



Project title: Apple dormancy break in the context of climate change

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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- Cultivar-specific models for predicting time of bud break have been developed for six apple cultivars in different climate change scenarios.

Background

Climate change is predicted to impact adversely on UK apple production, with warmer winters and an increased risk of late frost events of particular concern. Warmer temperatures will affect the dormancy cycle, which determines the timing and quality of bud break. Insufficient chilling can reduce and/or delay bud break (Petri and Leite, 2004), cause non-uniform flowering and, as a consequence, the production of smaller and abnormal fruits. At the same time, warmer spring temperatures can advance blooming dates, thereby increasing the risk of yield losses due to late frost events.

The dormancy cycle in apple trees is regulated solely by temperature (Heide and Prestrud, 2005), making the apple industry especially vulnerable to any changes in the climate. As chilling requirements vary between cultivars, in the short to medium term it is important to anticipate how different varieties are likely to respond to climate changes so that informed commercial planting decisions can be made over the next few decades. Whilst in the longer term it may be necessary to breed/select new varieties with reduced chill requirements.

Three main difficulties hinder the formulation of accurate predictions: (i) current chilling and heating models used for predicting bud break are not cultivar-specific, (ii) the models do not incorporate the climatic variability expected with global warming; and (iii) they often lack a link to biological principles as the physiological mechanisms behind dormancy break are not well understood. This project aims to investigate these three aspects with the final goal of developing an improved model for bud break prediction, which will be a useful tool to help to inform cultivar selection.

Using a combination of controlled environment experiments with excised shoots and potted trees, we investigated the relationship between temperature and time of bud break for six apple cultivars.

Summary

In the second year of this PhD we investigated the relationship between temperature and bud break of six apple cultivars. The effect of different chilling temperatures and durations of chilling were also studied. We developed cultivar-specific models incorporating winter chilling

and spring temperatures as factors determining time of bud break. These showed a cultivar-specific response to both factors as well as an interaction between chilling and spring temperature. Future work will continue investigating the effect of different chilling temperatures on bud break as well as some of the physiological mechanisms behind bud break.

Financial Benefits

This report summarises part of the work carried out in the second year of a four-year project, and so there are no direct financial benefits as yet. However, the project will provide key information for cultivar selection to the apple industry, a crucial decision for a crop with a lifespan of 15+ years and one that is highly susceptible to temperature changes predicted with global warming.

Action Points

There are no grower action points at this early stage of the project.

SCIENCE SECTION

Introduction

Global climate is changing as a consequence of an increase in greenhouse gas emissions due to anthropogenic activity (IPCC, 2014). If emissions continue growing unmitigated, mean winter and summer temperatures in the UK are predicted to increase by 2°C, and 3°C respectively, by 2060 (Murphy *et al.*, 2018). This would impact many apple cultivars currently being planted and have devastating consequences for the agriculture and food production industries which have been identified as “at risk” (IPCC, 2014).

Apple represents one of the biggest fruit crops in the UK, accounting for a value of production of over 165 million pounds for dessert and culinary varieties (Department for Environment, Food and Rural Affairs, 2018). In the UK, the potential impacts on agriculture of higher temperatures include a longer growing season with an earlier start, with an associated increased risk of late frost events (Harding *et al.*, 2015). The flowering stage in apple is particularly sensitive to changes in the climate and significant production losses have been reported in the past due to late spring frosts (Department for Environment, Food and Rural Affairs *et al.*, 2017). A particular concern of higher temperatures on apple production is the effect on dormancy (Campoy, Ruiz and Egea, 2011; Atkinson, Brennan and Jones, 2013). Perennial tree crops enter a dormant state during winter months which enables their survival in adverse environmental conditions. An absence of chilling temperatures can reduce and/or delay bud break (Petri and Leite, 2004), cause non-uniform flowering and, as a consequence, produce smaller and abnormal fruits. High temperatures in winter are negatively correlated with yield (Jackson and Hamer, 1980).

A reduction in winter chill (Sunley, Atkinson and Jones, 2006), combined with an increased risk of frost damage as a consequence of an earlier start to the growing season (Harding *et al.*, 2015) create an uncertain and concerning future scenario for apple production. It is important to anticipate how cultivars are likely to respond to these changes in the climate so that informed commercial planting decisions can be made over the next few decades.

The dormancy process has been artificially divided into three phases; paradormancy, endodormancy and ecodormancy (Lang, 1987). Whilst temperature and photoperiod regulate the transition between phases in most species (Garner and Allard, 1923), the only environmental cue determining dormancy induction and release in apple is temperature (Heide and Prestrud, 2005). During paradormancy or summer dormancy, terminal buds inhibit growth of axillary buds. Colder temperatures induce the transition towards endodormancy (Garner and Allard, 1923; Heide and Prestrud, 2005), when growth is prevented by internal

bud signals (Lang, 1987). Endodormancy is overcome by extended periods of chilling (Lang, 1987), known as chilling requirement (CR), which removes the physiological “blocks” that prevent growth. Trees remain ecodormant until environmental conditions are favourable for growth. Higher temperatures are needed to exit ecodormancy and promote bud development and blooming. The minimum amount of heat needed for bud break is known as the Heat Requirement (HR).

