C (12/12h) and 4.5 °C; however, some differences were observed between chilling at 2/-4 °C (8/16h) (Chapter 5) and chilling at -2.5 °C (Chapter 4). It should be noted that these temperature treatments are not fully comparable as they were not carried out simultaneously, slightly different methodologies were used, and -2.5 °C is not the mean of 2/-4 °C; nevertheless, more consistent results were perhaps expected. Current chilling models require hourly temperatures (Richardson et al., 1974; Fishman et al., 1987); if daily fluctuations do not have a significant effect on bud break, mean daily temperatures could instead be used, which would facilitate modelling, as hourly temperature data often has to be derived from maximum and minimum daily temperatures (Ritchie and Nesmith, 1991; Cesaraccio et al., 2004; Sunley et al., 2006; Luedeling et al., 2009d). Further research is

required to understand the effect on bud break of temperature fluctuations of different durations and should include temperatures within and outside the chilling temperature range.

Another key aspect that remains unknown is the time at which chilling accumulation begins, as current studies often identify an arbitrary date, or arbitrary markers such as leaf fall or the last day with negative chilling accumulation (see section *2.4.4 Selecting starting dates for chilling and heat accumulation*). Changes in bud and xylem sap carbohydrate dynamics (Chapter 6) did not provide evidence for a physiological marker that could be linked to the start of chilling accumulation; however, the first tissue/sap samples were not collected until October, when chilling accumulation had perhaps already begun. Whilst these observations could provide useful information on changes occurring earlier in the season, they would not help to establish a causal effect on dormancy induction. Research using transgenic plants exhibiting aberrant carbohydrate winter dynamics could provide relevant information to understand if a causal effect exists.

A contemporary approach used to determine starting dates for chilling and heat accumulation is Partial Least Squares (PLS) regression (Luedeling and Gassner, 2012; Guo et al., 2015a, 2015b; Drepper et al., 2020; Fernandez et al., 2021). PLS models time of bud break or blooming as a response to hourly temperatures and requires long-term climate and phenology datasets to identify if specific time periods contribute towards advancing or delaying blooming. However, an important limitation of this approach is that it is restricted to modelling bud break within the climatic conditions covered in existing datasets (Luedeling and Gassner, 2012); which may not always provide valuable insights in to how trees might respond to climate change scenarios. On the other hand, a range of climatic conditions can be simulated in control environment experiments by imposing specific treatments, providing the opportunity to observe different plant responses.

Linking chilling and heat accumulation

As time of bud break is determined by both chilling and heat accumulation, accurate models must include both temperature-driven processes. Whilst an accurate quantification of chilling accumulation is key, particularly in a climate change context and in regions already on the margins of sufficient winter chilling (Luedeling and Brown, 2011); heat accumulation in spring is the most important parameter influencing time of bud break and flowering in areas where there is currently sufficient chilling accumulation, such as in the UK, (Citadin et al., 2001; Guo et al., 2014). The predicted increases in temperatures due to climate change will affect both processes, and whilst most research has focused on winter chilling, new models that include both temperature-driven processes are needed to anticipate how cultivars will respond to the future climate. Results obtained in this PhD research programme provide new insights into how both processes influence bud break in apple, thereby improving the way their combined effect is modelled to predict time of bud break more accurately. Two key conclusions were reached: 1) there is an overlap between chilling and heat

accumulation (Chapter 4); 2) the base temperature for heat accumulation decreases with longer chilling (Chapter 5).

Whilst it was initially supposed that heat accumulation begins after endodormancy break (Richardson et al., 1974; Hänninen, 1990), a growing number of studies propose that both processes occur simultaneously in a partial or complete overlap (Harrington et al., 2010; Campoy et al., 2011b; Pope et al., 2014; Darbyshire et al., 2016b). Results obtained in Chapters 3 and 4 support the hypothesis of a partial overlap, as the optimum temperature for chilling accumulation increased with longer chilling (Chapter 4), and a gradual transition from endo to ecodormancy was noted in all studied cultivars (Chapter 3). Both observations can be explained with warm temperatures accumulating in a thermal time relationship, after only limited chilling has accumulated. An overlapping system is perhaps more realistic as this represents a more sophisticated and gradual transition from chilling to heat accumulation, instead of a simplistic and rather abrupt change once an arbitrary number of chilling units has accumulated.

Previous studies have suggested that longer chilling can reduce heat requirements to bud break (Ruiz et al., 2007; Darbyshire et al., 2013; Guo et al., 2014), indicating a clear correlation between both processes and highlighting the need to consider them simultaneously to develop more accurate models. The correlation between chilling and heat accumulation was also observed in Chapter 3, as the effect of warm temperatures declined with longer chilling. This observation was incorporated to the approach followed in Chapter 5 by creating a dynamic thermal time model in which the base temperature for heat accumulation was reduced as chilling increased, thereby accelerating the fulfilment of heat requirements. This approach provides an innovative solution to model the interaction between chilling and heat accumulation, incorporating the compensating mechanisms between both process that has previously been observed (Ruiz et al., 2007; Darbyshire et al., 2013; Guo et al., 2014).

As it becomes more evident that chilling and heat accumulation are not independent processes, investigating them in combination is crucial to make more accurate predictions in a climate change context. Furthermore, with a predicted reduction in future winter chilling and an increase in heat accumulation, it would be valuable for climate change adaptation to determine if a reversed compensating mechanism between chilling and heat accumulation also exists, i.e., if insufficient chilling could, to some extent, be compensated for by additional heat. Results obtained in Chapter 5 indicate a partial substitution might be possible, but only after a minimum amount of chilling, as previously suggested for sweet cherry (Kaufmann and Blanke, 2019). However, it is important to highlight that the results presented here focus on the effect of changes in chilling and heat accumulation on the *time* of bud break. Whilst time of bud break in apple and tree fruit crops is critical as it dictates the time available for fruit set and growth, other factors such as flower development, uneven blooming within a cultivar or successful pollination are as important (Jackson,

2003; Pardo and Borges, 2020). A suitable chill-to-heat ratio must ensure that none of these parameters are compromised, not just the time of bud break. Therefore, further research should focus on investigating the effect of a range of chilling and heat accumulations on flower development, degree of flowering uniformity within a cultivar, pollinator abundance and activity, and, ultimately, fruit yields, quality at harvest and storage potential.

Overall, results regarding the interaction between chilling and heat accumulation raise questions as to the suitability of using specific, and rather arbitrary, chilling requirement values to establish the climatic needs of a cultivar. Instead, a flexible approach that defines a more realistic transition from endo to ecodormancy and considers the correlation between both processes is required. Development of more accurate mechanistic models to describe chilling and heat accumulation must await advances in our understanding of the physiological mechanisms regulating both processes; but in the meantime, the approach presented in Chapter 5 provides the basis of a more realistic model. Future research should aim to define safe chill-to-heat ratios for a minimum level of bud break, based on profitable fruit production.

Implications for industry and adapting orchard management practices

Although predictions using existing chilling models anticipate a decline in winter chilling in the UK (Luedeling et al., 2011; Atkinson et al., 2013), the extent of this decline will be less severe than in other important global fruit growing regions, and so even under high emissions scenarios, studies predict there will be sufficient winter chilling at the end of the 21st century for most apple cultivars in the UK (Luedeling et al., 2011). However, these future winter chilling estimates were calculated using existing models (Luedeling et al., 2011), which do not accurately represent temperature contributions towards chilling accumulation (Chapter 5).

