Project Number:	SF 012 – GSK 194a
Project Title:	Winter Chill: Understanding vernalisation requirements for winter chill
Project Leader:	Gillian Fraser
Contractor:	Reading University
Report:	Final Report 2006
Start Date:	2002
End Date:	2006

DISCLAIMER

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

Chapter One.
General Introduction and Literature Review

1.1. *Ribes nigrum L.*

Ribes nigrum L. (blackcurrant) is native to Northern Europe and can be found in Asia, South America and North West Africa (Brennan, 1996). As a crop, *R. nigrum* has been cultivated for circa. 400 years.

1.1.1. An Historical Review

R. nigrum was first introduced into the United Kingdom from Holland by John Tradescant in 1611, and many 17th Century herbals record its medicinal properties (Brennan, 1996). It is thought that English settlers introduced the crop to North America in the early 17th Century, but by the 19th Century, the growth of *R. nigrum* in America was deemed illegal as its ability to act as a substitute host for *Cronartium ribicola* had decimated the country's timber production (Hummer and Barney, 2002).

Despite the recognised medicinal properties of *R. nigrum* fruit, the Royal Horticultural Society recognised only five cultivars in 1826 (Pennell, 2003). During the 19th Century, however, the number of listed cultivars increased significantly as a result of the introduction of open-pollinated seedlings from the early cultivars (Brennan, 1996). The breakthrough in the commercial market for the fruit occurred during the 2nd World War, when sweetened blackcurrant juice, in the form of Ribena, was distributed to maternity hospitals and schools to provide expectant mothers and children with a source of ascorbic acid (Vitamin C) (Anon, 1973).

Today, *R. nigrum* is an economically important crop in the United Kingdom, Germany, Russia, Poland, Scandinavia and New Zealand (Brennan, 1996). The fruit is most famous for its high ascorbic acid (Vitamin C) content, but berries also contain high concentrations of Vitamin A, potassium, phenolics, flavanoids and anti-oxidants (Hummer and Barney, 2002). In the UK, blackcurrants are grown primarily under contract to produce juice, but world-wide, blackcurrants are commonly used to produce pharmaceutical products (cough syrup, throat lozenges), jam, jelly, wine and liqueur (Green, 1971).

1.1.2. Classification of *Ribes nigrum* L.

Class	Angiospermae
Sub-class	Dicotyledoneae
Super-order	Rosidae
Order	Rosales
Family	Saxifragaceae/Grossulariaceae
Genus	*Ribes*
Species	*nigrum*

Much debate has surrounded the classification of *R. nigrum*. Whilst *Ribes* was originally placed within the Saxifragaceae family, further study positioned the genus in Grossulariaceae due to the plant's inferior ovaries, syncarpous gynoecium and fleshy fruit (Brennan, 1996; Hummer and Barney, 2002). Cullen (1997) placed *Ribes* in Grossulariaceae, after Escalloniaceae and before Pittosporaceae, stating that all three families were very closely related, via inherited characteristics, to Saxifragaceae. While *Ribes* is now recognised as a single genus, it is has been divided into several sub-genera. Again, much debate surrounds the sub-genera and while some taxonomists have produced only two sub-groups others have produced as many as nine (Brennan, 1996).

1.1.3. Botanical Description

Ribes nigrum can grow up to two metres in height and width (Clapham *et al*., 1995; Griffiths, 1997). The serrated leaves are lobed and can be up to 10cm in diameter (Westwood, 1978; Hummer and Barney, 2002). The upper surfaces of leaves are glabrous but slightly pubescent and both surfaces are covered with sessile aromatic glands (Hummer and Barney, 2002). Unlike *Ribes rubrum* and *Ribes sativum*, flowering racemes of *R. nigrum* are produced on two-year old wood. Racemes are drooping, pubescent, often glandular and although they are capable of bearing up to seventy flowers, they commonly produce only five to ten (Clapham *et al*., 1995; Brennan, 1996). One to four racemes are present in each bud (Wright, 1985). The perfect bell-shaped flowers measure eight millimeters in diameter (Clapham *et al*., 1995) and most commonly have five petals (Johnson, 1931) that are red to white on the inside and green on the outside (Plate 1.1; Griffiths, 1997). The petals are shorter than the sepals (Clapham *et al*., 1995) and usually five coloured sepals are present which are recurved at the tip (Clapham *et al*., 1995; Hummer and Barney, 2002). The hermaphrodite, actinomorphic,

inferior ovary is glandular and contains many ovules with two free, connate styles (Wright, 1985; Cullen, 1997).

