Project title:	Apple dormancy break in the context of climate change
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Project leader:	Mark Else (NIAB EMR), Julien Lecourt (NIAB EMR) and Paul Hadley (University of Reading)
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Key staff:	Carlota Gonzalez Noguer
Location of project:	NIAB EMR and University of Reading
Industry Representative:	NA
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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

- A preliminary model for studying time of bud break (TBB) has been developed using excised apple shoots.

Background and expected deliverables

Climate change is predicted to have an adverse impact 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.

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 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.

To define the relationship between forcing temperatures and bud break, heating requirements of a range of apple cultivars were investigated, using a combination of controlled environment experiments with excised shoots and monitoring of field-grown trees. Other variables that might have an influence on time of bud break were considered but temperature was the most important factor.

Summary of the project and main conclusions

In the first year of this PhD programme, we demonstrated that forcing temperature is the main determinant of time of bud break in apple shoots, although other factors such as cultivar and

bud type (floral or vegetative) also have a significant influence. A preliminary general model including forcing temperature (temperatures above 16 °C) was developed for all cultivars, and cultivar-specific models were generated for a range of cultivars. The initial results highlighted the importance of the cultivar-specific models, as varieties responded significantly differently to temperature. Future work will focus on combining the current model with chilling information for each cultivar, which is currently being investigated with excised shoots, potted trees and field grown trees. In summary:

- A preliminary model for studying time of bud break (TBB) has been developed using excised apple shoots. Results to date indicate that time of bud break is dictated primarily by forcing temperatures, followed by bud type (floral or vegetative) and cultivar
- Differences in TBB were observed between shoots on intact field-grown apple trees and excised shoots for a given cultivar. These could be due to insufficient chilling of excised shoots or an artefact resulting from using excised shoots
- Ongoing work is focusing on understanding the relationship between chilling temperatures and bud break to develop an improved model that can inform cultivar choice in different latitudes

Financial benefits

This report summarises the work carried out in the first 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 more than 30 years and one that is highly susceptible to temperature changes predicted with global warming.

Action points for growers

- 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 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 140 million pounds for dessert and culinary varieties (Defra, 2017). 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 registered in the past due to late spring frosts (Defra, 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 dormancy 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 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 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 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 ºC model”, considers 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 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 define the relationship between chilling accumulation and endodormancy release in a range of apple cultivars – develop a chill requirement model (Years 2 and 3)
2. To investigate the relationship between heat accumulation and bud break in a range of apple cultivars – develop a heat requirement model (Years 1, 2 and 3)
3. To combine the chill and heat requirement sub-models in a new model for predicting bud break (Years 3 and 4)
4. To investigate the accuracy of the new model for predicting bud break under future climate change scenarios (Year 4)
5. To investigate the physiological mechanisms regulating dormancy break (Years 3 and 4)

Materials and methods

Experiment 1 – Investigating the heat requirements of 16 apple cultivars under controlled environment conditions

Plant material and field sampling

One-year-old 40-50 cm long shoots from 16 different apple cultivars were used for Experiment 1. Shoots were collected between 25 January and 15 February 2018 from trees grafted into "M9" rootstocks. The cultivars used were: "Annaglo Gala (AG)", "Galaxy Gala (GG)", "Gala (GA)", "Royal Beauty Gala (RB)", "Royal Gala (RG)", "Bramley (BR)", "Braeburn (BB)", "Mariri Red Braeburn (MB)", "Queen Cox (QC)", "La Vera Cox (VC)", "Jonagold EMLA (JO)", "Jonagold Robijn (JR)", "Red Jonaprince (RJ)", "Red Love (RL)", "Tropical Beauty (TB)" and "Spatbluhender Taffetapfel (ST)". Shoots were collected from different growing locations (**Table 1**, Appendices); the relationship between cultivars and locations is specified in **Table 2** (Appendices) as well as information on planting year for each cultivar. Shoots collected from different locations were kept separate throughout the experiment.

Experimental design

After collection, shoots from the same variety and location were grouped in bundles, wrapped with a damp paper cloth and placed inside a plastic bag to avoid desiccation **(Figures 1 and 2)**. Shoots were then placed in a cold store at 4 °C ± 1 to ensure that the chilling requirement was satisfied (see discussion) before moving them into forcing conditions. Once a week, the damp paper cloth was changed and 1 cm of the base of each shoot was excised to avoid blockage of the vessels.

Figure 1. Bundle of shoots from the same variety (Photo taken on 26/01/2018)

Figure 2. Shoots wrapped with a damped paper cloth to avoid desiccation (Photo taken on 26/01/2018)

On 26 February 2018, shoots were moved into four growth chambers (Panasonic Versatile Environmental Test Chamber - MLR-352H) in which humidity, light and temperature could be adjusted. Four different forcing treatments were used, with equal humidity (70%) and

photoperiod (12 h dark/12 h light) but different constant temperatures: 16, 19, 22 and 25 °C. Environmental conditions inside the cabinets were monitored to ensure temperature remained within ± 1°C of the target value.

Apple branches were cut at the beginning of the experiment leaving only 10 buds on each shoot. Fresh weight, diameter and length of each shoot were recorded. Between 8 and 10 replicates were used for each cultivar-location and temperature treatment, with a total of 942 shoots. These were randomly distributed inside each chamber, standing in “Oasis” foam soaked with a mixture of tap water and bleach at 5 ml / litre of water (**Figure 3**). Once a week, the water was changed and 1 cm of the basal tip of each shoot was excised.

Buds were inspected daily for 42 (growth chambers at 22 and 25 °C) or 50 days (growth chambers at 16 and 19 °C), at which time shoots had become desiccated with no bud break occurring for at least 4 days. For each bud, the Green tip (stage 3) (**Figure 4**) stage of development was observed, as defined by Chapman and Catlin (1976), as well as the position of the bud within the shoot (positions 1 to 10, 1 being the apical bud and 10 the closer one to the base of the shoot, **Figure 5**) and the type of bud (vegetative or floral) (**Figures 6 and 7**).