Further research is required to validate the *Malus model* and calibrate it at a cultivar level before it is used for predictive climate change studies (see section *Limitations and future work*). Until then, predictions on how climate change will affect the studied cultivars should be viewed with caution. Nonetheless, the lower optimum temperature for chilling accumulation in “Braeburn Lochbuie”, “Galaxy Gala”, “Discovery” and “Dabinett” reported here (Chapters 4 and 5), suggests that the impact of warmer winters in this century might be greater than previously thought (Luedeling et al., 2011). To better illustrate the greater chill reductions expected with the *Malus Model*, annual winter chilling for the duration of this PhD (2017-2021) was calculated in Chill Fractions (*Malus Model*), Chill Portions (Dynamic model (Fishman et al., 1987)) and Chill Units (Utah model (Richardson et al., 1974)) (Table 7.1). Mean annual winter chilling accumulation between 2017 and 2021 was 1,332.6 CF (*Malus model*), 99.9 CP (Dynamic model) and 2,137.8 CU (Utah Model). Chilling accumulation was then calculated assuming a 2 °C temperature increase, as predicted with medium emission scenarios (Murphy et al., 2018). A 2 °C increase in winter temperatures would cause an 8.8% and 12.9% decline in CP and CU respectively, however, the impact on Chill Fractions would be greater, with a

reduction in chilling accumulated of 28.2% (Table 7.1). In a study using the Dynamic model to anticipate winter chilling decline under different climate change scenarios, a 13% reduction was predicted by the end of the 21st century in south-west England (Luedeling et al., 2011). Whilst values shown in Table 7.1 are only approximations, the percentage decline in CP compared to CF suggests that future reduction in winter chilling could be up to three times higher than previously reported (Luedeling et al., 2011), reaching a 40% loss by 2100. Even acknowledging the current limitations of, and the further work required with the *Malus model,* results in this thesis suggest that the predicted extent of winter chilling decline may have been significantly under-estimated. This finding will likely heighten existing concerns amongst UK apple producers since there would be insufficient chilling for most apple cultivars currently grown in the UK.

Table 7.1 – Annual chilling: observed accumulation of chilling per year from 15 October to 28 February; SD: standard deviation; Annual chilling +2 °C: estimated accumulation of chilling with a 2 °C hourly temperature increase; % reduction: difference in % between observed chilling accumulated and Annual chilling +2 °C. Chilling calculated in CF, CU and CP.

Model	Year	Annual chilling	Annual chilling +2 °C	% Reduction
Chill Fractions (*Malus model*)	2017/18	1430.9	1062.4	25.8
	2018/19	1313.7	938.8	28.5
	2019/20	1255.8	855.6	31.9
	2020/21	1330.0	974.5	26.7
	Mean ± SD	**1332.6 ± 72.8**	**957.8 ± 85.7**	**28.2 ± 2.7**
Chill Units (Utah model)	2017/18	2139.5	2009.5	6.1
	2018/19	2154.5	1905.0	11.6
	2019/20	2403.5	1900.0	20.9
	2020/21	1854.0	1611.5	13.1
	Mean ± SD	**2137.9 ± 224.7**	**1856.5 ± 171.0**	**12.9 ± 6.1**
Chill Portions (Dynamic model)	2017/18	98.1	89.2	9.1
	2018/19	101.1	93.6	7.4
	2019/20	105.8	96.2	9.0
	2020/21	94.7	85.4	9.9
	Mean ± SD	**99.9 ± 4.7**	**91.1 ± 4.8**	**8.8 ± 1.0**

Another important aspect that apple growers should consider when choosing cultivars for new plantings is their sensitivity to extended periods of unseasonably warm temperatures during dormancy, as highly sensitive cultivars will bud break too early and be at higher risk of frost damage (Augspurger, 2013; Pfleiderer et al., 2019). Results obtained in Chapter 3 provided information on the time it would take for buds of the studied apple cultivars to open, in the event of heatwaves of different mean temperatures and occurring from mid-winter until early spring. “Braeburn Mariri Red” appeared to be the most sensitive cultivar to warmer temperatures during ecodormancy, followed by “La Vera cox” and “Kingston Black”. These varieties are, therefore, at a higher risk of frost damage following a heatwave event, and growers choosing to plant these should consider frost

mitigation measures such as overhead sprinklers or heaters (Snyder and Melo-Abreu, 2005). On the other hand, the cultivars “Bramley”, “Galaxy Gala”, “Fuji Aztec” and “Jonagold Robijn” showed less susceptibility to winter heatwaves and might be safer options in areas with more frequent frost events.

A different experimental approach is needed to understand how “Dabinett” will adapt to climate change as experiments with excised shoots (Chapter 3) and potted trees (Chapter 5) did not provide conclusive results. It is possible that these observations are a consequence of an unusually high chilling requirement in this cultivar, or an extreme sensitivity to warm winter events, which could cause the uneven flowering reported in recent years by UK growers (Loraine Boddington, NACM, pers. comm.). To answer this question, future research could use potted trees in controlled environment experiments to replicate a range of winter conditions, including longer and colder treatments to determine chilling requirements, and warm temperature interruptions to understand if these are impacting “Dabinett”’s ability to flower.

In apple, a wide range of chilling requirements is available with existing cultivars, and low-chill varieties have been successfully bred and are cultivated in warmer climates (Hauagge and Cummins, 2000; Griesbach, 2007). Although cultivar selection for new plantings is not only determined by the climate, and other aspects such as soil characteristics or consumer preferences need to be considered, the existing range of chilling requirements provides the opportunity to grow cultivars that might be better suited to the predicted future climate in any given region. To select suitable cultivars, a climate analogues approach could be used, based on identifying regions with a current climate similar to that predicted in the future in the new planting area (Ramírez-Villegas et al., 2011).

Strategies for adaptation to climate change should prioritise proactive measures, such as selecting suitable cultivars, as these are more cost effective, more environmentally friendly, and involve lower labour requirements than reactive measures, such as the application of rest-breaking chemicals. Nevertheless, with rapidly changing climates and an increase in the frequency of extreme weather events, reactive measures such as the application of frost mitigation systems might be required in the UK (Snyder and Melo-Abreu, 2005). Other adaptation strategies might involve management practices to overcome dormancy, such as the application of rest-breaking chemicals, currently used in warmer growing regions like Kenya (Griesbach, 2007). One of the most commonly used chemicals, Hydrogen cyanamide (commercialised as Dormex™), has been shown to induce bud break and promote even blooming in areas with insufficient chilling for a range of fruit tree crops (Jackson and Bepete, 1995); however, due to its phytotoxicity (Siller-Cepeda et al., 2019), the chemical has already been banned in many countries. The combined used of the cytokinin Thidiazuron ([TDZ] N-phenyl-N-1,2,3-thiodiazol-5-il-urea) with mineral oil has been shown to induce even bud break in apricot and peach (Erez et al., 2008; Campoy et al., 2010), and might be an alternative solution in the

future. However, whilst phytotoxicity has not been reported, its effect is variable, depending on the timing and method of application (Erez et al., 2008; Campoy et al., 2010).