Plate 1.1. *R. nigrum* flower showing petals, anthers and styles

The small seeds have a moist outer coat and consist of a profuse endosperm surrounding a small embryo (Rendle, 1925). Fruit is in the form of a glabrous berry with a persistent calyx at the apex and a fleshy endosperm (Wright, 1985). It is dark purple to black in colour and measures 12-15mm diameter (Clapham *et al*., 1995; Brennan, 1996; Griffiths, 1997). The berries located at the distal end of the strig are reported to be smaller than those produced at the base of the strig (Hummer and Barney, 2002). The basic chromosome number is x=8 and cultivars are diploid 2n=16 (Keep, 1995).

1.1.4. Cultivar Details

The cultivars used in this study, 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' represent the three groups of *R. nigrum* – early, mid and late-flowering respectively. Breeding is conducted at the Scottish Crop Research Institute (SCRI) for GlaxoSmithKline (GSK).

'Ben Gairn' was produced from a cross between the SCRI's 'Ben Alder' (parentage 'Ben Lomond' and 'Ben More') and the Russian 'Golubka'. 'Golubka' was the progeny derived from a cross involving *Ribes dikuscha* which is resistant to blackcurrant reversion associated virus (BRAV) (Brennan, R., *Pers Comm*.). This resistance has been passed onto 'Ben Gairn'. One of the earliest fruiting cultivars, 'Ben Gairn' flowers circa. seven days before 'Ben Lomond' (the industry standard) and is harvested circa. eight days earlier. The early flowering character of this cultivar leaves it susceptible to spring frost damage. The ascorbic acid concentration is typically lower than some of the juicing varieties, but large berry size,

compact plant growth and dark juice colour result in this cultivar being a popular choice amongst the growers.

'Ben Hope' is the result of a complex cross between *R. nigrum* 'Westra' (X-ray mutant of 'Westwick Choice') and an HRI East Malling third generation backcross between *R. grossularia* and *R. nigrum*; Swedish 'Ojebyn' and the UK 'Goliath' (Brennan, R., *Pers Comm*.). 'Ben Hope' has a degree of resistance to the blackcurrant gall mite and is resistant to leaf spot and mildew. Although 'Ben Hope' flowers circa. two days after 'Ben Lomond', its harvest date is typically ten days later. Bush growth is vigorous and this may cause problems with harvesting machinery in mature plantations. Bushes generally produce high yields of medium-sized berries with good juicing qualities.

'Ben Tirran' was produced from a complex cross with the female parent Ben Lomond and the male produced from a cross between 'Seabrook's Black', 'Amos Black' and *R. rubrum* (Brennan, R., *Pers. Comm*.). It has good all-round resistance to foliar disease, especially American gooseberry mildew. It is one of the latest flowering cultivars and is harvested circa. seven days later than 'Ben Lomond'. Bushes produce high yields of large-sized berries with excellent juicing properties, but growers have recently reported bud burst and flowering abnormalities.

R. nigrum are grown commercially in rows with an average of 1410 bushes per hectare. Bushes are typically planted with 3m from the centre of one row to the centre of the neighbouring and 0.4m between plants within the rows. The typical yield from an established plantation would be in the range of 1.2 to 1.8 tonnes per hectare, although this is dependant on the cultivar and the incidence of late spring frosts.

1.2. Dormancy

It is not uncommon for organisms to enter a dormant state in response to unfavourable environmental conditions - animals hibernate, bacteria form dormant spores and amoebae produce cysts (Sussex, 1978). Similarly, in areas of the world that are subjected to harsh winter conditions, woody plants and trees must protect themselves to ensure their survival until the warmer spring approaches. Plants achieve this by entering a state of dormancy where growth is terminated and metabolic activity reduced.