Figure 3. Experimental set up inside a growth cabinet (Photo taken on 08/03/2018)

Figure 4. Green tip stage (Photo taken on 20/03/2018)

Figure 6. Example of a vegetative bud (Photo taken on 05/04/2018)

Figure 7. Example of a floral bud (Photo taken on 05/04/2018)

Figure 5. Numbering of the buds within a shoot (Photo taken on 21/03/2018)

Experiment 2 – Investigating the temperature response of bud break of five apple cultivars under field conditions and comparing it to responses of excised shoots in a controlled environment

Bud break of five apple cultivars, grown in the same field at NIAB EMR (51.287089, 0.445985), was monitored every other day from 22 March to 29 May 2018. The cultivars monitored were “Galaxy Gala (GG)”, “Bramley (BR)”, “La Vera Cox (VC)”, “Mariri Red Braeburn (MB)” and “Jonagold Robijn (JR)”. All trees were 4 years old at time of the experiment and were grafted on “M9” apple rootstock. Temperature was monitored and recorded by an Adcon system with telemetry installed on site and data were acquired with addVANTAGE Pro 6.4 Software.

Five trees of each cultivar were selected randomly and four branches within each tree chosen, each one growing towards a different cardinal point (North, South, East and West). From each branch, the 10 buds closer to the apical bud were monitored and the timing of the Green tip (stage 3) (**Figure 4**) stage of development was recorded, as defined by Chapman and Catlin (1976). A total of 200 buds per cultivar were monitored. The position of the bud within the shoot (positions 1 to 10, 1 being the apical bud and 10 the closer one to the base of the shoot, **Figure 5**) and the type of bud (floral or vegetative) (**Figures 6 and 7**) were also recorded.

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)

Experiment 1 – Investigating the heat requirements of 16 apple cultivars under controlled environment conditions.

The heat requirement of 16 apple cultivars was investigated by comparing the number of days to Green-tip (Time of bud break (TBB)) under four different forcing temperature treatments (16, 19, 22 and 25 °C). As perhaps expected, bud break occurred sooner at 25 °C compared to 16 °C in all cultivars (**Figure 8**). The mean time to bud break for each cultivar and temperature treatment is summarised in **Table 3**.

Table 3. Number of days to bud break from beginning of forcing, under each temperature treatment and for each cultivar. Data given as mean days to bud break +/- 1 standard deviation. Averages obtained from 8-10 replicates

	Days to bud break			
	Forcing temperature treatment			
Cultivar	**16 °C**	**19 °C**	**22 °C**	**25 °C**
Annaglo Gala	16 +/- 4	13 +/- 3	12 +/- 3	9 +/- 2
Gala	16 +/- 4	12 +/- 2	11 +/- 2	9 +/- 4
Galaxy Gala	22 +/- 4	16 +/- 5	15 +/- 3	12 +/- 4
Royal Gala	17 +/- 3	13 +/- 3	12 +/- 3	10 +/- 3
Royal Beauty Gala	18 +/- 5	13 +/- 3	11 +/- 3	9 +/- 2
Bramley	22 +/- 6	17 +/- 6	15 +/- 5	11 +/- 4
Queen Cox	20 +/- 4	16 +/- 4	14 +/- 3	12 +/- 4
La Vera Cox	21 +/- 4	18 +/- 3	15 +/- 3	11 +/- 3
Jonagold EMLA	15 +/- 5	13 +/- 4	13 +/- 5	9 +/- 5
Jonagold Robijn	20 +/- 5	22 +/- 8	18 +/- 4	15 +/- 5
Red Jonaprince	17 +/- 4	17 +/- 6	17 +/- 5	13 +/- 4
Red Love	20 +/- 4	15 +/- 4	13 +/- 4	9 +/- 3
Braeburn	12 +/- 3	10 +/- 3	8 +/- 3	8 +/- 2
Mariri Red Braeburn	16 +/- 5	15 +/- 3	15 +/- 3	10 +/- 4
Spatbluhender Taffetapel	39 +/- 6	31 +/- 6	24 +/- 4	19 +/- 3
Tropical Beauty	17 +/- 7	17 +/- 7	13 +/- 5	8 +/- 3