The level of reduction in greenhouse gases over the next few years will determine if rest-breaking chemicals will be required for apple production in the UK in the coming decades. Nonetheless, more sustainable growing practises to manage existing orchards should be prioritised. A study with apricots showed that shading trees during ecodormancy advanced harvest date by 2-3 days (Campoy et al., 2010). Microclimate manipulation by shading the orchard at critical times, such as during frost events or heatwaves, could be an effective management practise. For instance, sweet cherry production in the UK is gradually adopting covering systems, such as high tunnels and temporary nets (Withnall, 2015). Covered production protects cherries from birds, rain and frost damage, and can help regulate environmental conditions at critical stages, reducing the overall growing risk (Lang, 2009, 2014). Sweet cherry production has higher market returns than apple, making covered production economically feasible (Withnall, 2015). Whilst growing apple under cover is perhaps not profitable at present, these methodologies and management practices could be convenient in the future if uncovered, traditional production systems, become unviable due to climate change. Current research being carried out at Brogdale (Brogdale Collections, 2021) by the National Fruit Collection Trust and the University of Reading replicate covered apple production and provide an ideal system for future studies (The English Apple Man, 2018) (see section *Limitations and future work*).

Limitations and future work

Further work could broaden the application of the models developed here and provide more relevant information for apple production in the UK. As highlighted in previous sections, a first step should include validation of the *Malus model* and the approach presented in Chapter 5 with external datasets, including orchards from different climates. Calibration at a cultivar level should also be a priority to ensure accurate predictions, furthermore, it could help to identify varieties with similar behaviours.

A long-term climate change experiment at Brogdale (Brogdale Collections, 2021), provides an ideal setting to test and calibrate the models developed here, as it is an experimental system midway between a commercial orchard and a controlled environment. In the “Apples in a Warmer World” experiment (The English Apple Man, 2018), mature apple trees are grown under polytunnels replicating a range of future climatic conditions. The research presented here would benefit from a future collaboration with Brogdale (Brogdale Collections, 2021) to validate and calibrate the *Malus model* at their climate change experiment.

After validating and calibrating the model for a range of cultivars, future research should aim to identify safe chill-to-heat ratios (Chapter 5) that would ensure profitable fruit production for each variety. These ratios would provide a valuable tool to determine cultivar suitability to the future

climate in different regions. To make this information easily accessible, UK regional maps showing historical and future chill-to-heat ratios could be created and accompanied with information on the safe chill-to-heat ratios for each cultivar. Working in collaboration with existing weather forecasting companies (i.e., Weatherquest (2022) (UK)) could facilitate and improve the development of clear and comprehensive of maps. Growers, agronomists and apple producers could easily access this information; by cross-referencing the predicted chill-to-heat ratio in their area with the safe ratios for different cultivars they would be able to identify suitable varieties for their climatic characteristics.

Access to accurate and site-specific temperature data remains a matter of concern, as although many weather stations exist around the UK (Met Office, 2022), processing climatic data is time consuming and requires specialised skills. Furthermore, orchards are not always in proximity to weather stations, so temperature data is often extrapolated (Luedeling et al., 2009d), contributing to inaccuracies when estimating chilling and heat accumulation. Weather stations installed in orchards would produce more accurate data to monitor current chilling and heat accumulation at specific sites, which could then be compared with information from nearby weather stations and used to adjust estimates at a local level.

Chilling requirements reported for the same cultivar often vary between locations (Hauagge and Cummins, 1991a; Naor et al., 2003; Darbyshire et al., 2017). It would be interesting to determine if these differences occur when chilling is calculated with the *Malus model*, to understand if they are a consequence of inaccurate models, or if there are other parameters, not included in current models, that should be considered. Soil type and depth, water and nutrient availability and acquisition, rootstock, pests and diseases, effective pollination period, availability and activity of pollinators, and orchard practices such as pruning, canopy management, weeding strategies, etc., all influence growth and fruit production (Jackson, 2003; Naor et al., 2008; Ramírez and Davenport, 2013), and an effect on the dormancy cycle can be expected. Whilst air temperature is the main factor regulating dormancy, other factors could be driving the differences in chilling requirement observed between locations.

An important aspect to consider is that in all experiments carried out during this PhD programme, time of bud break was monitored instead of flowering time, and vegetative and floral buds were not accounted for separately. Whilst floral buds are of a greater interest as they are the basis for fruit production, young apple trees often bear low densities of buds and so both types of buds were considered at once and only the green tip stage (Chapman and Catlin, 1976) was assessed. Although chilling requirements of vegetative buds are higher than floral buds (Thompson et al., 1975; Naor et al., 2003; Campoy et al., 2011b), no differences in the effect of chilling or forcing temperatures were observed and therefore the *Malus Model* should be equally reliable for both bud types. Results obtained showed clear temperature effects on bud break, but further experiments should ensure

that the effectiveness of different temperature treatments remains consistent for flowering and final fruit production.

Air temperature is used as a single parameter to predict time of bud break and flowering in fruit trees (Legave et al., 2008; Parker et al., 2011; Pope et al., 2014; Chuine et al., 2016), but its effectiveness is not so evident in some cultivars (i.e., "Dabinett", Chapters 3 and 5), or perhaps current methodologies used to study chilling and heat accumulation are not suitable for these varieties. Results obtained in Chapter 6 indicate that further research on the composition of xylem sap during dormancy, could provide relevant information to distinguish apple cultivars with different chilling requirements. Developing accurate predictions of how different apple cultivars might respond to future climate change will be challenging until the physiological mechanisms regulating bud break are more fully understood. Whilst important advances have been made over the last few decades, such as the identification of candidate genes for dormancy regulation in apple (Mimida *et al.*, 2015; Wisniewski, Norelli and Artlip, 2015; Wu *et al.*, 2017); genetic or physiological markers have not yet been identified and it will take time until the implications of any findings are transferred to growing practices.

An interesting area that has received relatively little attention (but see Fernandez et al., 2020; Shellie et al., 2018), is the potential effect of water deficit stress on dormancy and bud break. Whilst reduced soil water availability can negatively impact on tree growth, yield and fruit size in apple (Naor et al., 2008; Bolat et al., 2014), Regulated Deficit Irrigation (RDI) can also be used in deciduous fruit trees to regulate vegetative growth, without a detrimental effect on fruit production if applied at an appropriate time and at the correct severity (Ebel et al., 1995). Interestingly, a study with "Elstar" apple trees showed that, after low-chilling accumulation, buds in shoots from mildly water-stressed trees developed faster and had a higher probability of flowering in spring than buds from irrigated trees (Fernandez et al., 2020). In grapevines, Shellie et al., (2018) observed a reduction in the amount of chilling required to exit endodormancy in deficit-irrigated vines, compared to irrigated vines. Understanding the link between water deficit stress and dormancy could inform the design of new orchard management practices that could be highly beneficial in a climate change context.