Chilling requirements vary greatly between apple cultivars (Hauagge and Cummins, 1991). Due to its importance for climate change adaptation, CR should be considered when selecting future cultivars and is a key trait to be included in breeding programmes. Statistical modelling is used to calculate CR and HR. The first chilling model developed, known as the “Chilling Hours model” or “below 7.2 °C model”, considers all temperatures below 7.2 °C to make an equal contribution to chilling accumulation (Weinberger, 1950) and does not take into account the effect of higher temperatures. This is a very simplistic approach to chilling accumulation modelling and it was soon demonstrated that not all temperatures contribute in the same way, and that higher temperatures have a negative effect on chill accumulation (Erez, Couvillon and Hendershott, 1979). Nowadays, the two most widely used models are the “Utah model” (Richardson *et al.*, 1974) and the “Dynamic model” (Fishman, Erez, and Couvillon, 1987). They both consider a different range of temperatures for chilling accumulation and a negating effect of higher temperatures; but the way in which the low and high temperatures interact differs. Both models have successfully predicted bud break in the studies used to develop them (Richardson *et al.*, 1974; Fishman, Erez, and Couvillon, 1987), but they have shown large inaccuracies when applied to low-chill varieties (Gilreath and Buchanan, 1981), to varieties and locations different from the ones used to parametrise the model, and when used under climate change scenarios (Legave *et al.*, 2008, 2013; Luedeling *et al.*, 2009).

Chilling models are combined with heating models to predict bud break and blooming dates. Many combinations of sub-chilling and sub-heating models have been compared for a range of species (Cesaraccio *et al.*, 2004; Legave *et al.*, 2008, 2013; Luedeling *et al.*, 2009; Chuine *et al.*, 2016; Darbyshire *et al.*, 2017). Results are varied and inconclusive, with model performance being highly variable depending on cultivar, location and time (Legave *et al.*, 2008, 2013; Luedeling *et al.*, 2009; Chuine *et al.*, 2016).

A chilling accumulation model capable of accurately predicting bud break for a range of cultivars, locations and climatic conditions is vital to guide cultivar selections in future plantings in the UK and overseas. The lack of accuracy in predicting time of bud break might be due to a missing link between model and biological parameters (but see Chuine *et al.*, 2016; Darbyshire *et al.*, 2017) as the physiological and molecular mechanisms regulating dormancy are still not fully understood. Changes in the balance of hormones are associated

with the dormancy process (Olsen, Junttila and Moritz, 1995; Olsen *et al.*, 1997, Li *et al.*, 2003, Cline, 2000; Ruttink *et al.*, 2007) but a direct regulatory effect has not yet been demonstrated. At a cellular level, several changes have also been observed during dormancy development; including the conversion from bound to free water in bud cells (Faust *et al.*, 1991), changes in the composition of lipids in cell membranes (Wang and Faust, 1990) and closure of plasmodesmata in cell walls, reducing cell-to-cell communication during dormancy (Rinne, Kaikuranta and van der Schoot, 2001). Differential gene expression throughout the dormancy-growth cycle has been reported in several studies (Ruttink *et al.*, 2007; Porto *et al.*, 2015). Although no genetic markers for chilling requirement have yet been developed, several studies have identified candidate genes for dormancy regulation in apple (Mimida *et al.*, 2015; Wisniewski, Norelli and Artlip, 2015; Wu *et al.*, 2017). The influence of environmental factors on dormancy and a close link with other physiological processes such as cold acclimation, make it difficult to be certain that changes observed in gene expression are linked to dormancy itself and not to other factors.

Project aim

To investigate the impacts of climate change on dormancy of apple trees and formulate recommendations for UK growers to inform cultivar selection for future plantings.

Objectives

1. To investigate the relationship between temperature and bud break in a range of apple cultivars (Years 1, 2 and 3)
2. To develop a model for predicting bud break in a range of apple cultivars (Years 3 and 4)
3. To investigate the accuracy of the new model for predicting bud break under future climate change scenarios (Year 4)
4. To investigate the physiological mechanisms regulating dormancy break (Years 3 and 4)

Materials and methods

Experiment 1 - Defining the relationship between temperature and bud break

One-year-old 40-50 cm long shoots from the following apple cultivars were used for this experiment: “Bramley”, “Galaxy Gala”, “Mariri Red Braeburn”, “La Vera Cox”, “Jonagold Robijn” and “Fuji Aztec”. Shoots were collected from trees grown in the same field at NIAB EMR. All trees had been grafted onto “M9” rootstocks and planted in 2014. Shoots were sampled between 1 and 1.8 m height of the tree, growing towards all cardinal points.

Seven different collections were done between October 2018 and March 2019 at which 40 shoots per variety were sampled. Due to scarcity of bud wood, only 20 shoots per collection from “Jonagold Robijn” and “Fuji Aztec” were sampled. Information on the date of each collection and number of shoots collected is provided in Table 1.

Table 1 – Collection dates and number of shoots collected per cultivar and collection

| Variety | Number of shoots collected | | | | | | |
|----------------------|------------------------------|------------------------------|------------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|
| | Date of collection | | | | | | |
| | 26 th Oct 2018 | 22 th Nov 2018 | 14 th Dec 2018 | 4 th Jan 2019 | 28 th Jan 2019 | 21 st Feb 2019 | 14 th Mar 2019 |
| Bramley | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Galaxy Gala | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Mairiri Red Braeburn | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| La Vera Cox | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Jonagold Robijn | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Fuji Aztec | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

After being collected, shoots were shortened to 30 cm length canes and individually labelled; varieties were differentiated with colour tapes. Shoots were placed into four growth chambers (Panasonic Versatile Environmental Test Chamber - MLR-352H) in which humidity, light and temperature were controlled. Apple varieties were randomly distributed inside each chamber, standing in 2.5 litre buckets with a mixture of tap water and bleach at 5 ml/litre of water. A plastic mesh was used to minimise contact between shoots (Figure 1). Once a week, the water mix was changed and 1 cm of the base of each shoot was cut to prevent vessel occlusion.