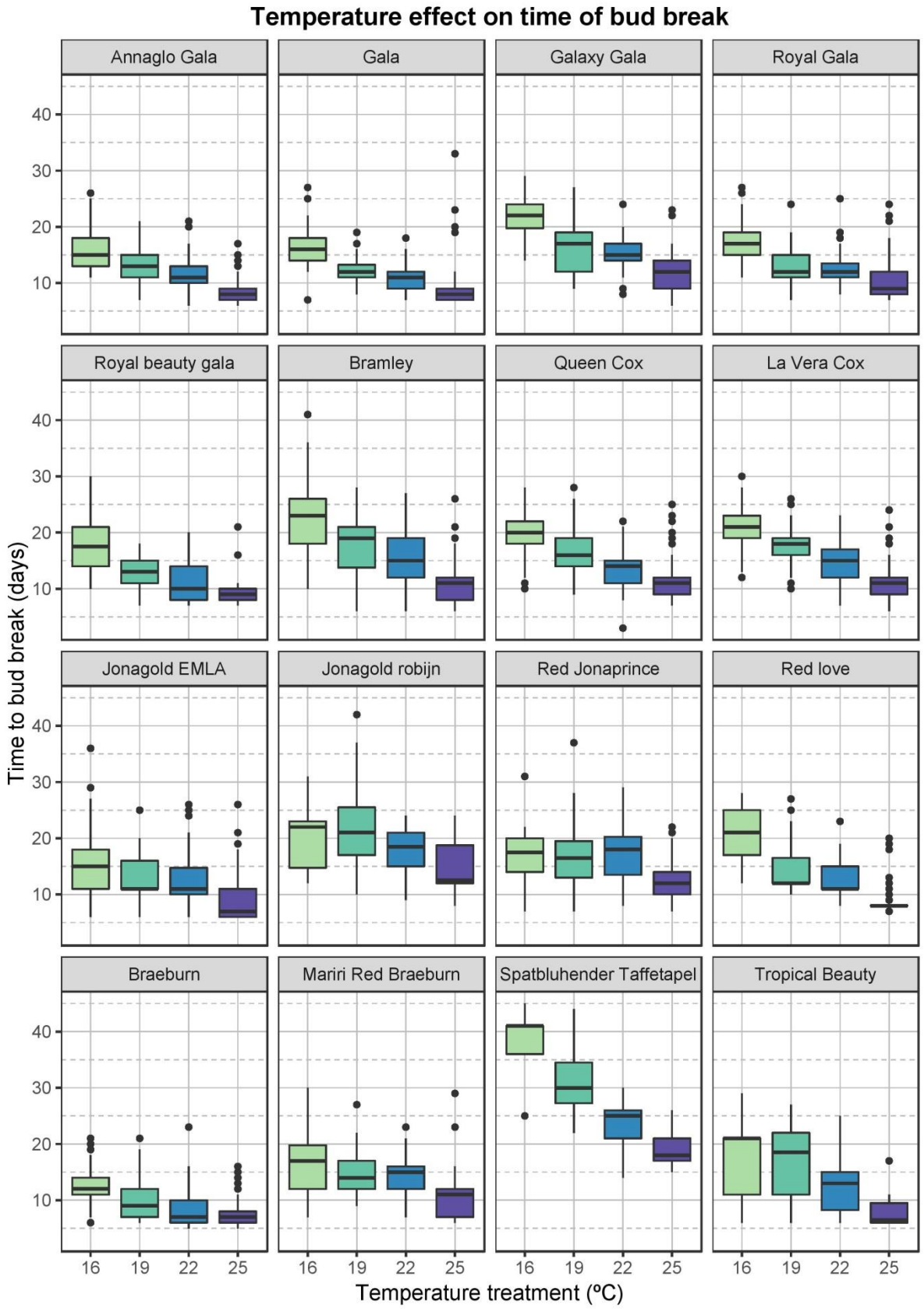

Figure 8. Effect of temperature treatment (x-axis) on days to bud break from beginning of forcing (y-axis). Each box corresponds to a different a cultivar

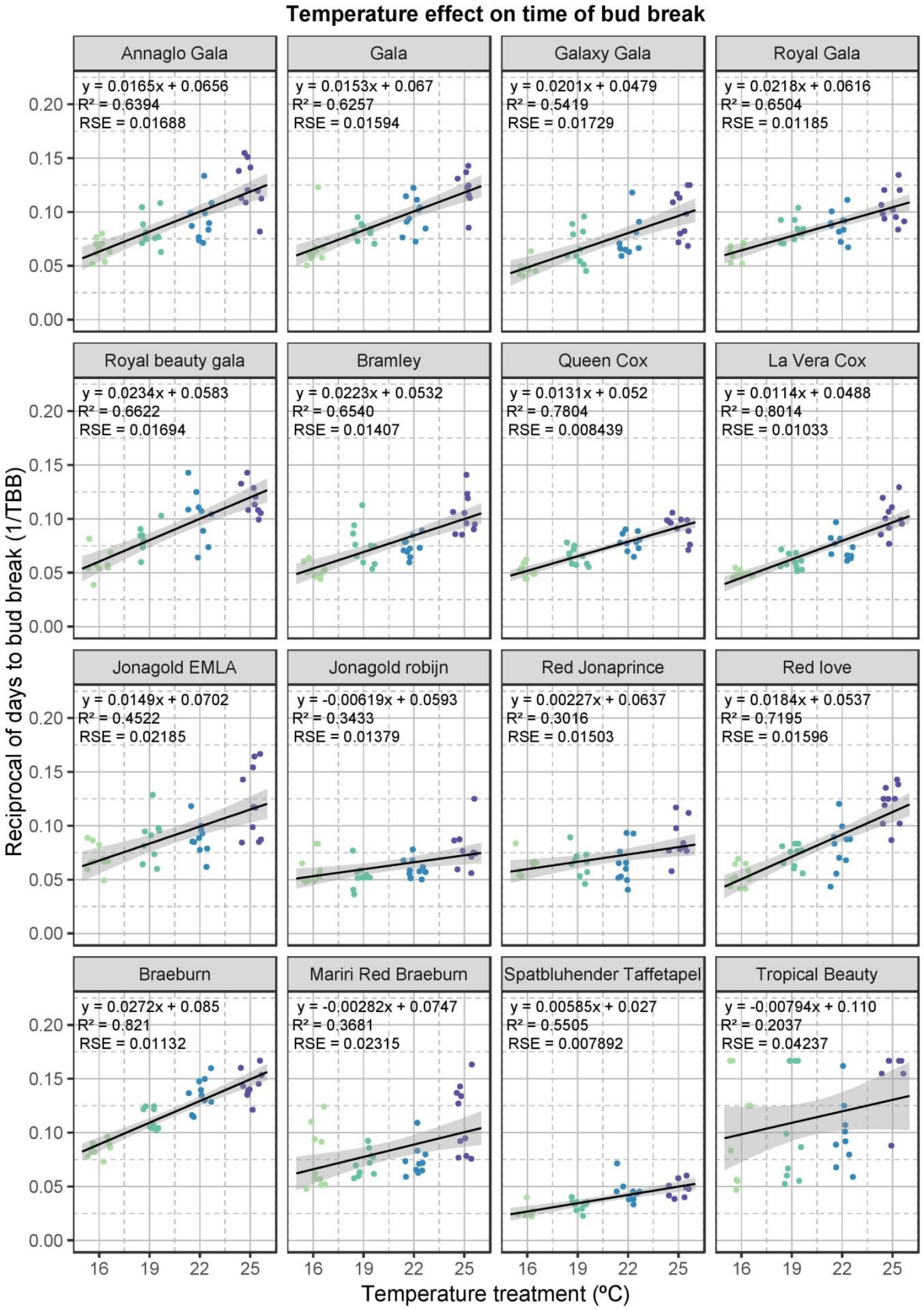

Figure 9. Cultivar-specific linear regressions between forcing temperature (x axis) and the reciprocal of the number of days from beginning of forcing to green-tip stage (y axis). Grey areas indicate 95% confidence intervals. Each dot represents the average of all buds from one shoot (8-10 replicates per cultivar)