A further impact of the changes in chilling and heat accumulation patterns due to climate change that has not been considered here, is a potential loss of flowering synchrony between cross-pollinating cultivars, and with pollinator emergence. As apple trees are self-incompatible, fruit production requires mixed plantings of compatible cultivars to ensure high levels of fruit set (Schneider et al., 2005; Ramírez and Davenport, 2013). Therefore, an overlap in flowering times between compatible cultivars is needed for insects to cross-pollinate (Jackson, 2003). A risk of asynchrony between cross-pollinating cultivars increases with climate change, as different cultivar responses to changes in chilling and heat accumulation have been observed (Chapters 3, 4 and 5).

Furthermore, as apple is an insect-pollinated crop, advances or delays in blooming times could also cause asynchrony with insect appearance, negatively impacting overall fruit production (Korösi et al., 2018).

Future work should focus on comparing the effect of changes in chilling and heat accumulation on cross-compatible cultivars. Other factors such as temperatures during fruit set and pollination should be considered to improve our understanding of the overall impact on final fruit production. As the effects of climate change become more evident, a holistic approach is required, including research on all factors influencing fruit production, and considered across key phenological stages.

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Appendix A – Extra Figures

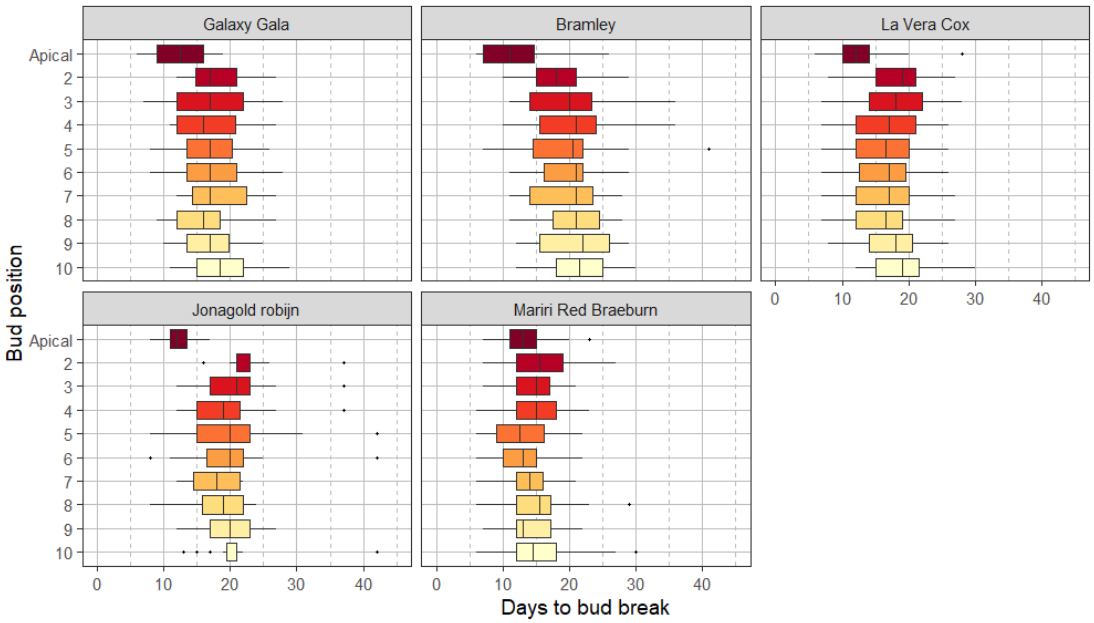

Figure A1 – Days to bud break of the 10 upper buds in an excised shoot, after being forced at 25 °C. Colours represent de proximity of a bud to the apex, with the darkest red being the apical bud. Boxplots show the median, first and third quartile, maximum and minimum values and outliers. Ten shoots were collected per variety in January 2018, as part of an experiment from the first year of this PhD (not presented). (Chapter 3)

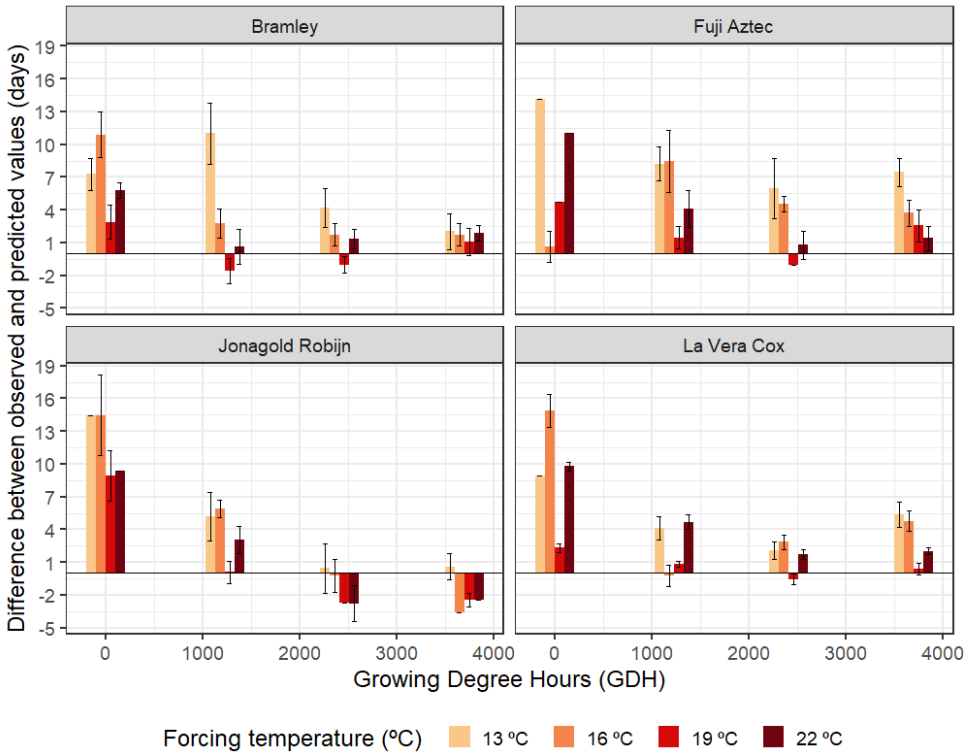

Figure A2 - Differences between observed and predicted days to first bud break of shoots that had accumulated different GDH in the field from the start of ecodormancy; and were forced at a range of forcing temperatures (13, 16, 18 and 22 °C). Each box represents an apple cultivar. Models were trained with 2018/19 data and tested with 2019/20 data. (Chapter 3)