Shoots were incubated with a humidity set at 90% and a photoperiod of 16 h light and 8 h of darkness. Light was supplied with 15 fluorescent lamps (FL40SSENW37), providing a photosynthetic photon flux density of approximately 300 $\mu\text{mol}/\text{m}^2\text{s}$. Different constant temperatures were used as treatments: 13, 16, 19 and 22 °C. Environmental conditions inside the cabinets were monitored with Lascar EL-USB-2 data loggers to ensure accurate environmental data was available for analyses.



Figure 1 - Shoots from collection 3 before being placed into forcing conditions. Picture taken on 14/12/18.



Figure 2 - Green tip stage. Picture taken on 20/03/2018

Shoots were inspected every 3-4 days for 6 weeks. For each assessment, the number of floral and vegetative buds reaching Green tip (stage 3 of development, as defined by Chapman and Catlin (1976)) (Figure 2) per shoot was recorded.

Experiment 2 - Investigating the effect of chilling temperature and duration of chilling on bud break of two apple cultivars

Two-year-old, well feathered (more than five branches) trees were used for this experiment. They measured between 1.8 and 2.5 m tall at the start of the experiment and were potted in 12 litre pots in a Sinclair pro compost mix containing bark, lime, coarse and medium peat, slow release fertiliser and wetter.

Two different apple cultivars were used: 80 “Mariri Red Braeburn” and 60 “Galaxy Gala”. Trees had been grafted in January 2017 and grown in the Netherlands (Botden & van Willegan nursery (51.633750, 5.957400)). They were lifted in November 2018 and transported to the UK in a non-insulated lorry.

After potting, trees were randomly placed on a sand bed for acclimation before starting any chilling treatment (Figure 3). A unique identifier number was given to each tree, which was individually labelled.

After acclimation in the sand bed, trees were moved into different chilling treatments. Four replicates of each cultivar were moved directly into the glasshouse at NIAB EMR in order to assess the initial depth of dormancy of the trees (Group 0, Figure 4).

Thirty-three trees were transported to the University of Reading in a non-insulated van (Figure 5). Trees were securely arranged to ensure minimum damage during transport.



Figure 3 - Acclimation of potted trees on a sand bed at NIAB EMR. Picture taken on 04/01/19



Figure 4 – Trees placed directly in the glasshouse at NIAB EMR after acclimation on a sand bed. Picture taken on 22/01/19.



Figure 5 - Transport of trees from NIAB EMR to the University of Reading. Picture taken on 23/01/19.

Between 29th of January and 4th of March, trees from all cold stores were gradually moved into the glasshouses, with five different removal events in total (Figure 6).

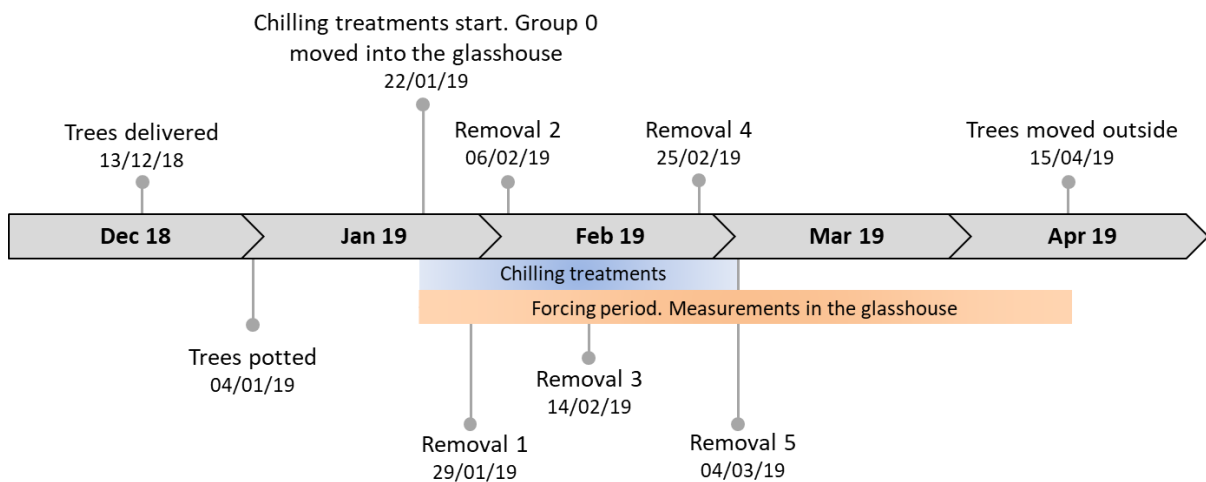


Figure 6 - Timeline potted trees experiment

Cold store facilities - Chilling treatments

Four different treatments were carried out: three artificial chilling treatments, provided in the dark, and one natural chilling provided under natural photoperiod.

Temperature and humidity were recorded hourly during all treatments. In Treatments 2, 3 and 4 they were monitored with a Decagon VP-4 temperature and humidity sensor, connected to a Decagon Em50G remote logger. In Treatment 1 it was monitored with a Lascar EL-USB-2 data logger.

Trees were randomly distributed inside each cold store or sand bed. 19 “Mariri Red Braeburn” and 14 “Galaxy Gala” received each treatment.

- Treatment 1: chilling at 2 °C, provided in a cold store located at NIAB EMR (East Malling). (Figure 7)
- Treatment 2: chilling at 7.5 °C, provided in a modified container with a cooling fan, located at NIAB EMR (East Malling). (Figure 8)
- Treatment 3: natural chilling. Trees were left in a sand bed at NIAB EMR (East Malling), receiving natural chilling.
- Treatment 4: chilling at 4.5 °C, provided in a cold store located at the University of Reading (Reading). (Figure 9)



Figure 7 – 2 °C cold store at NIAB EMR. Picture taken on 22/01/19.