1.2.1. Shoot Dormancy

Shoot dormancy is described as "a temporary suspension of visible growth of any plant structure containing a meristem" (Lang *et al*., 1987). Much confusion in this area of research can be attributed to authors employing different terminology and Lang *et al*. (1985) and Lang *et al*. (1987) attempted to standardise dormancy terminology. It is now widely recognised that three distinct types of dormancy exist - ecodormancy, paradormancy and endodormancy. Lang *et al*. (1987) listed 12 terms that have been used to describe ecodormancy, nine terms for paradormancy and 33 terms to describe endodormancy. This thesis will utilise the terminology adopted by Lang *et al*. (1987).

Plants enter ecodormancy as a result of being exposed to unfavourable environmental conditions such as extremes of temperature, drought and nutrient stress (Lang *et al*., 1987). If conditions alter to favour plant growth, ecodormancy may be suspended, but the longer a plant is in an ecodormant state the harder it is for environmental conditions to reverse the dormancy.

Paradormancy is regulated by physiological factors within the plant e.g. apical dominance (Lang *et al*., 1985). Tinklin and Schwabe (1970) stated that lateral buds of *R. nigrum* failed to break due to the terminal bud releasing an inhibitory substance, suggested to be abscisic acid (ABA). Apical buds also receive a greater proportion of nutrients than lateral buds (Crabbé and Barnola, 1996). As with ecodormancy, when the trigger for growth suspension is removed, e.g. the terminal bud, growth is promoted and lateral buds develop into vegetative shoots (Tinklin and Schwabe, 1970).

Endodormancy, regulated by physiological factors within the bud, is often preceded by eco- or paradormancy and is broken by the bud receiving a specific environmental stimulus, most

commonly chilling (Crabbé and Barnola, 1996). Other environmental factors e.g. photoperiod, may act as a partial substitute if chilling is lacking, but if the chilling requirement is not fulfilled, detrimental effects on bud burst and flowering become apparant (Campbell and Sugano, 1975).

The three types of dormancy may occur independently of each other (Figure 1.1) or they may co-exist (Crabbé and Barnola, 1996).

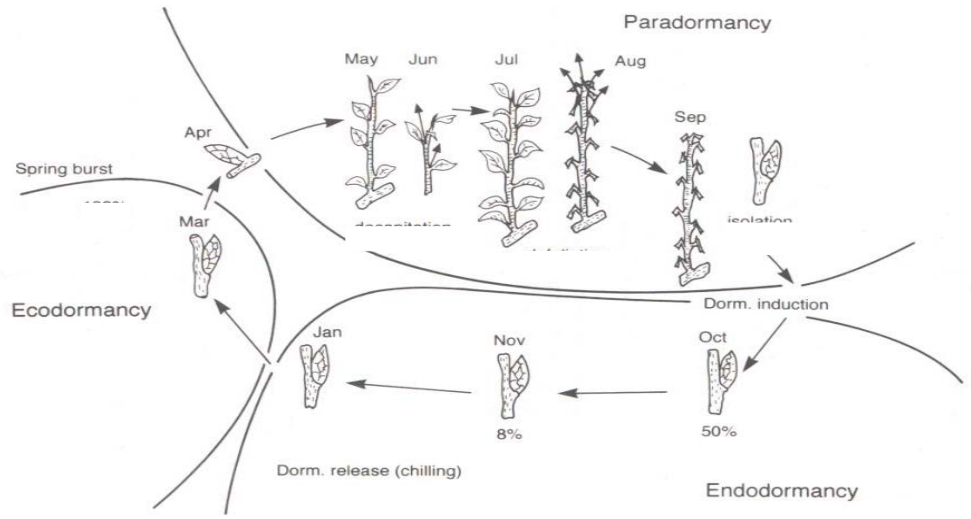


Figure 1.1. The relationship between eco-, para- and endodormancy.

(after Crabbé and Bernola, 1996)

1.2.1. Root Dormancy

Very little research has concentrated on root dormancy. O'Hare and Turnbull (2004) reported root activity in *Litchi chinensis* whilst the shoot was in a dormant state, but as the ambient temperature fell the rate of root growth decreased. Roots of conifers are thought to become ecodormant during the winter in response to low soil temperature (Bigras, 1996). If roots do not enter endodormancy it would explain why root growth continues after shoot growth has been suspended in autumn and recommences before bud burst occurs in spring. Without the need to fulfill a chilling requirement the only constraint to root growth would be unfavourable environmental conditions.