To explore in more detail the relationship between TBB and temperature, linear regressions between forcing temperature treatment and the reciprocal of the TBB were performed for each cultivar (**Figure 9**). Significant variability in the relationship between forcing temperature and time of bud break was observed between cultivars, graphically represented by different slopes of the fitted regression lines and by differences in the dispersion of the observations from each cultivar and temperature.

For each cultivar, the percentage of variability in time to bud break explained by temperature is represented by the R^2 value obtained for each regression line (found in each cultivar-box in **Figure 9**). Whilst temperature explained more than 70% of the variability in TBB of “Cox” cultivars, “Braeburn” and “Red Love”; temperature explained only 20% in “Tropical Beauty”. An important difference between sports of the same variety was observed between Braeburn and Mariri Red Braeburn.

Time to bud break was less in the apical bud of all varieties except in Red Love and Spatbluhender Taffetapel, where no pattern was observed in the opening order of the buds (**Figure 10**). On average, floral bud break occurred 3 days earlier than vegetative bud break (**Figure 11**).

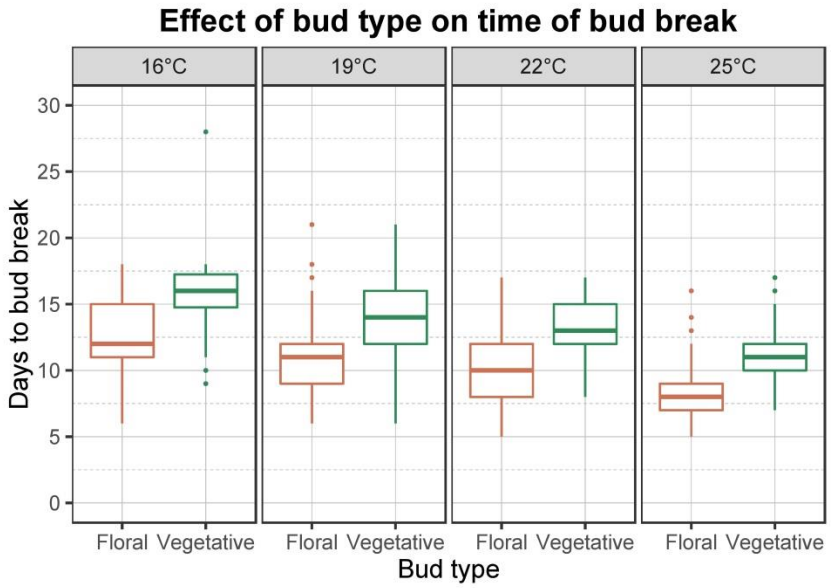

Figure 11. Effect of bud type (x axis) on days to bud break (y axis) from beginning of forcing. Each box represents a temperature treatment and boxplots floral (Orange) or vegetative (green) buds