Figure 8 - 7.5 °C cold store at NIAB EMR. Picture taken on 22/01/19.



Figure 9 - 4.5 °C cold store at the University of Reading. Picture taken on 23/01/19.

Between 3 and 5 trees from each variety and temperature treatment were moved into forcing conditions once a week (representing approximately accumulation of 200 chill units (Richardson, Seeley and Walker, 1974)). Five different removals were carried out for each temperature treatment. Information on the number of trees moved and date of removal can be found on Table 2.

Table 2 – Number of trees moved into the glasshouse at NIAB EMR (Treatments 1, 2 and 3) and the University of Reading (Treatment 4) at each removal event. Braeburn corresponds to “Mariri Red Braeburn” and Gala to “Galaxy Gala”.

| Treatment | Number of trees moved into the glasshouse | | | | |
|-------------------------------|---|-----------------------|-----------------------|-----------------------|-----------------------|
| | Date of removal | | | | |
| | Removal 1 29/01/19 | Removal 2 06/02/19 | Removal 3 14/02/19 | Removal 4 25/02/19 | Removal 5 04/03/19 |
| T1: chilling at 2 °C | 5 Braeburn | 5 Braeburn 3 Gala | 5 Braeburn 4 Gala | 4 Braeburn 4 Gala | 3 Gala |
| T2: chilling at 7.5 °C | 5 Braeburn | 5 Braeburn 3 Gala | 5 Braeburn 4 Gala | 4 Braeburn 4 Gala | 3 Gala |
| T3: natural chilling | 5 Braeburn | 5 Braeburn 3 Gala | 5 Braeburn 4 Gala | 4 Braeburn 4 Gala | 3 Gala |
| T4: chilling at 4.5 °C | 5 Braeburn | 5 Braeburn | 5 Braeburn | 4 Braeburn | 3 Gala |

| | | | | | |
|--|--|--------|--------|--------|--|
| | | 3 Gala | 4 Gala | 4 Gala | |
|--|--|--------|--------|--------|--|

Glasshouse facilities - Forcing treatment

Due to the separate locations of the chilling treatments, the impracticability of weekly transporting trees from one location to another and the possible effect that would have had on the trees; two different heated glasshouses were used for the forcing treatments. However, forcing conditions were the same in both glasshouses: 20 °C and natural lighting. Temperature and humidity in both glasshouses were recorded hourly with a Decagon VP-4 temperature and humidity sensor, connected to a Decagon Em50G remote logger. Trees were randomly located inside each glasshouse, distributed in groups of four to maximise space for data collection.

- Glasshouse at NIAB EMR: a 25 m² compartment was used for forcing conditions (Figure 10). Automatic irrigation was provided. Trees from chilling treatments 1, 2 and 3 were moved into this glasshouse.
- Glasshouse at the University of Reading: approximately 6 m² (Figure 11) were used from a >50 m² glasshouse. Trees were manually watered daily. Trees from chilling treatment 4 were moved into this glasshouse.



Figure 10 – Glasshouse compartment at NIAB EMR. Picture taken on 05/03/19.



Figure 11 - Glasshouse space at the University of Reading. Picture taken on 05/03/19.

When trees were transferred to the glasshouse, four branches were marked per tree, each one growing towards a different cardinal point (North, South, East and West). Branches were selected according to the following criteria: age (1-year-old growth), growing orientation, length and position of growth within the tree (between 1.2 and 1.8 m height). Branches were marked with colour ribbons and the total number of buds per branch counted.

For each branch, the following measurements were carried out once a week in both glasshouses:

- Number of open* floral buds
- Number of open* vegetative buds
- Apical bud open (yes/no)

**Buds at Green tip stage 3 of development, as defined by Chapman and Catlin (1976) (Figure 2)*

For each tree, the following was monitored twice a week:

- Appearance of the first flower
- 10% bloom
- 50% bloom

Results

All graphs and analyses were performed with the statistical software R: A Language and Environment for Statistical Computing (Version 1.1.463) (R Core Team, 2018). Data obtained with all experiments is currently being analysed so only part of the results is presented in this report.

Experiment 1 - Defining the relationship between temperature and bud break

The relationship between temperature and bud break was investigated in six apple cultivars by comparing the number of days to first bud break (Days to Green-tip (Figure 2)) under four different forcing temperature treatments (13, 16, 19 and 22 °C) and after accumulating different amounts of chilling.

Chilling was calculated and compared for three different models: the below 7.2 °C model (Chilling Hours) (Weinberger, 1950), the Utah model (Chill Units) (Richardson, Seeley and Walker, 1974) and the Dynamic model (Chill Portions) (Fishman, Erez, and Couvillon, 1987). Chilling was calculated with the *chillR* package (Luedeling, 2019). Data presented throughout this report corresponds to chilling accumulation as calculated with the Utah model as it explained more of the variability in time of bud break.

