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Report:	Annual report, December 2020
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Key staff:	Carlota Gonzalez Noguer
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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.

Carlota Gonzalez Noguer

PhD Student

University of Reading and NIAB EMR

Signature  18 January 2021

Report authorised by:

Dr Mark Else

Head of Department, Crop Science and Production Systems

NIAB EMR

Signature  Date 15 January 2021

Professor Paul Hadley

Director

Centre for Horticulture, School of Agriculture, Policy and Development, University of Reading

Signature  Date 15 January 2021

CONTENTS

GROWER SUMMARY

Headline

- The optimal temperature for chilling accumulation in apples trees is cultivar-specific and varies depending on the amount of chilling previously accumulated.
- Winter dynamics of soluble sugar profiles in different parts of the tree show potential as physiological markers to differentiate between dormancy stages.

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 episodes.

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, 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.

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 underpinning winter chill requirement and 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 longer-term cultivar selection.

Summary

In the third year of this PhD the effect of different chilling temperatures and duration of chilling on bud break of two apple cultivars was investigated. Freezing temperatures appeared to make a strong contribution to chilling accumulation, which had not previously been reported. Datasets are

being analysed and will be used to develop cultivar-specific chilling models in the final year of the project. A second experiment investigating winter dynamics of soluble sugar profiles showed peaks of sorbitol at different timepoints during the winter. Further work will continue to determine the potential of using soluble sugar profiles as physiological markers of dormancy break.

Financial Benefits

This report summarises part of the work carried out in the third year of a PhD and so there are no financial benefits yet. However, the project will provide key information for cultivar selection to the apple industry.

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 any apple cultivars currently being planted and could have devastating consequences for the agriculture and food production industries which have been identified as "at risk" (IPCC, 2014).

Apple production in the UK is valued at £165 million 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 and 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 registered 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 its effect on dormancy (Campoy, Ruiz and Egea, 2011; Atkinson, Brennan and Jones, 2013). Perennial tree crops enter a dormant state during winter months which ensures their survival in adverse environmental conditions. A decrease in chilling 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 UK apple production. It is important to anticipate how cultivars are likely to respond to these changes so that informed commercial planting decisions can be made over the next few decades.

The dormancy process has been artificially divided in to 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 air 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 could be a key trait in future breeding programmes. Statistical modelling has been used to calculate CR and HR. The first chilling model developed, known as the "Chilling Hours model" or "below 7.2 ºC model", considered all temperatures below 7.2 ºC 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 quantifying chill accumulation, 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 between models. Both models successfully predicted bud break in the studies used to develop them (Richardson *et al.*, 1974; Fishman, Erez, and Couvillon, 1987), but large inaccuracies resulted when they were applied to low-chill varieties (Gilreath and Buchanan, 1981), to varieties and locations different from the ones used to parametrise the models, 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 the 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).

Fluctuations in tissue concentrations of soluble sugars during winter have also been linked to the dormancy process and identified as potential physiological markers (Ito, Sakamoto and Moriguchi, 2012; Kaufmann and Blanke, 2017; Fernandez *et al.*, 2019). The rationale behind this idea is that, to survive winter, trees accumulate and store sugars, often in the form of starch. In spring, starch is transformed into soluble sugars and transported to support bud break and development.

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 any changes in gene expression are linked to dormancy itself and not to other factors.

Materials and methods

Experiment 1 - Investigating the effect of chilling temperature and duration of chilling on bud break of two apple cultivars

One-year-old potted trees were used for this experiment, all grafted on M9 rootstock and from the following two apple varieties: “Braeburn Lochbuie” and “Discovery”.

Trees were purchased as bare root trees from Lodder-Unterlagen GmbH (Dülmen, Germany). Trees were grafted in winter 2018, grown near Freiburg im Breisgau (South-West Germany 47.9938, 7.8273) for approximately a year (**Figure 1**) and hand lifted on 17th of October 2019.

Figure 1 - Trees growing in the field before being lifted. Photo taken on 09/12/2019

Figure 2 - Bare root trees on arrival to the University of Reading. Photo taken on 04/11/2019

Trees were transported to the UK in a non-refrigerated lorry and arrived in Reading on 29 October 2019 (**Figure 2**). All trees measured between 95 and 110 cm height on arrival. On 4 November, trees were potted into 3 litre pots containing a peat-based compost (Sinclair pro compost mix consisting of bark, lime, coarse and medium peat, slow-release fertiliser and wetter).