1.3. Initiating Dormancy

This thesis will concentrate on the effects of endodormancy and any reference to dormancy should be taken in this context, unless stated otherwise.

Environmental factors and plant hormones are thought to be involved in inducing dormancy (Noodèn and Weber, 1978; Crabbé and Bernola, 1996). However, although it has been accepted that environmental changes are involved in dormancy induction, scientists are divided over the role of hormones.

1.3.1. Environmental Factors

Environmental changes e.g. photoperiod, temperature and drought, indicate that unfavourable climatic conditions are approaching and plants respond accordingly to ensure their survival.

Although it was reported in 1914 that dormancy could be initiated by transferring plants from long day to short day conditions, it was not until 1923 that the connection between photoperiod and dormancy was hypothesised (Noodèn and Weber, 1978). Dormant buds of *Picea abies* (Heide, 1974a), *Ceiba pentandra, Bombax buonopozense* and *Gmelina arborea* (Thomas and Vince-Prue, 1997) were formed after being transferred from long day to short day conditions. Following transfer from long day (24-hour photoperiod) to short day (9-hour photoperiod) conditions, growth of apical meristems were inhibited and protective scales formed around new buds to prevent low temperature and dehydration damage (Thomas *et al*., 1966; Whalley and Cockhull, 1976). When returned to long-day conditions, bud growth resumed (Thomas *et al*., 1966). Noodèn and Weber, 1978 suggested that plants could be classified into one of four categories (Table 1.1.).

Table 1.1. The suggested response of plants to photoperiod and dormancy

Dormancy induced by short days	Dormancy induced by long days	Photoperiod insensitive	Do not enter dormancy
Acer pseudoplatanus	*Allium cepa*	*Liquidamber styraciflua*	*Euonymus alata*
Betula pubescens	*Kleinia articulata*	*Paulownia tomentosa*	*Prunus spp.*

(after Noodèn and Weber, 1978)

The authors also classified *Prunus* spp. into the final category, despite the first weighted chill unit model, which determines the chilling requirement for over-coming dormancy, being constructed using *Prunus persica* 'Redhaven'.

Little research has concentrated on the optimum wavelength for inducing dormancy, but initial work suggested red light was more effective than blue light, suggesting that phytochrome was the photoreceptor (Noodèn and Weber, 1978, Thomas and Vince-Prue, 1997). Phytochrome exists in two forms – P_r absorbs red light and is transformed to P_{fr}, which in turn absorbs far red light and converts back to P_r (Campbell, 1996). This reversible reaction is responsible for many of the fundamental processes in plants, including seed germination, flowering and stomatal opening (Campbell, 1996). In some species e.g. *Cornus florida*, *Hildegardia bartei* and *Quercus* spp., changes in photoperiod were thought to induce cold-hardiness and dormancy (Thomas and Vince-Prue, 1997). As in flowering, the duration of darkness is thought to be the important factor in dormancy on-set, demonstrated by delayed dormancy in *Acer palmatum*, *Platanus occidentalis* and *Taxus cuspidata* as a result of night breaks (Thomas and Vince-Prue, 1997).

The first correlation between temperature and dormancy was reported in the 1800's, when dormant buds were formed in response to decreasing autumn temperature and plants exposed to continually warm winter temperatures failed to grow the following spring (Noodèn and Weber, 1978). Heide (1974a), however, suggested that the response to decreasing temperature was a safety mechanism, capable of initiating dormancy in the absence of other environmental/hormonal stimuli. Endoodormancy of *Robina pseudoacacia* was triggered when the photoperiod fell to 12-hours, but when exposed to photoperiods in excess of this, dormancy was induced by low temperature (Vince-Prue, 1975). Similarly, temperature has been reported to substitute for photoperiod in the process of dormancy induction, and vice versa (Noodèn and Weber, 1978).

1.3.2. Hormonal Control of Dormancy

The correlation between abscisic acid (ABA), originally known as 'dormin' or 'abscisin' and dormancy is a contentious subject that has been debated amongst researchers for decades.