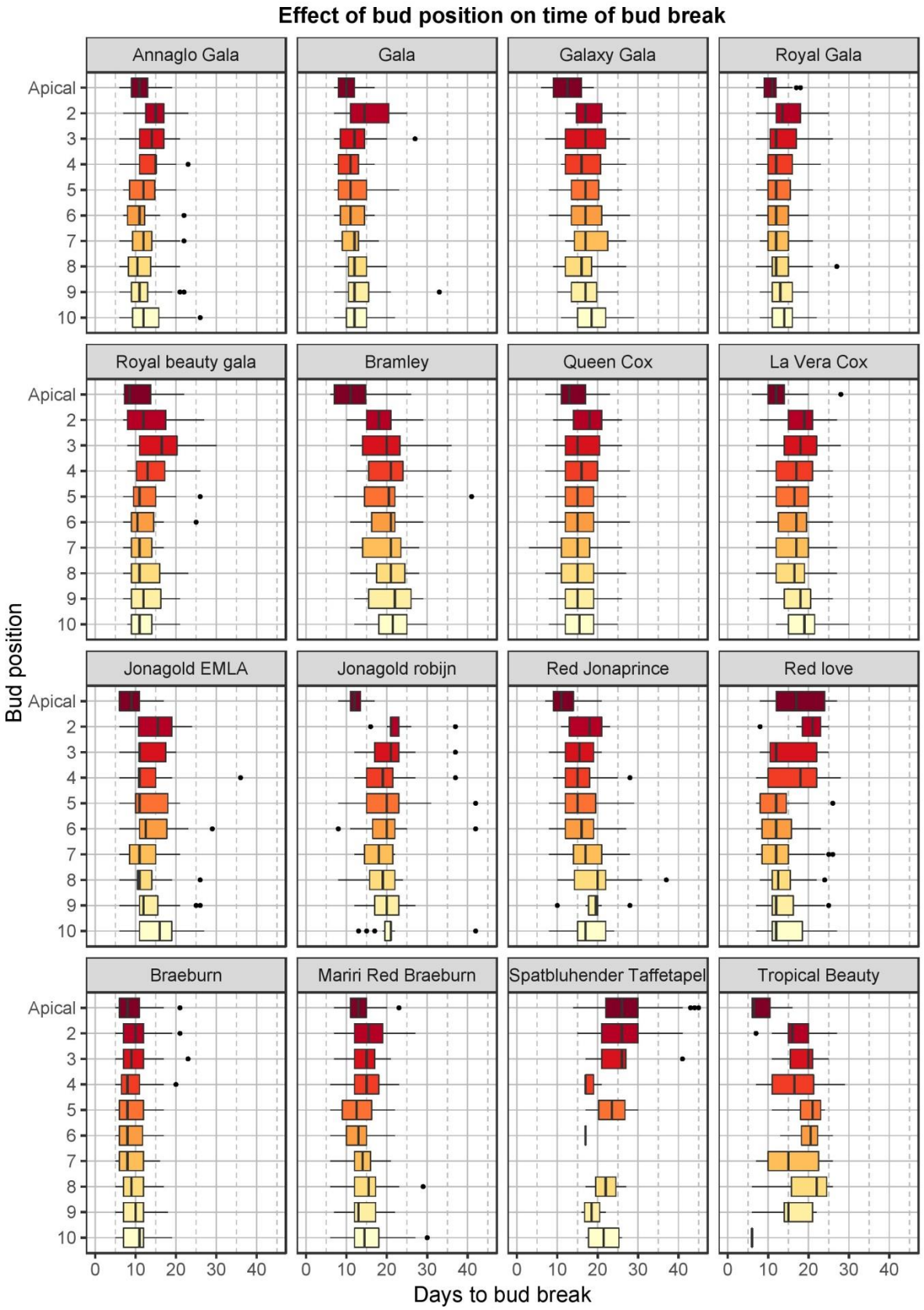

Figure 10. Days to bud break from forcing (x axis) for each bud within a shoot, from apical bud to bud 10 (furthest away from the apical bud) (y axis). Different boxplot colours are used for each bud position; darker red represents the apical bud, the lighter the colour the further away from the apical bud

A Generalised Linear Mixed Effect Model (GLMM) was used to explore the importance of different variables in determining time of bud break (R package "lme4", (Bates *et al.*, 2015)). This type of model permits the incorporation of any variability between shoots which cannot be controlled and that is not accounted for in any of the measured factors. Nine variables were included in the initial model (fixed effects: temperature, variety, shoot length, shoot diameter, shoot weight, bud type, bud position and year of planting; random terms: shoot ID and location). The initial model can be represented as:

Initial model:

$$\textit{Time of bud break} \sim \textit{temperature} + \textit{variety} + \text{shoot length} + \text{shoot diameter} + \text{shoot weight} + \textit{bud position} + \textit{bud type} + \textit{year of planting} + (1 \mid \textit{location/shoot ID})$$

The information theoretic approach (Burnham and Anderson, 2002) was used to compare all possible models containing those variables. The best model was identified based on the Akaike Information Criterion (AIC) (Akaike, 1974) (R package "MuMIn" (Barton, 2018), see Appendices for R script) and contained all the initial variables except shoot diameter and shoot length; as these did not have a significant effect on time of bud break. The final model can be represented as:

Final model:

$$\textit{Time of bud break} \sim \textit{temperature} + \textit{variety} + \text{shoot weight} + \textit{bud position} + \textit{bud type} + \textit{year of planting} + (1 \mid \textit{location/shoot ID})$$

Visual model validation was performed (R package "LMERConvenienceFunctions", (Tremblay and Ransijn, 2015)) to ensure all assumptions of the model were satisfied (normal distribution of residuals, homoscedasticity and no collinearity) (**Figure 12**, Appendices). When looking at the contribution to the overall model of each variable, individual term deletions indicate that, in this model, temperature is the most important variable in determining time of bud break, followed by bud type, cultivar, position of the bud within the shoot, year of planting and finally, fresh weight of the shoot. This is determined by looking at the AIC value obtained when removing each individual parameter from the model. Higher AIC values are linked to worse model performance (Akaike, 1974). **Table 4** (Appendices) shows the differences in AIC values when removing each variable.

The GLMM indicates significant differences in time of bud break between some varieties, but not others; these are summarised in **Table 5**. Tropical Beauty is a known early flowering variety; its time of bud break appears to be significantly different to all the other varieties. Time of bud break in Annaglo Gala is different to that of Gala, Royal Gala and Royal Beauty

Gala, although they are all sports from the same variety. Similarly, significant differences were also detected between Braeburn and Mariri Red Braeburn.

The adjusted R^2 for the overall model is 0.39, indicating that almost 40% of the variability in time of bud break is explained by the variables included, however, 60% of the variability is not explained by the model.

Table 5. Differences in time of bud break between apple cultivars as reported by the GLMM. Significant differences are indicated by * at different confidence levels: '*' 0.001 '**' 0.01 '*' 0.05 'ns' not significant**

	AG	GG	GA	RB	RG	BR	BB	MB	QC	VC	JO	JR	RJ	RL	TB	ST	
AG		ns	***	**	***	***	***	***	**	*	Ns	ns	ns	ns	***	ns	**AG**
GG	ns		ns	ns	ns	**	*	***	ns	ns	Ns	**	ns	ns	***	ns	**GG**
GA	***	ns		ns	ns	ns	*	ns	ns	ns	Ns	***	ns	ns	***	*	**GA**
RB	**	ns	ns		ns	ns	Ns	*	ns	ns	Ns	***	ns	ns	***	*	**RB**
RG	***	ns	ns	ns		ns	Ns	ns	ns	ns	Ns	***	*	ns	**	**	**RG**
BR	***	**	ns	ns	ns		Ns	ns	ns	*	*	***	**	ns	***	**	**BR**
BB	***	*	*	ns	ns	ns		ns	ns	**	**	***	**	ns	**	**	**BB**
MB	***	***	ns	*	ns	ns	Ns		ns	**	**	***	***	ns	**	**	**MB**
QC	**	ns	ns	ns	ns	ns	Ns	ns		ns	ns	**	ns	ns	***	*	**QC**
VC	*	ns	ns	ns	ns	*	**	**	ns		ns	*	ns	ns	***	ns	**VC**
JO	ns	ns	ns	ns	ns	*	**	**	ns	ns		ns	ns	ns	***	ns	**JO**
JR	ns	**	***	***	***	***	***	***	**	*	ns		*	ns	***	ns	**JR**
RJ	ns	ns	ns	ns	*	**	**	***	ns	ns	ns	*		ns	***	ns	**RJ**
RL	ns	ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns		***	ns	**RL**
TB	***	***	***	***	**	***	**	**	***	***	***	***	***	***		***	**TB**
ST	ns	ns	*	*	**	**	**	**	*	ns	ns	ns	ns	ns	***		**ST**
	AG	**GG**	**GA**	**RB**	**RG**	**BR**	**BB**	**MB**	**QC**	**VC**	**JO**	**JR**	**RJ**	**RL**	**TB**	**ST**	