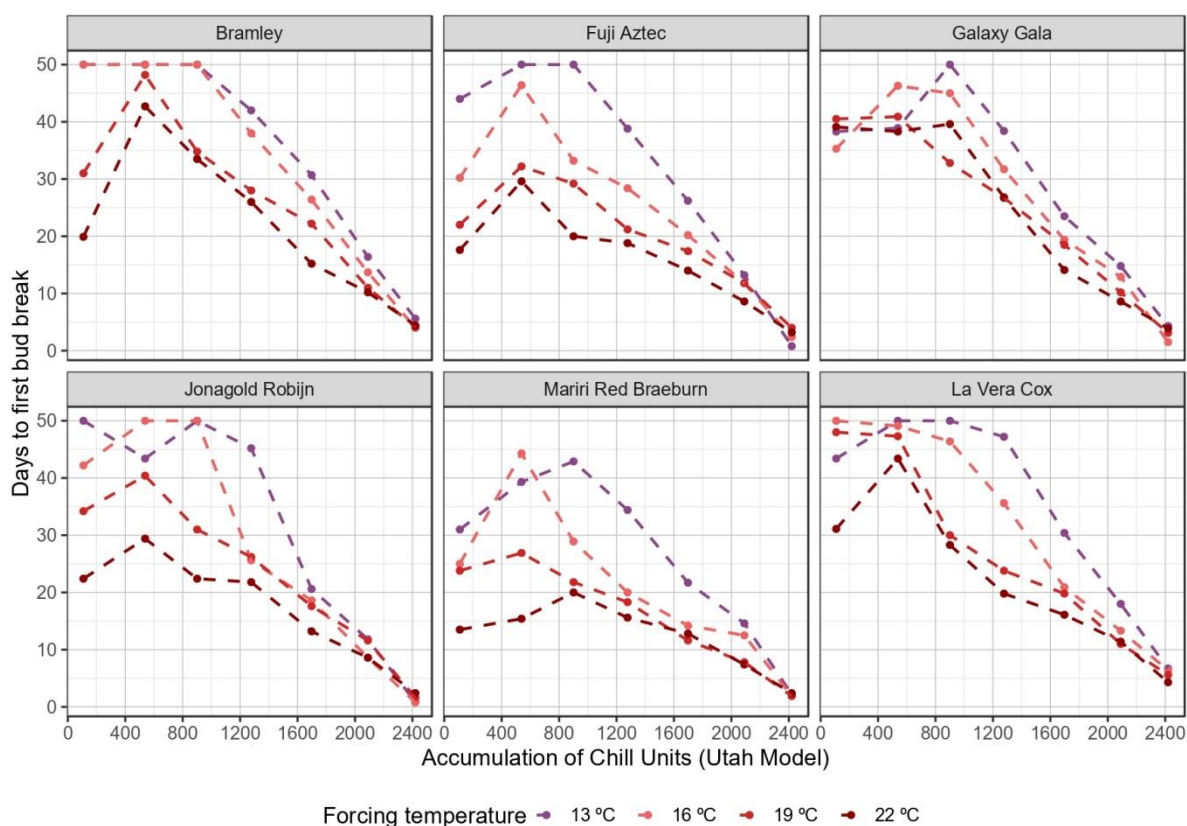


Figure 12 – Number of days to first bud break (y-axis) for shoots that had received different amounts of chilling (x-axis). Each box represents a different cultivar. Forcing temperature treatments are represented by four different colours and each dot is the average of ten shoots.

Changes in time of bud break throughout the winter indicate two distinguishable phases (Figure 12). At the beginning of autumn, as trees accumulated chilling, time to bud break increased and differences were observed between forcing temperatures. In all varieties, time to bud break reached a maximum around December, when trees had accumulated between 800 and 1200 Chill Units. Afterwards, more chilling reduced time to bud break and differences between forcing temperatures were less evident. At the end of the winter to beginning of spring, when trees had accumulated more than 2000 Chill Units (Richardson, Seeley and Walker, 1974), almost no differences could be observed in time of bud break between forcing temperatures (Figure 12).

Percentage of bud break after 6 weeks under forcing temperatures increased with more chilling accumulated, but differences were apparent between varieties (Figure 13). Whilst approximately 75% bud break was achieved in the last collection (2400 Chill Units) of Galaxy Gala and Mariri Red Braeburn, less than 50% was obtained for Bramley and Jonagold Robijn. At the beginning of winter, percentage of bud break was less than 25% in all varieties. Some differences in percentage of bud break at different forcing temperatures were observed and these vary between varieties and throughout the winter.