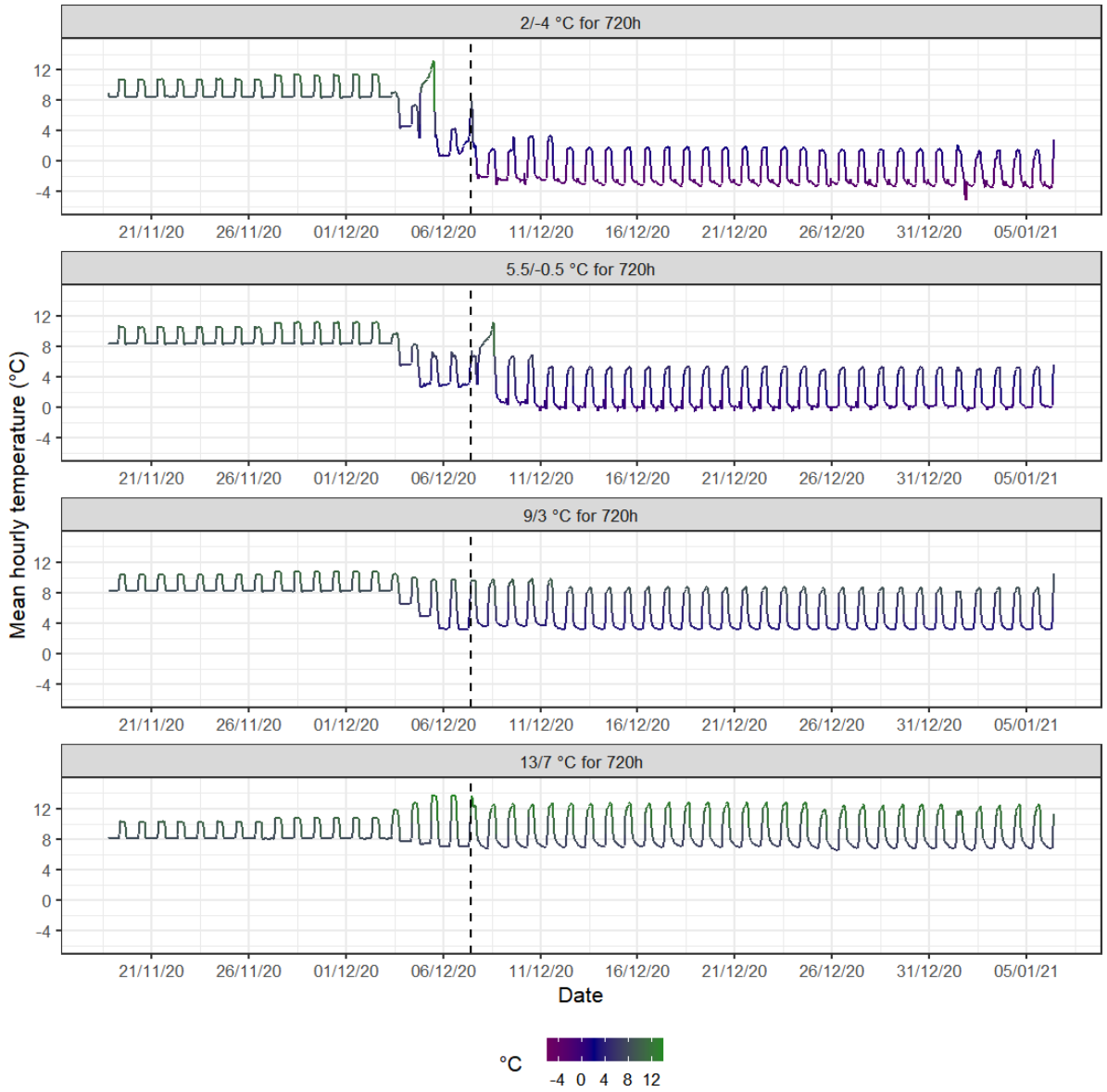

Figure A3 - Hourly temperatures for treatments C1-720, C2-720, C3-720 and C4-720 (Chapter 5), as recorded with data loggers inside the growth cabinets. Vertical line indicates the end of the acclimation period.

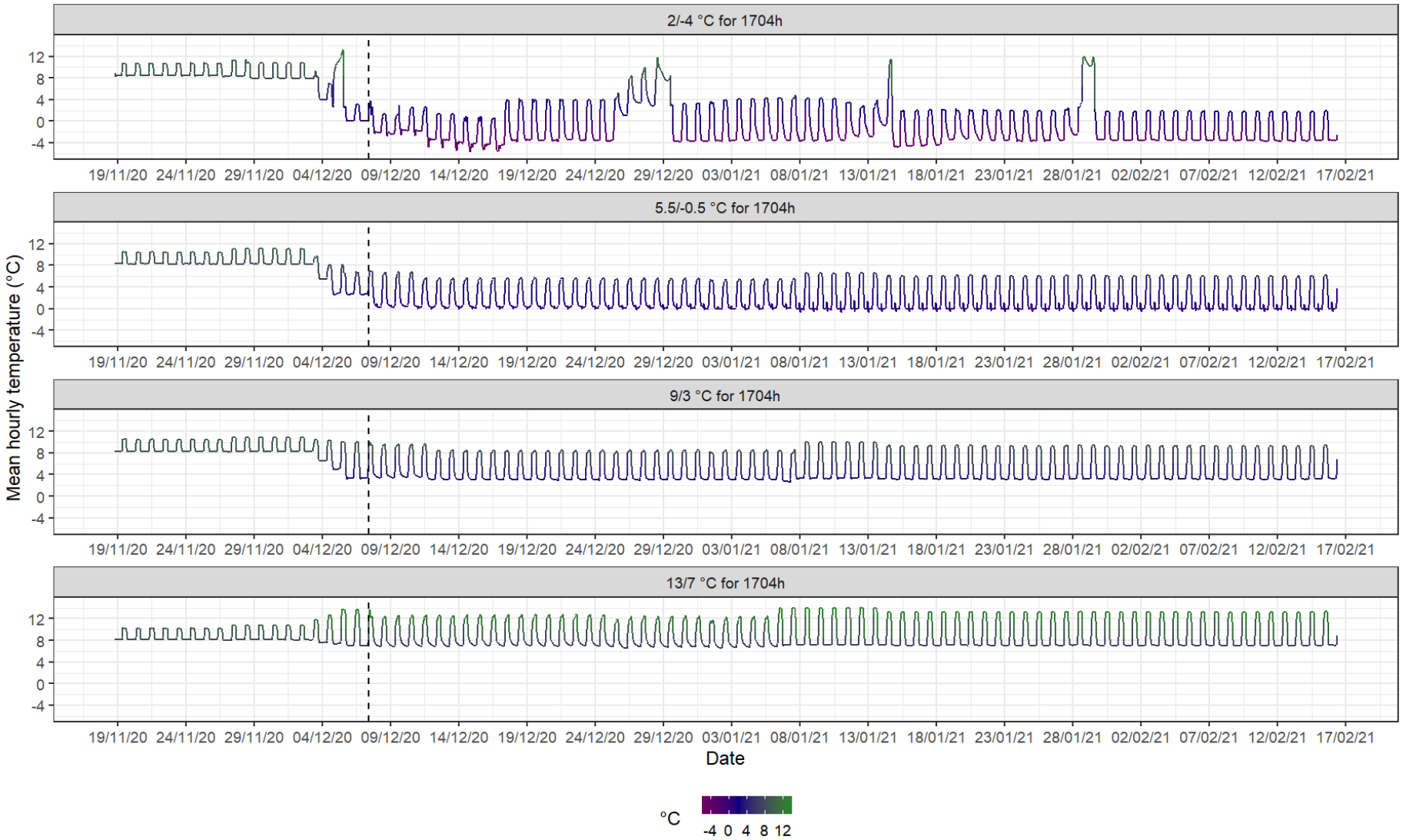

Figure A4 - Hourly temperature for treatments C1-1704, C2-1704, C3-1704 and C4-1704 (Chapter 5), as recorded with data loggers inside the growth cabinets. Vertical line indicates the end of the acclimation period.