Experiment 2 – Investigating the temperature response of bud break of five apple cultivars under field conditions and comparing it with their response under a controlled environment

Bud break in the field was analysed by comparing the heat accumulated at time of bud break between cultivars. Heat accumulation was calculated using the Growing Degree Days function (Anderson, Richardson and Kesner, 1986), with 4 ºC as a baseline temperature for all varieties.

The proportion of open buds (see **Equation 1**) at different heat accumulations was compared between cultivars (**Figure 13**) and with excised shoots obtained from these same cultivars (used in Experiment 1). All cultivars required less than 300 h heat requirement in the field, whilst more variability was observed under controlled environments.

Equation 1:

$$\textit{Proportion of buds at Heat accumulation "X"} = \frac{number\ of\ \text{open}\ buds\ at\ heat\ accumulation\ "X"}{number\ of\ open\ buds\ at\ the\ end\ of\ experiment\ 2*}$$

*The final percentage of opened buds at the end of Experiment 2 was always higher than 75%

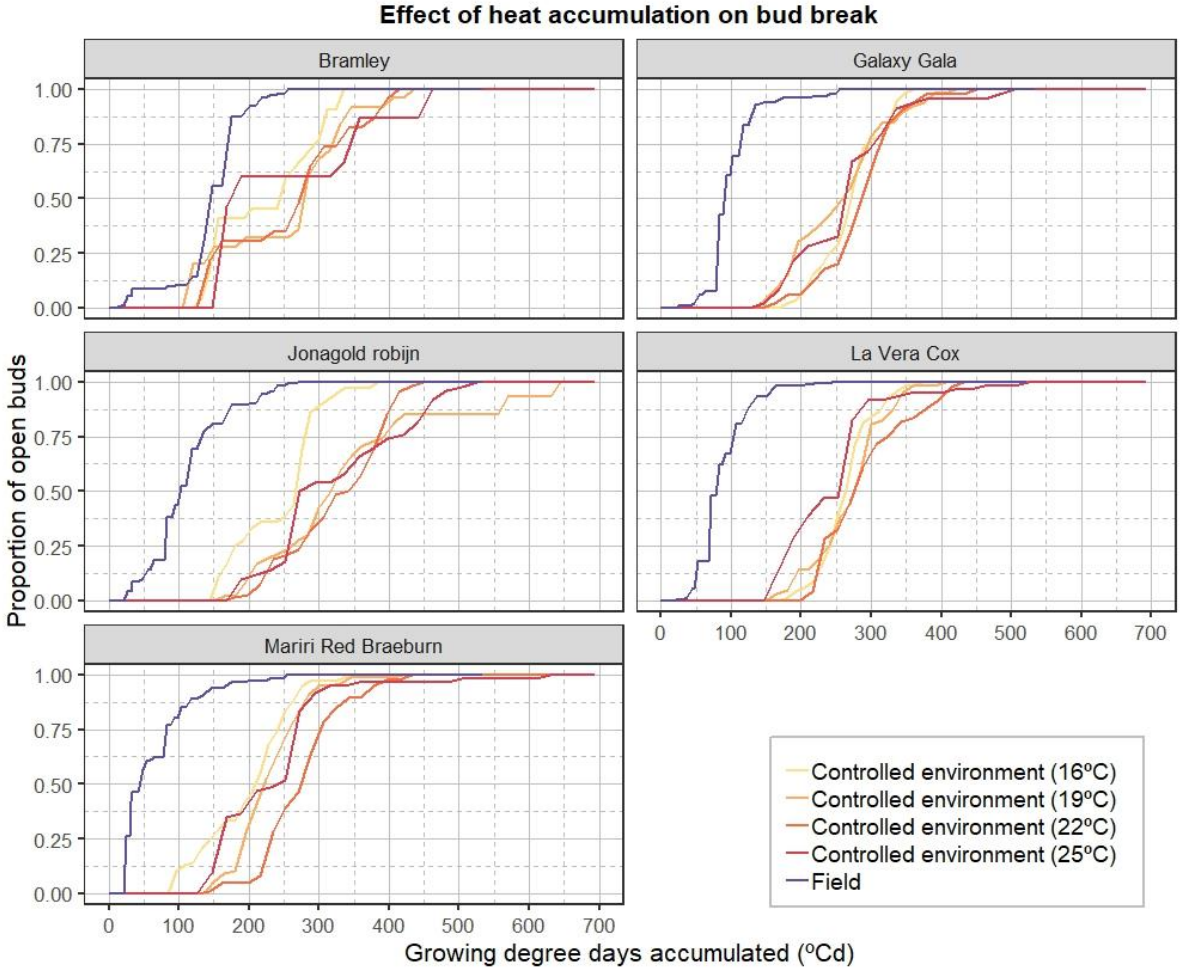

Figure 13. Proportion of open buds at different heat accumulations. Each box represents a cultivar and coloured lines the different experiments as indicated on the legend

Bramley showed the most abrupt response to heat accumulation, with most buds not opening until more than 150 h of heat had been accumulated. The response was more gradual in Jonagold, Mariri Red Braeburn, La Vera Cox and Galaxy Gala. The temperature response in the field (blue line) was significantly different for all cultivars when compared to results from

excised shoots in the growth cabinets (**Figure 13**). All excised shoots required more heat under controlled environments compared to the field.