After a series of *Ribes nigrum* and *Prunus cerasus* experiments, Tinklin and Schwabe (1970) and Mielke and Dennis (1978) concluded that an inhibitory substance was translocated from leaves to bud scales and into the centre of the buds, thus preventing bud burst. The inhibitory substance, which was reported to move not only basipetally on defoliated stems, but also between stems on the same bush, was identified as ABA (Tinklin and Schwabe, 1970). In support of this, extracts of *Malus domestica* bud scales, when applied to active buds *in vitro*, inhibited bud growth (Swartz *et al*., 1984). The biological activity of the extract, when compared with water and 0.5µM ABA, was recorded as 'intermediate' and it was concluded that ABA was partly responsible for maintaining dormancy (Swartz *et al*., 1984). Bud ABA concentration increases as dormancy is initiated and decreases to a minimum in spring (Figure 1.2), and this trend led to the hypothesis that dormancy induction/release was dependant on the presence/absence of ABA (Tinklin and Schwabe, 1970).

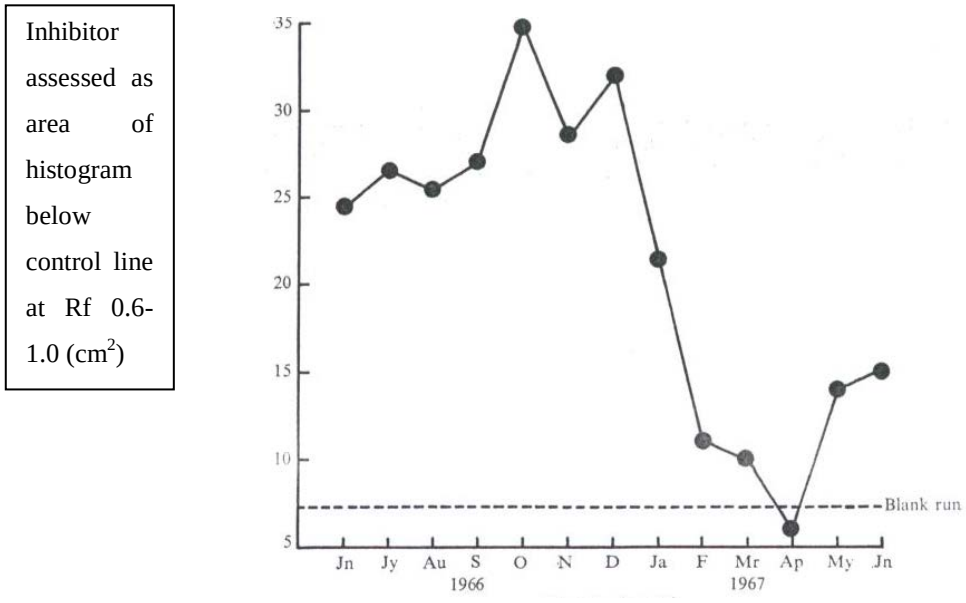


Figure 1.2. Inhibitor concentration from June 1966 to June 1967.

(after Tinklin and Schwabe, 1970)

In support of a correlation between ABA and dormancy release, ABA concentration decreased in *Pyrus pyrifolia* 'Nijisseiki' buds as dormancy progressed towards spring (Tamura *et al*., 1992). In parallel to the decrease in ABA, the concentration of a 'gibberellin-

like' substance, a 'cytokinin-like' substance and indoleacetic acid increased and it was concluded that the decrease in ABA concentration was closely related to a release from bud dormancy. A similar result was reported for *M. domestica* (Bondock *et al*., 1995). The increase and subsequent decrease of ABA in dormant *Prunus persica* buds, as reported by previous authors, however, was deemed to be coincidental and unrelated to the dormant state of the buds (Ramina *et al*., 1995).

Exogenous application of benzylaminopurine, naphthaleneacetic acid and gibberellic acid terminated *Garcinia mangostama* bud dormancy (Wiebel *et al*., 1992) and gibberellin concentration increased in *Ribes nigrum* buds as dormancy progressed towards spring (Vince-Prue, 1975). Ramina *et al*. (1995) hypothesised that these hormones induced dormancy breaking or were a result of dormancy breaking.