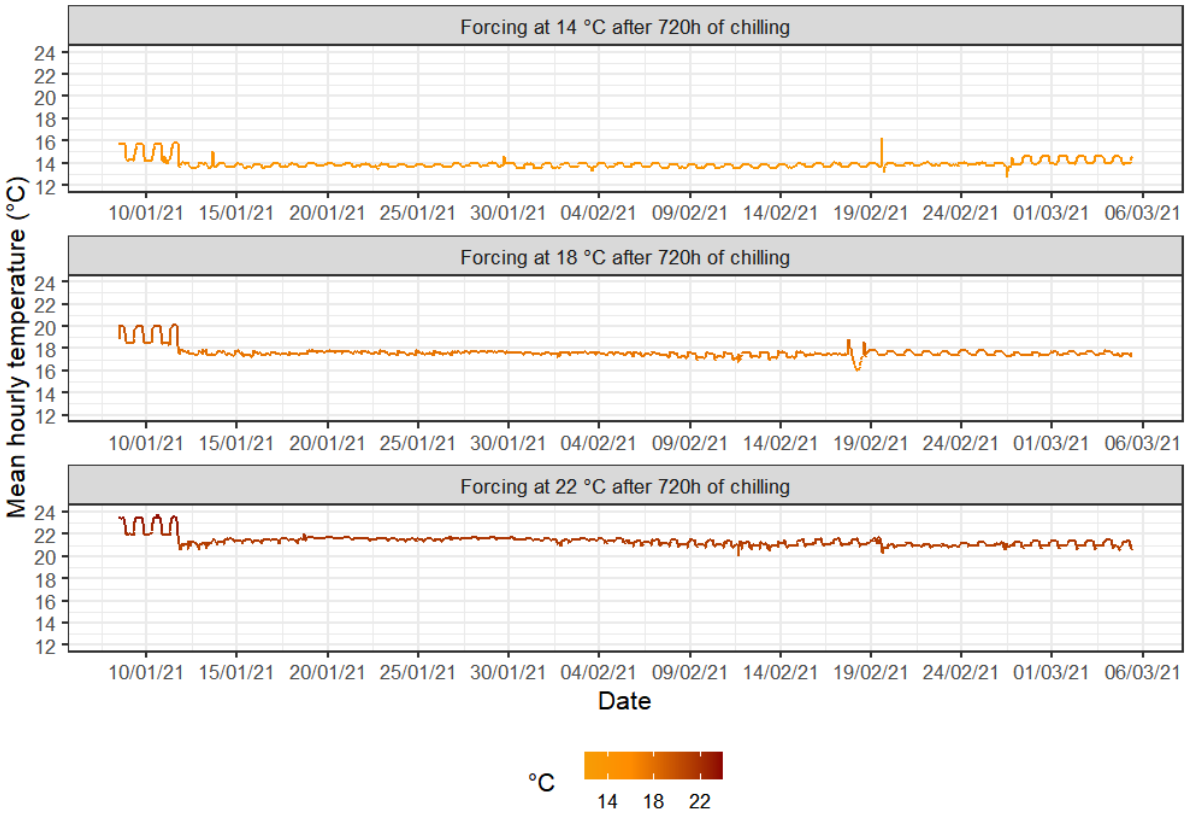

Figure A5 - Hourly temperatures for treatments F1, F2 and F2 after 720h of chilling (Chapter 5), as recorded with data loggers inside the growth cabinets.

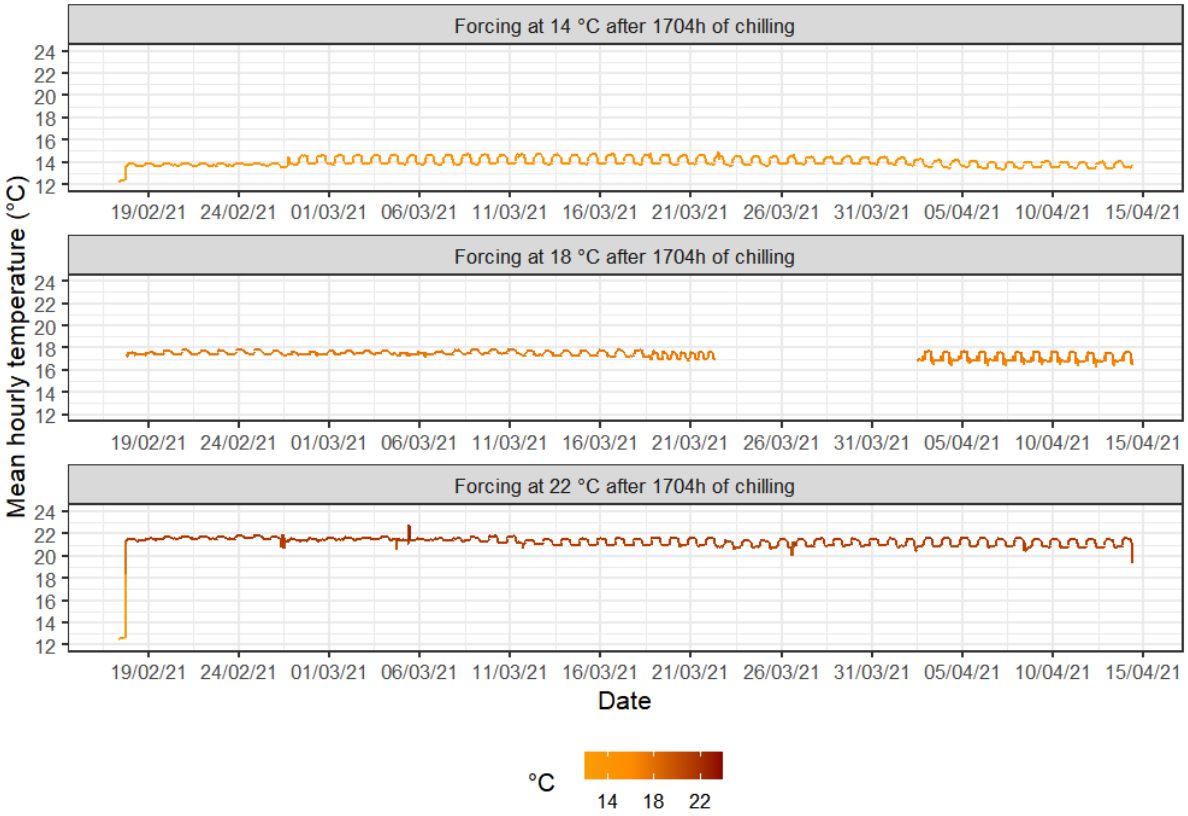

Figure A6 - Hourly temperatures for treatments F1, F2 and F2 after 1704h of chilling (Chapter 5), as recorded with data loggers inside the growth cabinets.