Data from Experiment 2 is currently being analysed but preliminary statistical analyses show heat accumulated as the main variable influencing time of bud break in the field.

Discussion

Under a controlled environment, the temperature treatment applied for forcing bud break appears to be the main driver determining time of bud break. As shown with the results of the GLMM, 60% of the variability in TBB remains unexplained, but only heat temperatures were investigated in these experiments, and it was assumed at the outset that CR was satisfied in all cultivars. All shoots had received more than 1200 h below 8 °C by the beginning of the experiment, and most apple varieties are estimated to require less than 1000 h (Hauagge and Cummins, 1991). However, the different response in bud break under controlled environment compared to that in the field, and the high number of days to bud break obtained with some cultivars (**Table 3**) suggest that perhaps the CR had not been met in all cultivars. Approximately 700 h from the more than 1200 h received by all excised shoots were artificially provided during the storage in a cold store. This procedure might have affected the chilling accumulation and will not be repeated in future experiments. Year 2 experiments are focusing on investigating CR of a subset of the cultivars included in Year 1 experiments, shoots are being collected from intact trees at different times during the winter months and placed straight into forcing conditions. Incorporating chilling accumulation in the final model is likely to improve performance, as chilling is the main environmental cue regulating dormancy in apple trees (Heide and Prestrud, 2005).

When all cultivars are analysed together, variety is the third most influential variable on TBB. The results of the cultivar-specific linear regressions show that more of the variability in TBB can be explained when models are individually developed for each variety. Other factors such as type of bud also affected TBB; and including these in the cultivar-specific models may improve their accuracy.

The importance of other factors apart from forcing temperatures influencing bud break is supported by the results of Experiment 2, where all cultivars had been exposed to the same climate and had been grown under the same orchard management practices, but still showed some differences in time of bud break. Data from this experiment are currently being analysed to try to understand if other factors such as bud type, bud position or chilling had an effect on bud break.

Some differences in bud break were observed between excised shoots and those on intact trees in the field. Although these differences could be due to insufficient chilling received by the excised shoots, the evidence that shoot weight is an important factor in determining bud break under controlled environments suggests that perhaps excised shoots should not be used to represent those on intact trees. Excised shoots have been used in many dormancy studies (i.e. Hauagge and Cummins, 1991; Cook and Jacobs, 1999; Campoy *et al.*, 2012), and since the results obtained here cast doubt on the validity of this approach, Year 2 experiments have been designed to investigate dormancy using a range of plant materials, including excised shoots, potted trees and trees grown in the field.

The results obtained with these experiments reaffirm the previously reported vulnerability of dormancy to climate change (Luedeling *et al.*, 2011; Luedeling, 2012). Since temperature is the main driver for bud break, any changes affecting this environmental factor are likely to have important impacts on bud break and, therefore, on apple production. A better understanding of how different cultivars are likely to respond to the predicted climate changes is needed and future experiments will focus on this.

Further statistical analyses of the Experiments described in this report are currently being undertaken in order to improve the model by incorporating chilling accumulation and clustering varieties with similar responses to temperature.

Conclusions

These conclusions are the result of one experiment and should not be extrapolated more widely at this stage. However, preliminary results have highlighted several important aspects:

(i) The importance of temperature as a key driver of bud break

(ii) Differences in bud break between apple cultivars, even when grown under the same environmental conditions and growing practices

(iii) The existence of other important factors regulating bud break

(iv) The potential need for improved methodologies for studying dormancy break that more closely resemble responses in intact field-grown trees.

Knowledge and Technology Transfer

The student attended and presented at:

- The AHDB PhD conference, 26-27 November 2018, Solihull (Flash presentation)
- The annual CTP-PhD meeting, 20 July 2018, NIAB EMR, East Malling
- The CTP Student Open Day, 31 October 2019, NIAB EMR, East Malling

- The Department of Crops Science seminar at the University of Reading, January 2019

Carlota will attend and present a poster at the 3rd Agriculture and Climate Change Conference (Budapest, Hungary), 24-26 March 2019.

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Appendices

Table 1. Locations from which shoots were collected

Location	Location Coordinates (Latitude, Longitude)	Location code
Agrii plot	51.287089, 0.445985	A
East Egham	51.287465, 0.455250	B
South Park	51.288454, 0.443069	C
East Egham far side	51.285984, 0.457194	D
Wiseman	51.286500, 0.465491	E
Bradsley Farm	51.194093, 0.549148	F
Brogdale	51.295704, 0.876633	G

Table 2. Apple cultivars, location and year of planting

Cultivar	Cultivar code	Location(s) of collection	Location code	Year of planting
Annaglo Gala	AG	Bradsley Farm	F	2014
Galaxy Gala	GG	Agrii plot	A	2014
Gala	GA	East Egham far side	D	2009
Royal Beauty Gala	RB	Agrii plot	A	2014
Royal Gala	RG	East Egham	B	1998
		Wiseman	E	1995
Bramley	BR	Agrii plot	A	2014
		East Egham far side	D	2000
		Bradsley Farm	F	1986
		Brogdale	G	1977
Braeburn	BB	East Egham far side	D	2009
		Bradsley Farm	F	2014
Mariri Red Braeburn	MB	Agrii plot	A	2014
Queen Cox	QC	East Egham	B	1998
		Wiseman	E	1995
		Bradsley Farm	F	2003
La Vera Cox	VC	Agrii plot	A	2014
		Brogdale	G	2002
Jonagold EMLA	JO	Brogdale	G	2002
Jonagold Robijn	JR	Agrii plot	A	2014
Red Jonaprince	RJ	Agrii plot	A	2014
Red Love	RL	South Park	C	2012
Tropical Beauty	TB	Brogdale	G	1977
Spatbluhender Taffetapel	ST	Brogdale	G	1977