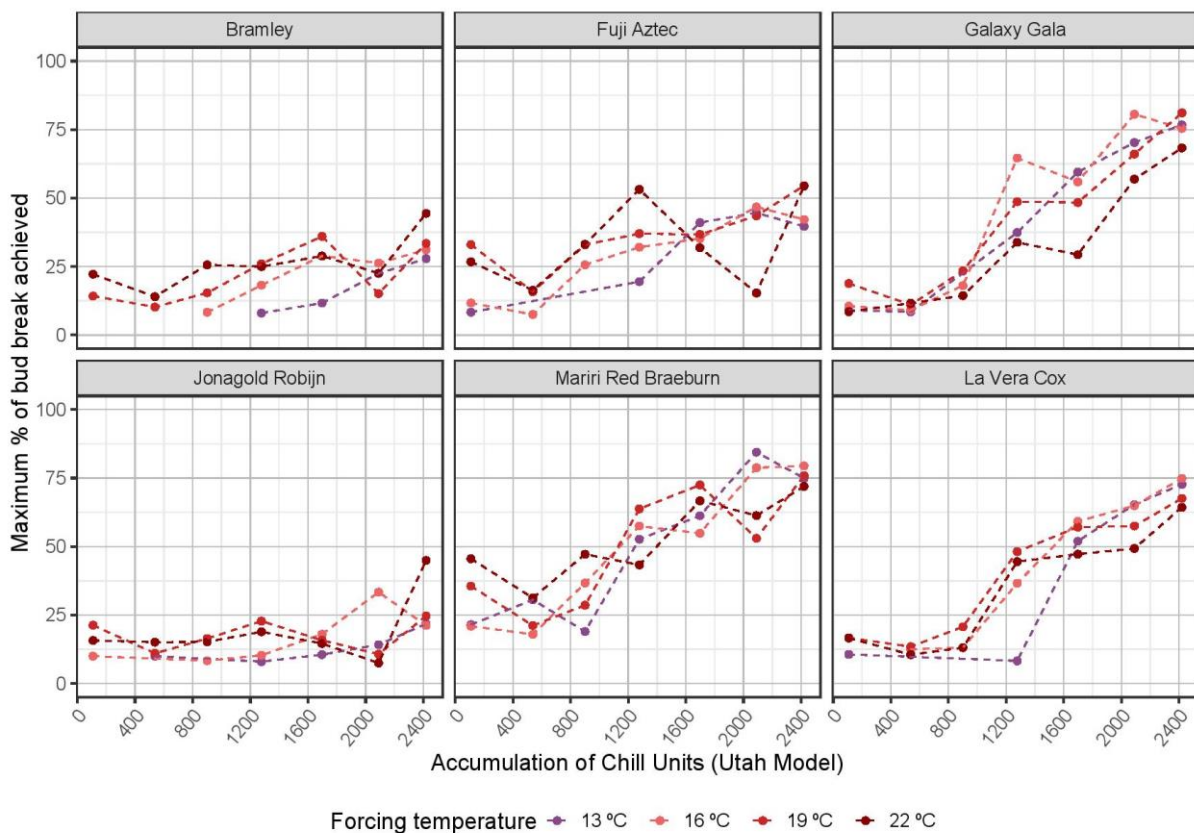


Figure 13 - Maximum % of bud break achieved after 42 days under forcing conditions (y-axis), for shoots that had received different amounts of chilling (x-axis). Each box represents a different cultivar. Forcing temperature treatments are represented by four different colours and each dot is the average of ten shoots.

Cultivar-specific models were developed in order to define mathematically the relationship between temperature and bud break and investigate the combined effect of chilling and spring temperature (represented by forcing temperature treatments). The best models were selected based on Akaike Information Criterion (Akaike, 1974).

Final model structure:

$$\text{Days to bud break} = a - bW - cW^2 - dS + eWS$$

Where **W** represents Winter chilling; **S** Spring temperature; and **a, b, c, d** and **e** are Cultivar-specific parameters.

Final model structure indicated that days to bud break declined curvilinearly with increased chilling and linearly with increasing spring temperature. The impact of spring temperature declined linearly with increased winter chilling. A 3D graphical representation of the Mariri Red Braeburn model is shown in Figure 14, where both observed and modelled data is represented. These cultivar-specific models explained between 67 and 87% of the variability in time of bud break, depending on the cultivar.

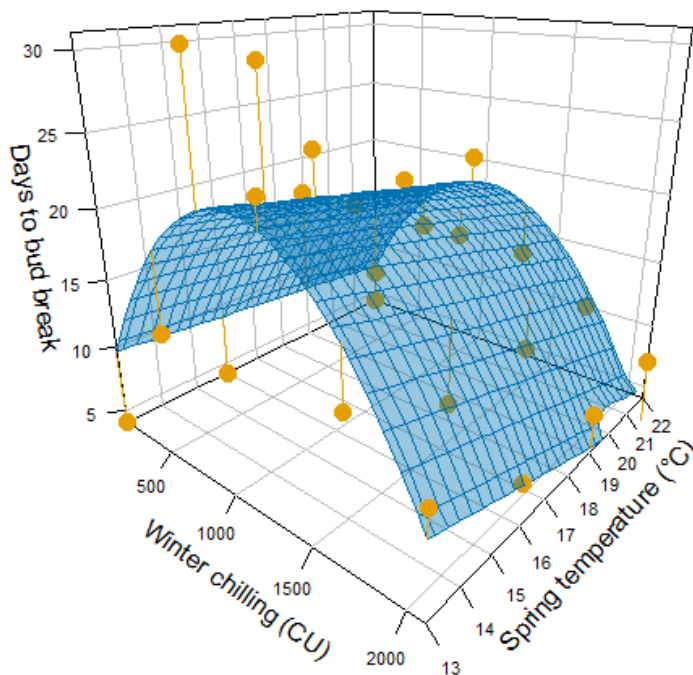


Figure 14 - 3D graph representing the bud break response (*y-axis*) to winter chilling (calculated in Chill Units, *x-axis*), and forcing temperatures (representing spring temperature, *secondary x-axis*) of Mariri Red Braeburn (●): Observed values. Blue surface: modelled values

Experiment 2 - Investigating the effect of chilling temperature and duration of chilling on bud break of two apple cultivars

The effect of chilling temperature and duration of chilling was investigated in two apple cultivars by comparing the number of days to first bud break (Days to Green-tip (Figure 2)) under forcing conditions after trees were previously exposed to 4 different chilling treatments (2, 4.5, 7.5 °C and natural chilling) and a range of chilling durations.

Preliminary results indicate time to bud break was reduced with increased chilling for all chilling treatments. This was particularly true for Mariri Red Braeburn but less evident in Galaxy Gala (Figure 15). Maximum percentage of bud break was lower than 30% for all varieties and treatments and no clear relationship between percentage of bud break and chilling temperature or chilling duration was observed (Figure 15). Some difficulties encountered during the development of this experiment could explain the low percentage of bud break obtained (see Discussion).