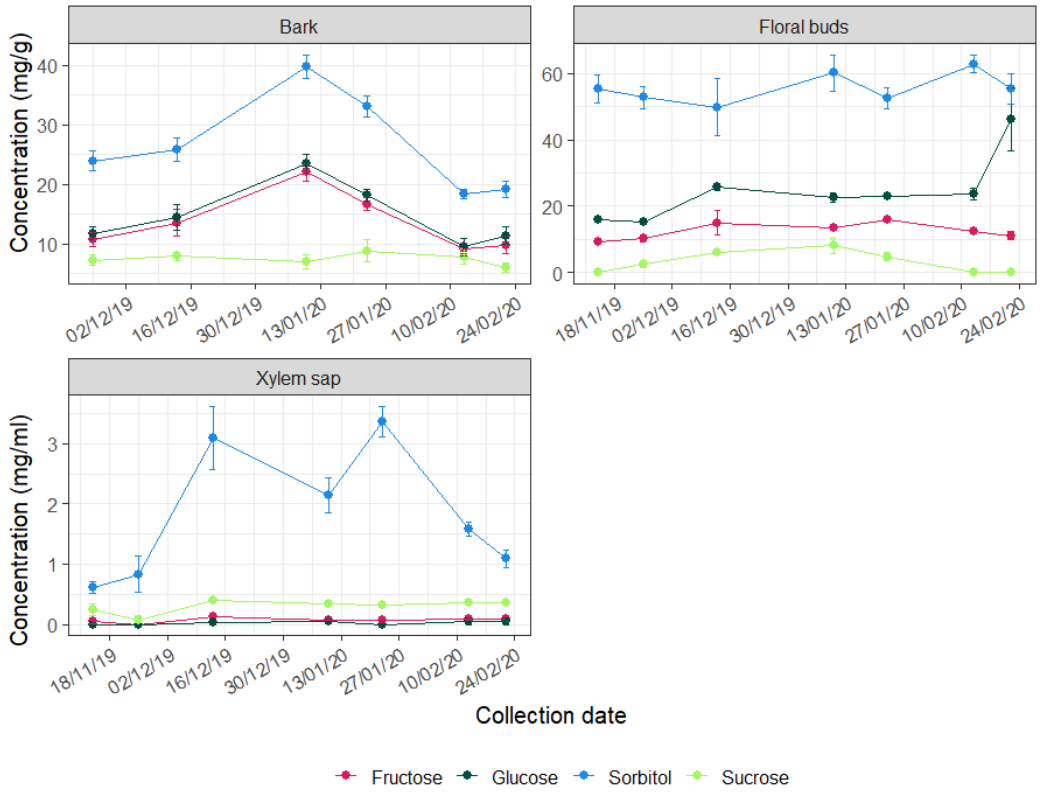

Figure A7 – Concentration of carbohydrates in bark, floral buds (top figures, mg of carbohydrate in g of dry bud) and xylem sap (bottom, mg of carbohydrate in a ml of xylem sap) of the cultivar "Braeburn Mariri Red". Samples collected between 2019 and 2020 as a preliminary experiment to test the methodology. Each point represents the mean of five replicates, error bars show ±1 SE from the mean (Chapter 6).

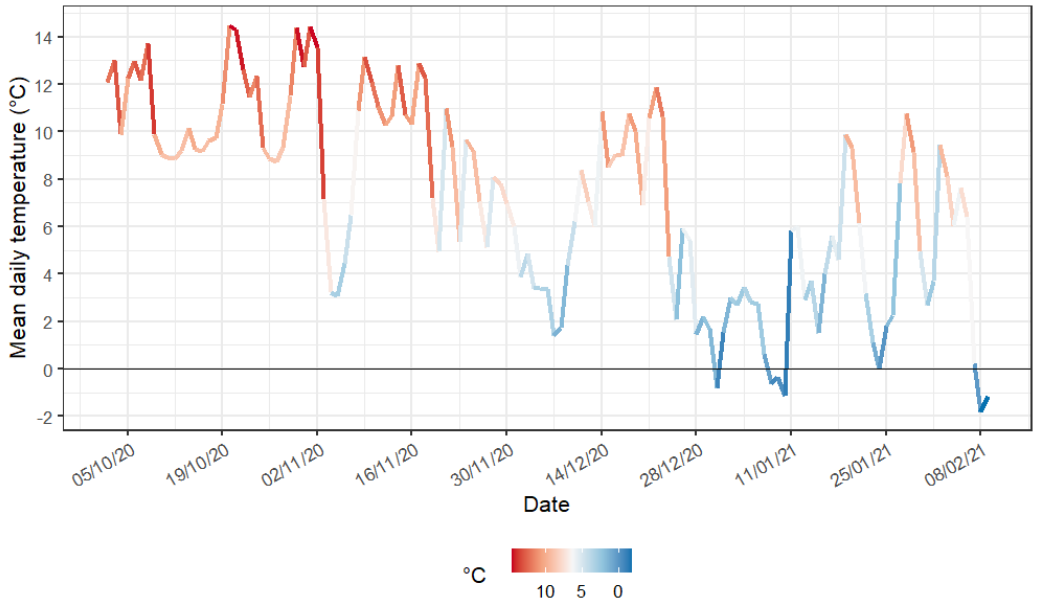

Figure A8 – Mean daily temperature (°C) in the field during the dormancy period (October 2020 to February 2021) (Chapter 6).

Appendix B – Extra Tables

Table B1 – Date of first bud break, 50% bud break, and number of days in between, for a range of apple cultivars. Data recorded during two years, dates are the average of five trees growing in a field at NIAB EMR (Chapter 3).

Year	Cultivar	Date of first bud break	Date of 50% bud break	Days between first and 50% bud break
2018/19	Bramley	23/03/2019	20/04/2019	28
	Fuji Aztec	21/03/2019	05/04/2019	15
	Galaxy Gala	19/03/2019	27/03/2019	8
	Jonagold Robjin	16/03/2019	13/04/2019	28
	Braeburn Mariri Red	16/03/2019	28/03/2019	12
	La Vera Cox	27/03/2019	08/04/2019	12
2019/20	Bramley	25/03/2020	14/04/2020	20
	Fuji Aztec	26/03/2020	07/04/2020	12
	Galaxy Gala	30/03/2020	13/04/2020	14
	Jonagold Robjin	12/03/2020	13/04/2020	32
	Kingston Black	05/04/2020	25/04/2020	20
	Braeburn Mariri Red	19/03/2020	01/04/2020	13
	La Vera Cox	27/03/2020	11/04/2020	15
	Dabinett	27/04/2020	*	NA

*50% bud break was not observed within the assessment period (1/3/20 to 15/5/20)

Table B2 – Results from Cultivar-specific mixed models on days to first bud break for dessert apple cultivars. Models include 3 variables: forcing temperature, GDH accumulated and the interaction between both variables. GDH was calculated from satisfaction of chilling requirements. Results presented: variable estimates, standard error (SE), t-value and p-value indicating significance level of each variable. Analysis of Variance Table with Satterthwaite's method. Models trained with 2018/19 data from collections 4-7 (Chapter 3).