After potting, trees were placed randomly in a glasshouse at 15 +/- 3 °C for acclimation before starting any chilling treatment. A unique identifier number was given to each tree, which was individually labelled.

After 16 days of acclimation in the glasshouse, trees were moved into the different chilling treatments. Four replicates of each variety (group Control) were left in the glasshouse, where the temperature was increased to 18 +/- 4 °C. These trees did not receive any chilling in order to be able to assess the initial depth of dormancy of the trees.

Chilling treatments

Chilling treatments were provided in controlled environment growth cabinets (Fitotron 3 Wiss Technik UK Ltd.) at the University of Reading (**Figures 3 and 4**), eight different temperature treatments were used: -4, -2.5, -1, 1.8, 4.5, 7.2, 10 °C and day/night alternating temperature of 7/2 °C (12/12h). All treatments received a 12/12h photoperiod using warm-white high frequency fluorescent tubes providing a radiation intensity of approximately 100 µmol/m^2. Humidity was not actively controlled during chilling due to the limitations 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 uniform conditions were maintained across all treatments. In all growth cabinets, temperature and humidity were recorded hourly with dataloggers (Tinytag Plus 2 TGP-4500).

Trees were chilled for different durations between November 2019 and March 2020. Four trees from each variety and treatment were moved into the glasshouse to force bud break at three different time points:

- Removal 1: 6th of January 2020 – 1080 h of chilling
- Removal 2: 4th February 2020 – 1776 h of chilling
- Removal 3: 2nd March 2020 – 2424 h of chilling

Forcing treatment

A forcing treatment was imposed in a glasshouse at the University of Reading (**Figure 5**). Temperature was kept at 18 +/- 4 °C and artificial lighting 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 located inside the glasshouse.

Figure 3 - Start of chilling treatment at -2 °C. Photo taken on 21/11/2019

Figure 4 - Start of chilling treatment at 7 °C. Photo taken on 21/11/2019

Figure 5 – Start of forcing in the glasshouse. Photo taken on 07/01/2020

Temperature and humidity were hourly recorded with a Decagon VP-4 temperature and humidity sensor at canopy height, connected to a Decagon Em50G remote datalogger. Trees were drip irrigated for 3 minutes twice a day, using emitters with a flow rate of 2L/h.

Data collection

To determine the effect of different temperature treatments on the dormancy status of the trees, data were collected on tree vigour and bud break. For each tree, the total number of spurs, terminal and axillary buds were counted at the beginning of the chilling treatment. After one and 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. Homogeneity was measured on a scale from 1 to 5, with five representing equal bud break in all parts of the canopy (**Figures 6**).

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 7**) were recorded, differentiating between spurs, terminal and axillary buds.

Figure 6 - Assessment of bud break homogeneity. Lowest level (1) on the left and highest homogeneity (5) on the right. Photo taken on 13/03/2020

Figure 7 - Green tip stage. Photo taken on 20/03/2018

Experiment 2 – Soluble sugar analysis

This experiment was carried out to investigate winter dynamics of soluble sugar profiles in apple trees, to determine whether this information could be used to inform the development of the variety-specific chilling models.

Plant material

Thirty-five three-year-old potted apple trees of Mariri Red Braeburn were used in this experiment. All trees were 200 - 250 cm high, were grafted on M9 rootstock and grown in 12 L pots in a peat-

based compost (Sinclair pro compost mix). Trees were purchased from Frank P Matthews Ltd (Tenbury Wells, Worcestershire, UK) in December 2018 and were used in a chilling experiment during winter 2018/19 (see SF TF 170_Annual_Report_2019 CTP_FCR_2017_2). This experiment finished in April 2019, after which trees were grown on an outside sand bed.

Experimental design and sample collection

Buds, bark and xylem sap were collected on seven occasions between November 2019 and February 2020. On each sampling date, branches and the main trunk were excised from five trees, placed in plastic bags and transported to the laboratory for processing. Only two trees were sampled at a time to minimise the amount of time between collection, sample processing and storage at -80 °C.