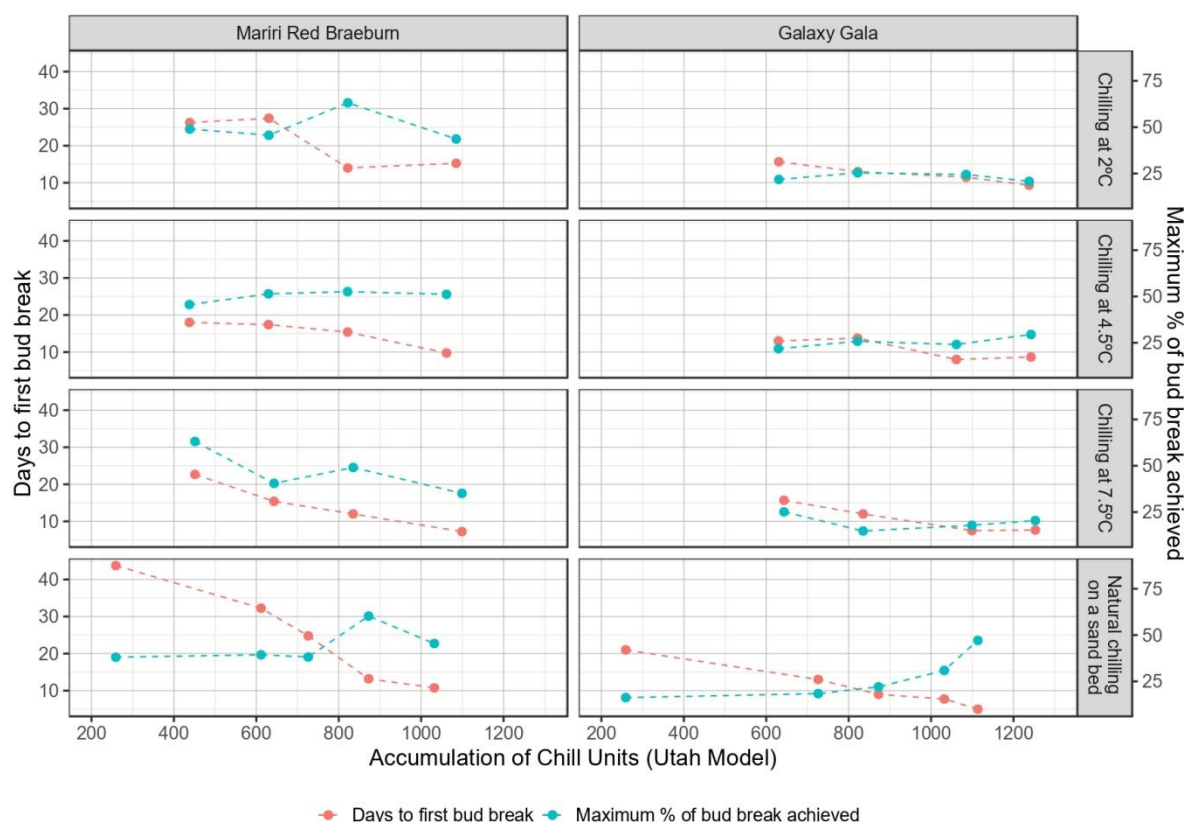


Figure 15 - Number of days to first bud break (*left y-axis*) and maximum % of bud break achieved (*right y-axis*) of potted trees that had received different amounts of chilling (*x-axis*). Each column represents a variety and each row a chilling treatment received. Each dot is the average of 3-4 trees.

Further analyses are being carried out but a positive linear relationship between chilling accumulation and time of bud break was observed for both varieties and all temperature treatments (Figure 26).

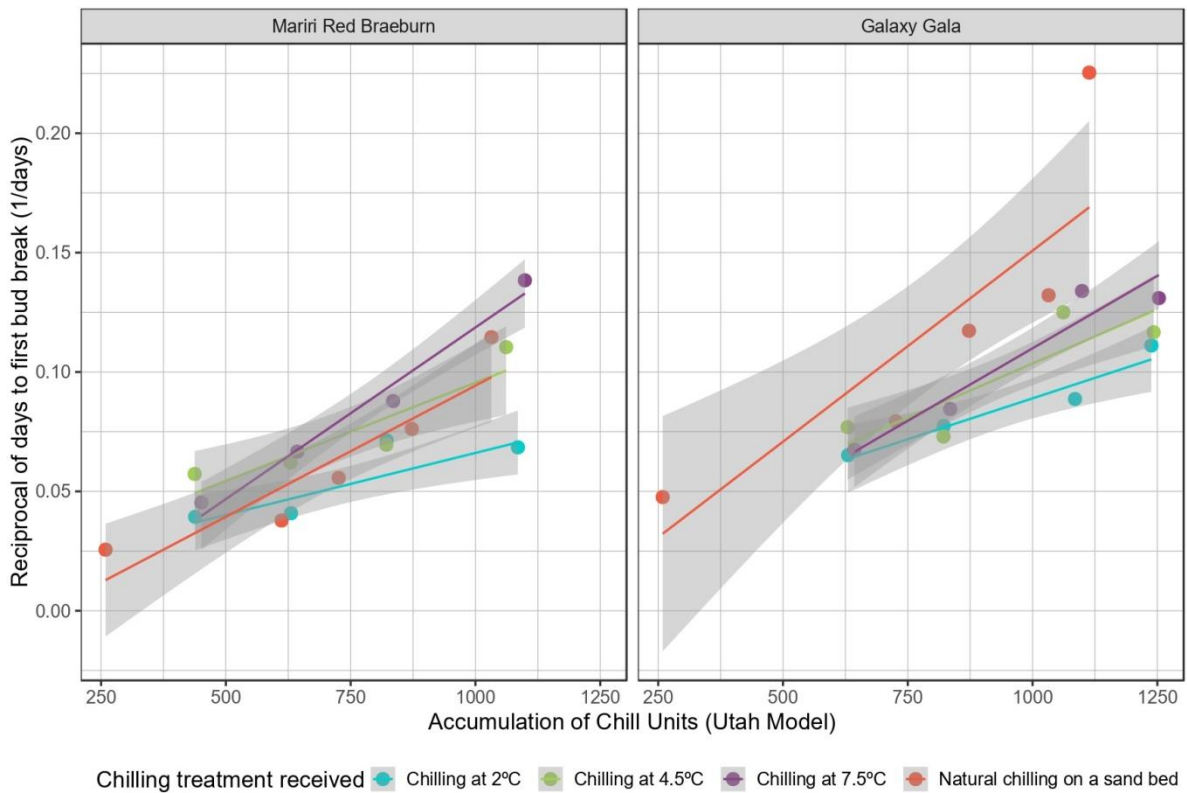


Figure 26 - Linear regressions between chilling accumulated (*x-axis*) and the reciprocal of days to first bud break (*y-axis*) for 4 chilling treatments, represented by different colours. Each box represents a cultivar and each dot is the average of 3-4 trees.