Cultivar	Variable	Estimate	SE	t-value	P-value
Bramley	Intercept	4.66e+01	4.86e+00	9.597	
	Forcing temperature	-1.83e+00	2.10e-01	-8.702	6.559e-15 ***
	GDH accumulated	-2.17e-02	3.29e-03	-6.577	2.231e-05 ***
	Forcing temp * GDH	7.30e-04	1.46e-04	5.007	1.585e-06 ***
Fuji Aztec	Intercept	4.422e+01	4.887e+00	9.048	
	Forcing temperature	-1.929e+00	2.543e-01	-7.586	1.867e-05 ***
	GDH accumulated	-0.0212546	0.0036687	-5.793	1.781e-05 ***
	Forcing temp * GDH	0.0007699	0.0001894	4.064	0.001047 **
Jonagold Robijn	Intercept	4.25e+01	8.17e+00	5.206	
	Forcing temperature	-1.71e+00	4.17e-01	-4.107	0.002132 **
	GDH accumulated	-2.06e-02	5.82e-03	-3.535	0.002664 **
	Forcing temp * GDH	7.02e-04	2.97e-04	2.366	0.033252 *
La Vera Cox	Intercept	4.91e+01	5.78e+00	8.494	
	Forcing temperature	-2.15e+00	2.88e-01	-7.484	2.11e-05 ***
	GDH accumulated	-2.14e-02	3.76e-03	-5.699	0.0001162 ***
	Forcing temp * GDH	7.66e-04	1.87e-04	4.09	0.0021693 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

Table B3 – Model equation, R2 and Residual Standard Error (RSE) of the linear regressions performed to find the optimum chilling temperature for each cultivar and duration of chilling (1,080; 1,776 and 2,424h). Optimum and effective chilling temperatures were calculated as described in Section 4.2.4 Data analyses. Results presented for the linear regressions of the optimum temperature, 0.1 °C above and 0.1 °C below the optimum (Chapter 4).

Braeburn Lochbuie										
Hours of chilling	**Real and effective chilling temperatures (°C)**								**Model Equation**	**R^2**	**RSE**
	Real chilling temperature	**-4**	**-2.5**	**-1**	**1.75**	**4.5**	**7.25**	**10**			
1080	Effective temp at optimum temperature (0.8 °C)	4.8	3.3	1.8	0.95	3.7	6.45	9.2	y = -0.00177x + 0.0502	0.8026	0.002706
	Effective temp at optimum +0.1 °C (0.9 °C)	4.9	3.4	1.9	0.85	3.6	6.35	9.1	y = -0.00179x + 0.0503	0.802	0.00271
	Effective temp at optimum -0.1 °C (0.7 °C)	4.7	3.2	1.7	1.05	3.8	6.55	9.3	y = -0.00174x + 0.0501	0.8012	0.002716
1776	Effective temp at optimum temperature (4.4 °C)	8.4	6.9	5.4	2.65	0.1	2.85	5.6	y = -0.00423x + 0.0609	0.7951	0.006679
	Effective temp at optimum +0.1 °C (4.5 °C)	8.5	7	5.5	2.75	0	2.75	5.5	y = -0.00413x+ 0.0605	0.7944	0.00669
	Effective temp at optimum -0.1 °C (4.3 °C)	8.3	6.8	5.3	2.55	0.2	2.95	5.7	y = -0.00431x + 0.0612	0.7942	0.006693
2424	Effective temp at optimum temperature (6.1 °C)	10.1	8.6	7.1	4.35	1.6	1.15	3.9	y = -0.00597x + 0.0947	0.8300	0.01018
	Effective temp at optimum +0.1 °C (6.2 °C)	10.2	8.7	7.2	4.45	1.7	1.05	3.8	y = -0.00587x + 0.0944	0.8293	0.0102
	Effective temp at optimum -0.1 °C (6.0 °C)	10	8.5	7	4.25	1.5	1.25	4	y= -0.00606x + 0.0949	0.8298	0.01018
Discovery											
Hours of chilling	**Real and effective chilling temperatures (°C)**								**Model Equation**	**R^2**	**RSE**
	Real chilling temperature	**-4**	**-2.5**	**-1**	**1.75**	**4.5**	**7.25**	**10**			
1080	Effective temp at optimum temperature (-2.5°C)	1.5	0	1.5	4.25	7	9.75	12.5	y = -0.00208x + 0.04	0.8493	0.004508
	Effective temp at optimum +0.1 °C (-2.4°C)	1.6	0.1	1.4	4.15	6.9	9.65	12.4	y = -0.00285x + 0.0495	0.6199	0.008314
	Effective temp at optimum -0.1 °C (-2.6°C)	1.4	0.1	1.6	4.35	7.1	9.85	12.6	y = -0.00206x + 0.0401	0.8446	0.004577
1776	Effective temp at optimum temperature (-0.4°C)	3.6	2.1	0.6	2.15	4.9	7.65	10.4	y = -0.0028x + 0.0493	0.6204	0.008308
	Effective temp at optimum +0.1 °C (-0.3°C)	3.7	2.2	0.7	2.05	4.8	7.55	10.3	y = -0.00285x + 0.0495	0.6199	0.008314
	Effective temp at optimum -0.1 °C (-0.5°C)	3.5	2	0.5	2.25	5	7.75	10.5	y = -0.00274x + 0.0491	0.6203	0.008309
2424	Effective temp at optimum temperature (1.4 °C)	5.4	3.9	2.4	0.35	3.1	5.85	8.6	y = -0.00384x + 0.07	0.8479	0.004763
	Effective temp at optimum +0.1 °C (1.5 °C)	5.5	4	2.5	0.25	3	5.75	8.5	y = -0.00385x + 0.07	0.8463	0.004789
	Effective temp at optimum -0.1 °C (1.3 °C)	5.3	3.8	2.3	0.45	3.2	5.95	8.7	y = -0.00382x + 0.0699	0.8469	0.004779

Table B4 – Chilling temperature contributions in the *Malus model* (Chapter 5).

Below optimum	
Chilling temperature (° C)	**CF per hour**
-10	0
-9.5	0.0625
-9	0.1250
-8.5	0.1875
-8	0.2500
-7.5	0.3125
-7	0.3750
-6.5	0.4375
-6	0.5000
-5.5	0.5625
-5	0.6250
-4.5	0.6875
-4	0.7500
-3.5	0.8125
-3	0.8750
-2.5	0.9375
-2	1

Above optimum	
Chilling temperature (° C)	**CF per hour**
-1.5	0.9668
-1	0.9334
-0.5	0.9001
0	0.8667
0.5	0.8334
1	0.8000
1.5	0.7667
2	0.7333
2.5	0.7000
3	0.6666
3.5	0.6333
4	0.5999
4.5	0.5666
5	0.5332
5.5	0.4999
6	0.4665
6.5	0.4332
7	0.3998
7.5	0.3665
8	0.3331
8.5	0.2998
9	0.2664
9.5	0.2331
10	0.1997
10.5	0.1664
11	0.1330
11.5	0.0997
12	0.0663
12.5	0.0330
13	0

Table B5 – Time of bud break of studied cultivars in 2020 and 2021, (*) indicates data not available as trees were grubbed before bud break (Chapter 6).

Year	Cultivar	Date of first bud break
2020	Bramley	25/03/2020
	Fuji Aztec	26/03/2020
	Galaxy Gala	30/03/2020
	Jonagold Robjin	12/03/2020
	Kingston Black	05/04/2020
	Braeburn Mariri Red	19/03/2020
	La Vera Cox	27/03/2020
	Dabinett	27/04/2020
2021	Bramley	20/03/2021
	Fuji Aztec	*
	Galaxy Gala	16/03/2021
	Jonagold Robjin	*
	Kingston Black	27/03/2021
	Braeburn Mariri Red	10/03/2021
	La Vera Cox	28/03/2021
	Dabinett	01/04/2021
	Anna	06/02/2021