Xylem sap extraction

Xylem sap was extracted using a vacuum pump following the method developed by Bollard (1953). For each tree, sap was extracted from the main trunk and/or between one and four branches. All branches measured more than 60 cm in length and 1–2 cm thickness. The protocol described below was followed for each branch:

- A scalpel was used to remove approximately 8 cm of bark and phloem at the base of the branch.
- All terminal buds and spurs were removed (see Bark and buds collection section)
- The branch was pushed through a previously perforated rubber bung, ensuring 2 cm of the base extruded below the bung to allow sap to drip (**Figure 8**). A tight fit between the branch and the bung was required to create a strong vacuum.
- The bung and the branch were inserted in the top hole of a metal cylinder with a narrow side tube; and a plastic bottle for sap collection was inserted in the bottom hole (**Figure 9**). Using a plastic tube, a vacuum pump was connected to the narrow side metal tube of the cylinder (**Figure 10**).
- The pump was turned on and an approximately 4 cm piece of the distal part of the branch was cut to release xylem tension and prompt sap to drip from the proximal cut surface. This 4 cm piece was discarded.
- Three cm pieces of branch were cut at regular intervals (10-30 s), which maintained a flow of xylem sap from the proximal end of the branch.

Figure 8 - Branch with phloem removed, inserted in a rubber bung for sap extraction. Photo taken on 18/03/2020

Figure 9 - Metal cylinder with bottle for sap extraction. Photo taken on 18/03/2020

Figure 10 - Set up for xylem sap extraction. Photo taken on 25/11/19

Bark and buds collection

The 3 cm branch sections cut during sap extraction were collected for analyses of soluble sugars in the bark. These were re-cut to keep only the internode part of the branch, while ensuring a minimum distance of 0.5 cm from the nearest bud. Between 5 and 8 pieces of internode bark, of different lengths and thicknesses, were collected from each tree.

Ten apical or spur buds were collected from each tree before beginning the sap extraction. Bud scales were carefully removed with the aid of a scalpel and tweezers.

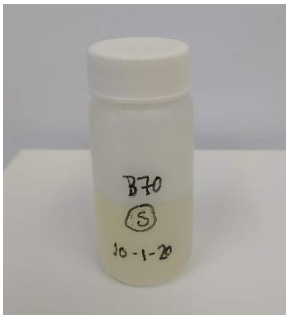

Figure 11 – Extracted xylem sap. Photo taken on 20/01/2020

Figure 12 – Buds with scales removed. Photo taken on 20/01/2020

Figure 11 – Pieces of bark. Photo taken on 20/01/2020

All xylem sap, bark and bud samples (**Figures 11, 12 and 13**) were immersed in liquid Nitrogen immediately after collection and stored at -80 °C until needed for analysis of soluble sugars.

Analysis of soluble sugars

Soluble sugars in bark and flower buds were analysed according to a modified protocol of Kaufmann and Blanke, 2017. Bud samples were freeze-dried and then ground using a pestle and mortar. Powdered samples were dissolved in distilled water (0.05g DM in 1.5 ml HPLC H_2O) and the extract was stirred for 1 h, incubated in a heated water bath at 60 °C for 1 h and then centrifuged at 4643 RCF for 30 min (Centrifuge Sigma 4-16kg Henderson Biomedical). The supernatant was analysed for fructose, sorbitol, glucose and sucrose using a high performance liquid chromatograph (HPLC) (Waters e2695), with a refractive index (RI) detector, an Amino Column (Luna 5µm NH2 100A, 250 x 4.6mm) set at 40 °C and a mobile phase of 80:20 Acetonitrile (MeCN) water and a flow rate of 1.80 ml/min.

The same protocol was followed with bark samples, but these were ground using an electric grinder and 0.2 g DM samples were dissolved in 5 ml HPLC H_20 water.

Soluble sugars in xylem sap were extracted following a modified protocol (Ito, Sakamoto and Moriguchi, 2012; Kaufmann and Blanke, 2017). Sap samples were defrosted in a water bath at 25°C for approximately 1 h, after which 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 above.

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 sets obtained from all experiments are currently being analysed and so only some results are presented here.