Discussion

Experiments described in this report were designed to investigate some questions that originated from the experiments of the first year of this PhD project (SF/TF 170 Annual Report 2018 CTP_FCR_2017_2). Improving on the methodology developed in Year 1, different modifications were implemented; no artificial chilling was provided to excised shoots and all shoots measured the same length. As demonstrated in Year 1, cultivar-specific models explained more of the variability in time of bud break than a global model incorporating all varieties. Therefore, only cultivar-specific models have been developed this year. In order to investigate the combined effect of chilling and spring temperatures, multiple collections were done throughout the winter and four different spring temperatures (forcing) were used.

Cultivar-specific models with winter chilling and spring temperature as independent variables explained more than 77% of the variability in time of bud break in all varieties studied. Increased chilling reduced time to bud break, as previously reported (Thompson, Jones and Nichols, 1975; Cook and Jacobs, 2000; Naor *et al.*, 2003). However, model structure indicated that time of bud break declined curvilinearly with increased chilling, suggesting a more complicated relationship between chilling accumulation and bud break. Warmer spring temperatures reduced time to bud break but the effect of spring temperatures decreased as more chilling accumulated. It is generally agreed that after chilling requirements have been satisfied, a certain number of hours above a threshold temperature are required to induce bud break. However, different threshold temperatures for both chilling and heat accumulations can be found in the literature, ranging from 12 to 15 °C (Naor *et al.*, 2003; Heide and Prestrud, 2005). We demonstrated that as early as February (Figure 14), buds at 13 °C opened almost at the same time as those at 22 °C, suggesting 13 °C should be considered above the chilling temperature threshold. The combined effect of cold and warm temperatures during chilling accumulation has been previously reported, and it is incorporated in the Dynamic model (Fishman, Erez and Couvillon, 1987). Although chilling calculations with the Utah model fitted our dataset better, results obtained also show an interaction between chilling and warm temperatures. Their combined effect in reducing time of bud break will be further investigated in the next two years.

Overall, results obtained with excised shoots indicate that in a projected climate change scenario, with warmer winters and warmer springs, reduced chill accumulation followed by an increase in spring temperatures could induce bud break earlier, increasing the chances of damage due to late frost. Further analyses are being performed but these models provide the basis for a more predictive understanding of the effects of climate change on time of bud break.

Some difficulties were encountered during the development of the potted tree experiment due to the unexpectedly large size of the trees. The spatial planning in the cold stores and glasshouses was carried out for trees of approximately 1.5 m height and having five branches each; however, on arrival trees measured more than 2 m and had more than ten long branches, taking a lot more space than expected. Even if care was taken, several branches were broken during transport of trees from one treatment to another and many branches were on top of each other in the glasshouses, hindering data collection and potentially affecting final results. The over-crowding of trees in the glasshouse at NIAB EMR caused other problems such as aphid infestation.

The low percentage of bud break obtained during this experiment could be partly due to the difficulties described. However, another possible factor hindering bud break is the effect of apical dominance, which would inhibit bud break of non-apical buds. A study with potted apple trees showed that bud break on trees placed horizontally during forcing conditions was more than double compared to vertical trees (Naor *et al.*, 2003). Taking these concerns into consideration, different modifications will be incorporated to the methodology next year.

Although some differences in the effect of chilling temperature on bud break can be observed, these are less pronounced as previously reported (Thompson, Jones and Nichols, 1975; Naor *et al.*, 2003). A clear linear relationship between chilling duration and bud break was shown and further analyses are being carried out.

Conclusions

Further analyses are being performed and other experiments were carried out. These are preliminary conclusions and should not be extrapolated more widely at this stage. However, some important aspects observed to date:

- (i) The importance of temperature as a key driver of bud break
- (ii) The relationship between temperature and bud break changes throughout the dormancy period and is cultivar-specific
- (iii) Both chilling and warmer temperatures influence time of bud break. There is an interaction in their effect on time of bud break, which changes as winter progresses and is cultivar-specific.
- (iv) Whilst temperature is the main factor determining time of bud break in all cultivars, incorporating other factors in future models could greatly improve bud break predictions, especially for cultivars where more variability was observed.

Knowledge and Technology Transfer

The student attended and presented at:

- 3rd Agriculture and Climate Change Conference (Budapest, Hungary), 24-26 March 2019 – Poster presentation
- David Miller Award Event (RHS Wisley, UK), 14 October 2019 – Oral presentation
- International Conference on Integrative Plant Physiology (Sitges, Spain), 27-29 October 2019 – Poster presentation
- Crops Science PhD Symposium, University of Reading, 5 November 2019 – Oral presentation

Awards received:

- Second best poster at the 2018 Berry Gardens Research and Agronomy Conference, 6 December 2018
- David Miller Travel Award from the SCI Horticulture Group, May 2019. Award received for attending the International Conference on Integrative Plant Physiology.

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