R script for model selection and validation

```
library(MASS)
library(lme4)

#initial model
model1 <- glmer (TBB ~ temp
                 + var.code
                 + bud.type
                 + bud.num
                 + scale(year.planting)
                 + scale(weight.g)
                 + scale(diameter.cm)
                 + scale(length.cm)
                 + (1|location/shoot),
                 na.action = na.pass,
                 family = poisson,
                 data = CE1.nona)

summary(model1)

#Model selection, use dredge() function from the MuMIn package
library(MuMIn)
library(arm)
stdz.model <- standardize(model1, standardize.y = FALSE)
model.set <- dredge(stdz.model)
top.models <- get.models(model.set, subset = delta <2.0) #show models within 2 AIC
top.models #the best model is “final.model”

final.model <- glmer (TBB ~ temp
                 + var.code
                 + bud.type
                 + bud.num
                 + scale(year.planting)
                 + scale(weight.g)
                 + (1|location/shoot),
                 na.action = na.pass,
                 family = poisson,
                 data = CE1.nona)

drop1(final.model, test="Chi")

#visual model validation
library(LMERConvenienceFunctions)
mcp.fnc(model1)
```

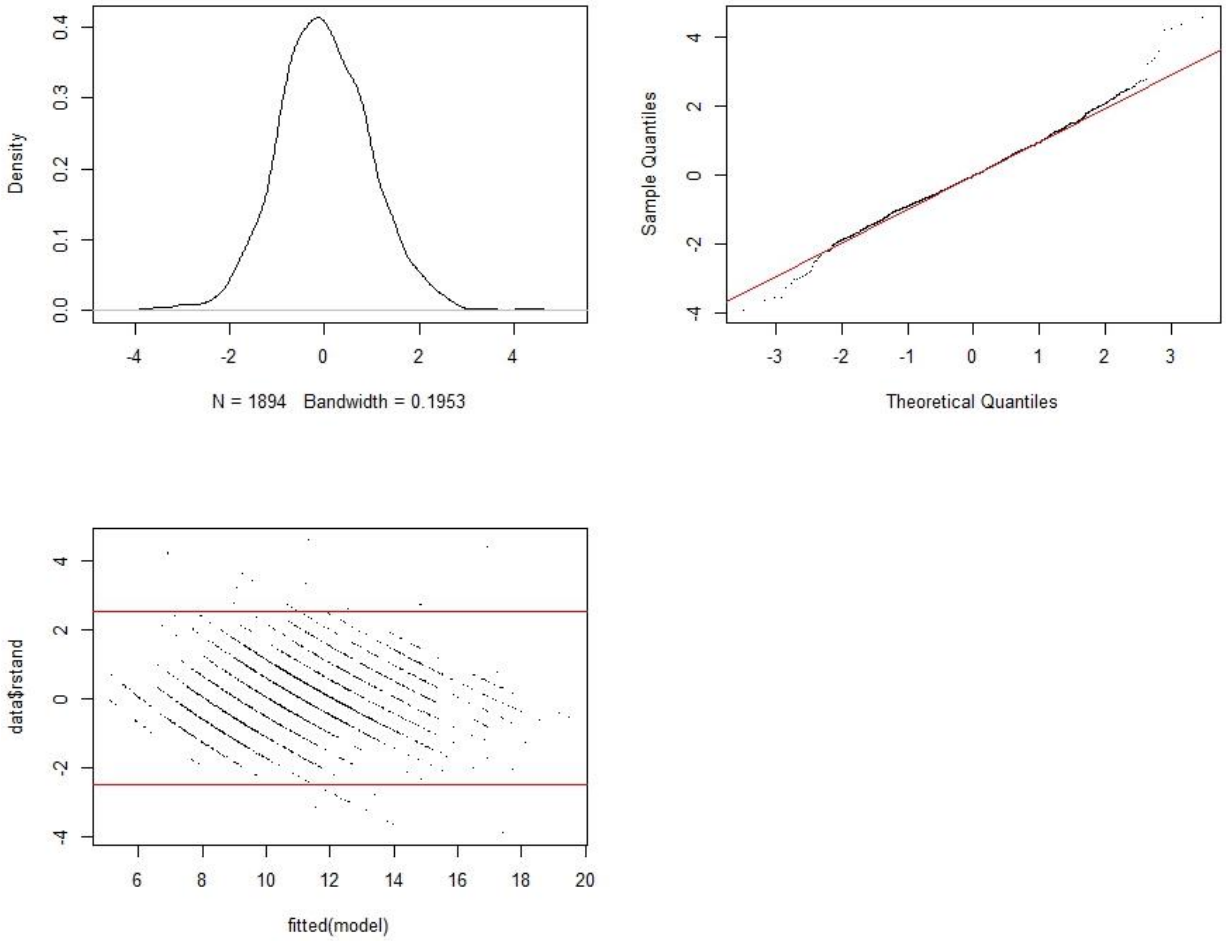

Figure 12. Graphical validation of the model obtained with the "LMERConvenienceFunctions" R package (Tremblay and Ransijn, 2015). Top-left plot shows a normal distribution of residuals, top-right a Q-Q plot and bottom-left plot represents the distribution of residuals versus the fitted values

Table 4. AIC value from the overall model when deleting single variables, higher AIC values are associated with low model performance. Values obtained with the plot1() function from the "lme4" package (Bates *et al.*, 2015) (see Appendices for R script). Signif. codes: '*' 0.001 '**' 0.01 '*' 0.05**

Variable removed from the model	AIC value without the variable	Pr(Chi)
Temperature	9140.3	<2.2e-16***
Bud type	8918.7	<2.2e-16***
Cultivar	8807.4	<2.2e-16***
Position of the bud within the shoot	8755.2	<2.2e-15***
Year of planting	8693.4	0.0006972***
Weight of the shoot	8685.9	0.0439620*