Experiment 1 - 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 7**) under forcing conditions after trees were previously exposed to seven different chilling treatments (-4, -2.5, -1, 1.75, 4.5, 7.25 and 10 °C and natural chilling) and three durations of chilling (45, 74 and 101 days).

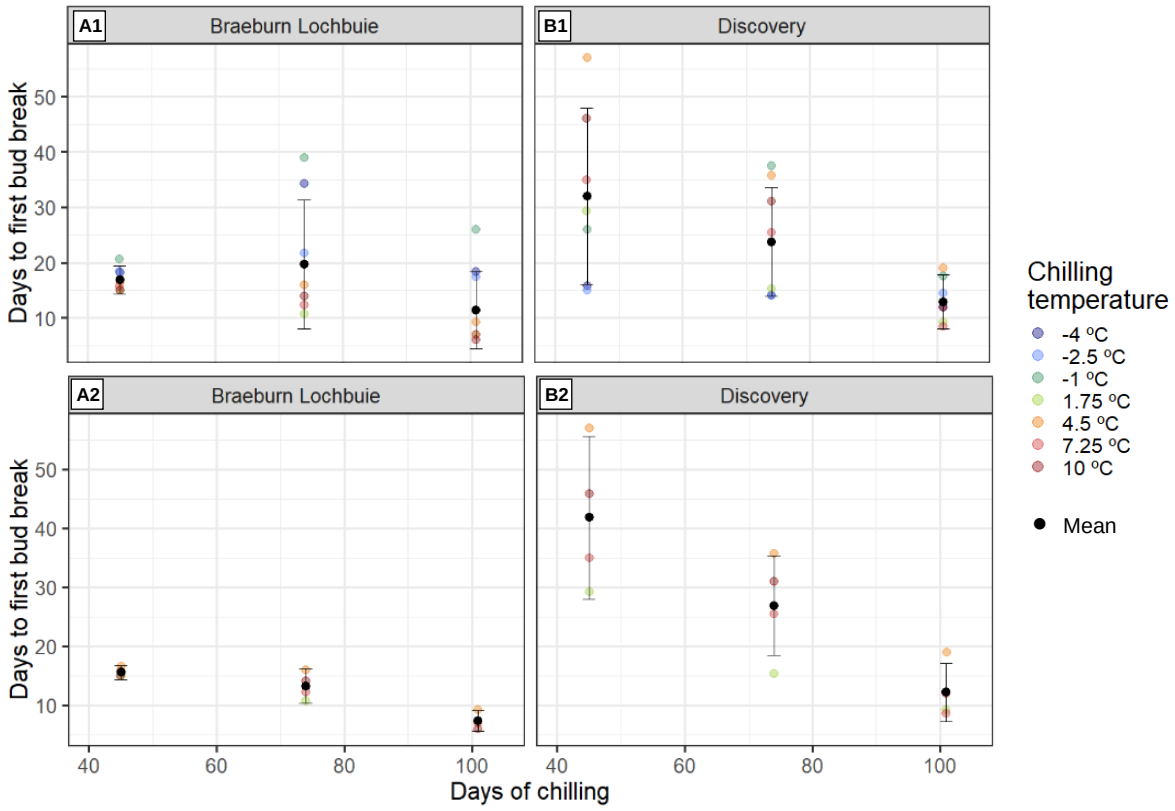

Figure 14 – Days to first bud break after 45, 74 and 101 days of chilling for Braeburn Lochbuie (A1, A2) and Discovery (B1, B2). Chilling temperature treatments are represented with different colour dots. A black dot (●) shows the mean +/- SD for all temperatures. Figures A1 and B1 show all treatments. Figures A2 and B2 omit temperatures below freezing.

Days to bud break decreased with increased length of chilling for Discovery (**Figure 14B**) but this pattern was less clear in Braeburn Lochbuie (**Figure 14A**). For this variety, the average number of days to bud break for all chilling temperatures was slightly higher after 74 days, compared to those

trees that received less chilling (45 days) (**Figure 14 A1**). When treatments below zero were omitted time to bud break decreased with longer chilling for both varieties (**Figure 14, A2 and B2**).

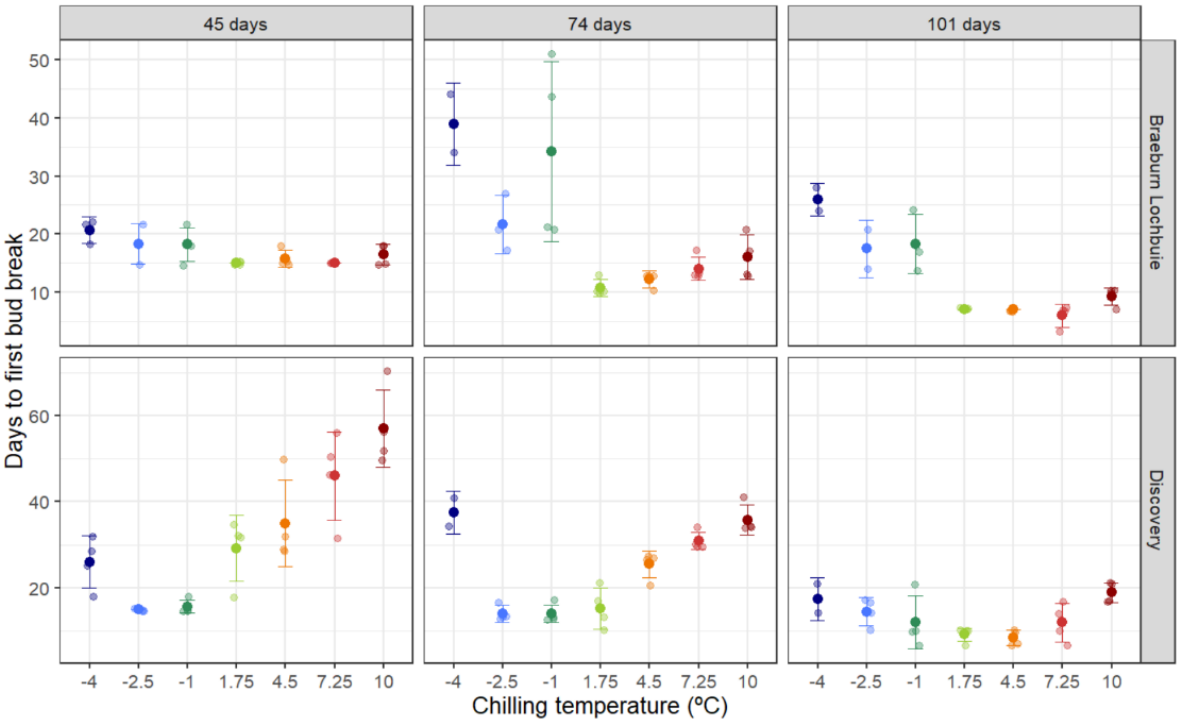

Figure 15 – Days to first bud break in trees chilled at different temperatures (colour dots) for 45, 74 and 101 days. Faded dots represent individual replicates for each treatment. Bolder dots show the mean +/- SD.

Overall, differences in the time of bud break between chilling temperatures were observed but these varied depending on cultivar and decreased with longer chilling (**Figure 15**). Whilst clear differences between temperatures were observed for Discovery, these effects were less evident in Braeburn Lochbuie. In Discovery, after 45 and 74 days of chilling, the number of days to bud break was lowest in trees chilled at -2.5 °C, and increased linearly at higher and lower temperatures. After 101 days of chilling, the effects of different chilling temperatures on time to bud break were minimal in Discovery (**Figure 15**).

The opposite trend was observed in maximum percentage of bud break, which increased with longer chilling in both varieties (**Figure 16**). The effect of different chilling temperatures on percentage of bud break was less evident than on time of bud break, and it varied depending on cultivar and length of chilling received (**Figure 17**).

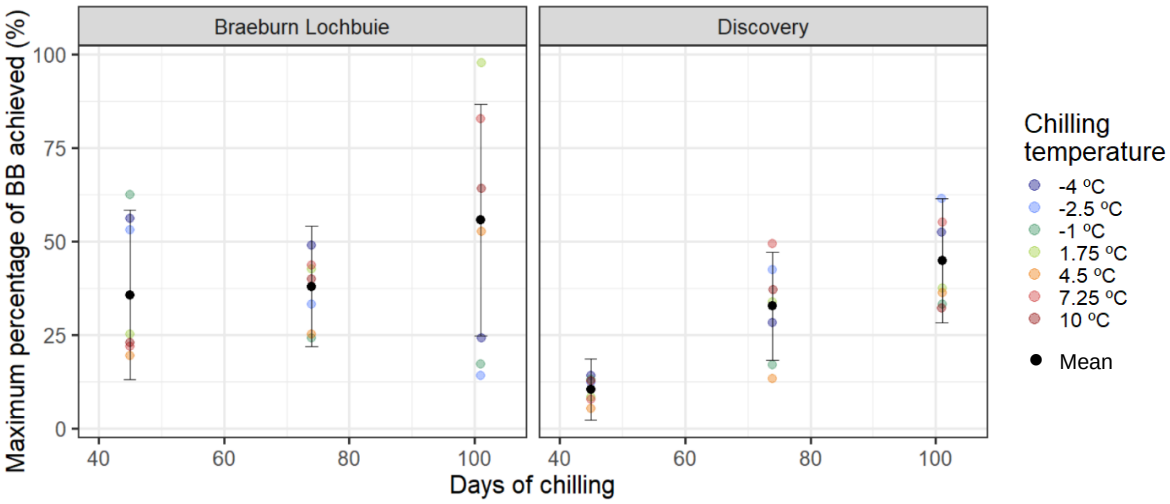

Figure 16 – Maximum percentage of bud break achieved in trees chilled at different temperatures (colour dots) for 45, 74 and 101 days. Mean percentage of BB for all temperatures is shown in black (●) +/- SD.

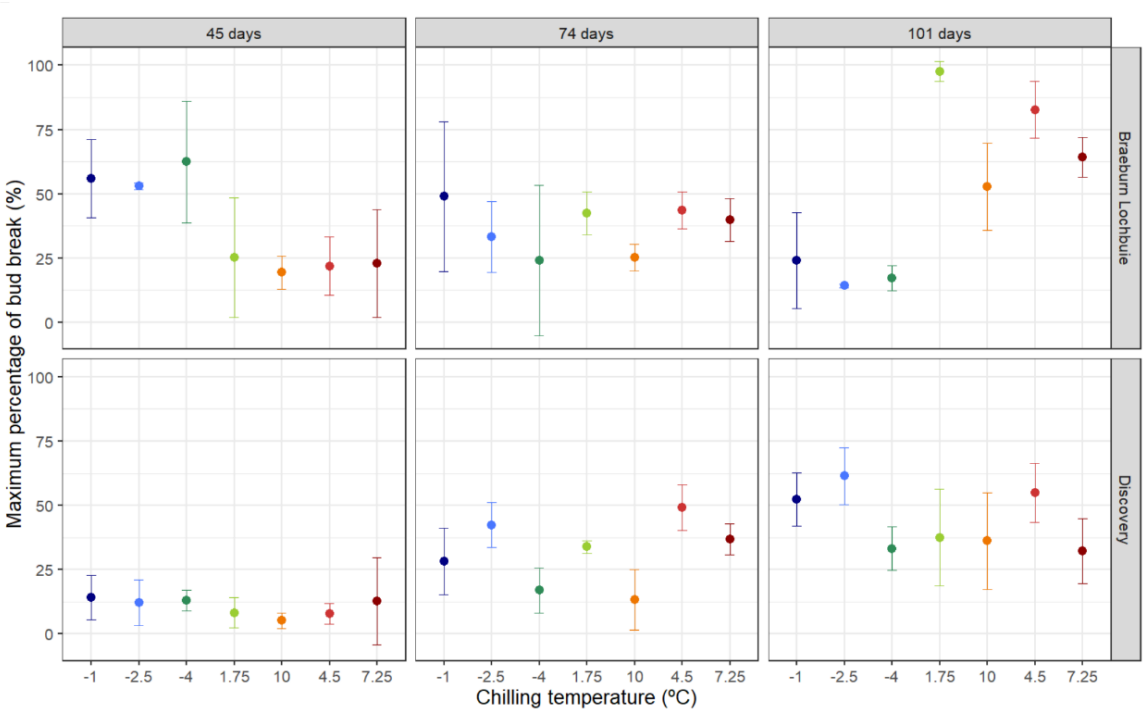

Figure 17 - Mean maximum percentage +/- SD of bud break achieved in trees chilled at different temperatures (colour dots) for 45, 74 and 101 days.

Experiment 2 – Soluble sugar analyses

Concentrations of soluble sugars (fructose, glucose, sorbitol and sucrose) in apple buds, bark and xylem sap were quantified at different times throughout the winter. No statistical analyses have been performed on this data yet, results below are only a graphical interpretation.

On each sampling date, sorbitol was found at higher concentrations in all plant tissues measured (**Figure 18**). The presence of other sugars was detected in all tissues and sap throughout the winter, being higher in bark and buds than in xylem sap, were concentrations of glucose, sucrose and fructose were minimal (**Figure 18C**).

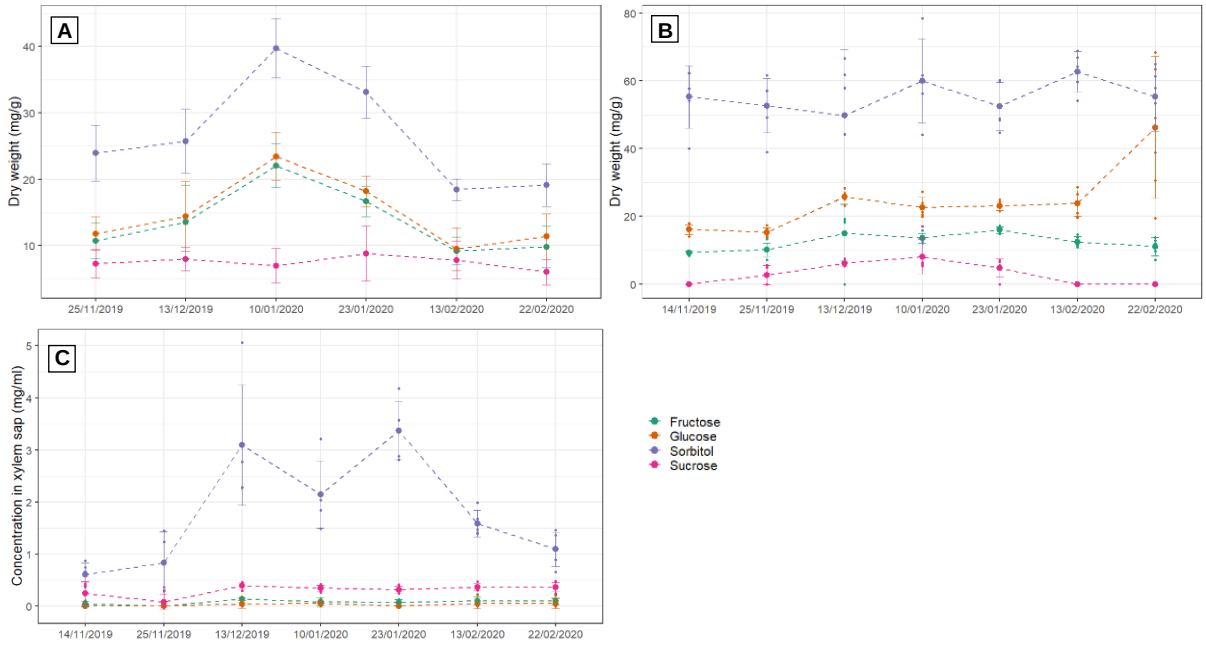

Figure 18 – Mean +/- SD concentration of soluble sugars in apple bark (A), buds (B) and xylem sap (C) at different timepoints during the winter.

In the bark, a clear peak in the concentration of sorbitol, glucose and fructose was apparent around mid-January, which coincided with a slight decrease of sucrose (**Figure 18A**). At the same time, a sharp dip in sorbitol was found in xylem sap (**Figure 18C**) and an increase in apple buds (**Figure 18B**).

During the last time point at the end of February, an abrupt increase of glucose was detected in apple buds (**Figure 18B**).

Discussion

The experiments reported here represent only a small part of this PhD project which includes a much broader approach to investigating the research questions. Previous annual reports have highlighted the importance of developing cultivar-specific models and the need to elucidate the combined effect of chilling and spring temperature on time of bud break. Experiments outlined here focus on the effect of different chilling temperatures on bud break and represent the first attempt to identify a physiological marker for dormancy break.

Preliminarily analyses show a clear temperature-dependant response on time of bud break for Discovery, with an optimum temperature for chilling accumulation of around -2 ^{o}C. The most widely used chilling models, initially developed for peach, consider temperatures around 6 ^{o}C to be optimal for chill accumulation (Richardson, Seeley and Walker, 1974) whilst studies with apple have suggested 2 ^{o}C to be optimum for the variety Jonathan (Thompson, Jones and Nichols, 1975). To our knowledge, this is the first dormancy study with fruit trees that includes freezing temperatures. Predictions using published models are not accurate when used under climate change scenarios or if applied in areas or to fruit trees different than the ones used for calibrating the model (Legave *et al.*, 2008, 2013; Luedeling *et al.*, 2009; Chuine *et al.*, 2016). These results show significant differences between Discovery and Braeburn Lochbuie and highlight the importance of understanding cultivar-specific responses to chilling. In the case of Discovery, a new chilling model incorporating below zero temperatures would be a better representation of this varieties' response to chilling.

Whilst an optimum chilling temperature is apparent in Discovery, this is less clear for Braeburn Lochbuie indicating that dormancy break in this variety might be less influenced by chilling temperature and, instead, be mostly determined by the duration of chilling. No previous studies have looked at the chilling responses of Braeburn Lochbuie and further research is required to better understand its weak response to different chilling temperatures. However, the lower variability between chilling temperatures could be an advantage for growing Braeburn under climate change conditions as warmer temperatures during winter might have a weaker effect on time of bud break, compared with other varieties.

Results obtained from the carbohydrate analyses identified sorbitol as the main sugar found in buds and sap during the winter, as previously reported in cherry and pear (Ito, Sakamoto and Moriguchi, 2012; Kaufmann and Blanke, 2017). However, the dynamics of changes in sugar concentrations observed during the winter differ from published studies.

In apple buds, sorbitol concentrations remained constant until mid-December, after which two peaks were observed. In previous studies, sorbitol concentration in buds was much lower but it also increased to a peak during mid-winter (Ito, Sakamoto and Moriguchi, 2012; Kaufmann and Blanke, 2017). For cherry, this increase of sorbitol has been suggested as a possible indicator of the start of endodormancy (Kaufmann and Blanke, 2017). In their experiment, bud samples were collected from October whilst our first sample was collected in November, which could explain why we do not have a clear increase in Sorbitol. Future experiments will start at the beginning of autumn.

A clear peak of sorbitol was observed in xylem sap at the beginning of January, as similarly reported in a study with pear (Ito, Sakamoto and Moriguchi, 2012). The timing of this increase in sorbitol concentration could be an indicator of endodormancy break as it coincides with an abrupt decrease on days to bud break when forcing branches under controlled environments (data not shown). On 10 of January, an increase in sorbitol was also seen in buds whilst a simultaneous decrease was observed in xylem sap. Although further research is needed, these changes in sorbitol concentrations could indicate the transport of sugars from xylem sap to buds, in preparation for bud break.

Overall, clear differences in the response to chilling temperatures were observed between varieties and winter dynamics of soluble sugars show potential to be used as markers to differentiate between dormancy stages. Further experiments have been designed and are being carried out to include earlier sample collections and a wider range of cultivars.

Conclusions

Further analyses are being performed and data will be interpreted in combination with results from other experiments. These are preliminary conclusions and cannot be extrapolated more widely at this stage. However, some important aspects have been observed to date:

(i) Optimal temperature for chilling accumulation is cultivar-specific and varies depending on the amount of chilling previously accumulated.

(ii) Winter dynamics of soluble sugar profiles in different parts of the tree show potential for use as physiological markers to differentiate between dormancy stages.

Knowledge and Technology Transfer

The student attended and presented at:

- AHDB Tree Fruit Panel meeting (NIAB EMR), 26 November 2019 – Oral presentation
- CTP summer event, 4 August 2020 – Online oral presentation
- Crops Science PhD Symposium (University of Reading), 3 November 2020 – Online oral presentation

Awards received:

- Student Prize from the Worshipful Company of Fruiterers (£1000), received in July 2020

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