Bud scales not only form a barrier around buds and protect against low temperature, but they also prevent bud growth as a result of competitive absorption of nutrients (Abbott, 1969). Removal of bud scales has promoted bud burst in several crops, including *Malus domestica* (Swartz *et al*., 1984), *Ribes nigrum*, (Tinklin and Schwabe, 1970), *Vitis vinifera* 'Zinfandel' (Iwisaki and Weaver, 1977), *Pseudotsuga menziesii* (Roberts *et al*., 1974) and *Pyrus serotina* 'Kosui' (Yotsuya *et al*., 1984). Similarly, the mode of action of the dormancy-breaking chemical DiNitroOrthoCresol (DNOC) was suggested to be bud scale degradation (Abbott, 1969). However, physiological injury to a bud may promote premature bud burst (Plancher, 1983b) and removal of bud scales may constitute such an injury.

1.4. Chill Unit Models

Several chill unit models have been developed to quantify the optimum chilling temperature and duration for ending dormancy. The Two-Step model considers the effect of cycling warmer temperatures with chilling temperatures, the Degree Growth Stage Model describes the annual growth cycle of a species in relation to temperature and the Utah model assigns relative importance to actual field temperatures.

1.4.1. Two Step Model

This model takes into consideration the cycling of cold chilling temperatures followed by warmer temperatures (Lang, 1989), and has been utilised in countries where winter temperatures are not uniformly cold e.g. South Africa, where large differences between day

and night temperature have rendered other chill unit models ineffective (Allan, 1999). Temperature and bud burst data of *Prunus persica* 'Redhaven' was modeled based on Figure 1.3, and using the equation:

$$A \underset{b}{\overset{a}{\leftrightarrow}} B \xrightarrow{c} C$$

where A – dormancy phase

B – product of low temperature exposure

C – product of B

a – chilling negation reaction, initiated by high temperature (16°C to 24°C)

b – chilling reaction initiated by low temperature (0°C to 13°C)

c – conversion of B to C at moderate temperature (13ºC to 15ºC)

(after Erez and Couvillon, 1987)

At chilling temperatures, reaction *b* takes place, but reaction *c* cannot progress until warmer temperatures (13°C) are experienced. If, however, plants are exposed to excessively high temperatures (>16°C), reaction *a* occurs. According to this theory, uneven and limited bud burst is experienced at 0^{o}C as reaction *b* occurs but reaction *c* is inhibited (Erez and Couvillon, 1987). By alternating 0^{o}C with temperatures conducive to reaction *c*, plants receive the chilling required for even bud burst.

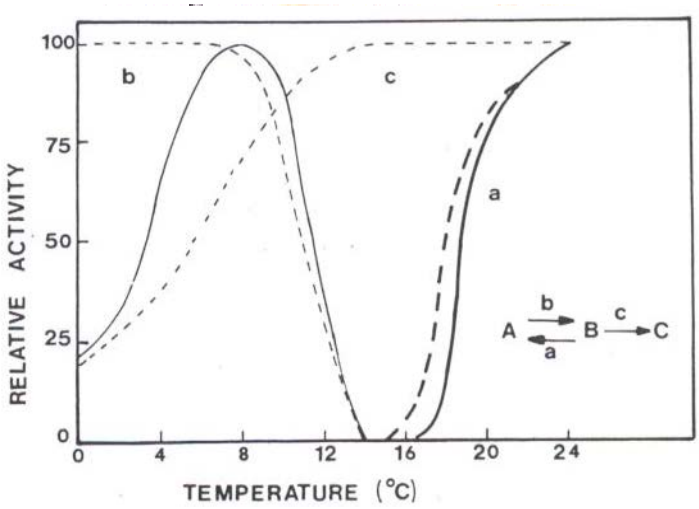


Figure 1.3. The temperature-dependant two stage model

(after Erez and Coullivon, 1987)

The two-step model was successfully used to determine *Prunus persica* 'Redhaven's' chilling satisfaction, (Erez *et al*., 1998). When used to determine the date of bud burst of *P. persica* 'Starcrest' and 'Baby Gold 9', this model was less reliable than other commercially-available models (Barba and Melo-Abreu, 2002). Little work has been conducted on the optimum chilling duration required for each step of this process, despite the model being developed over 15 years ago. The absence of large day/night temperature differentials experienced in the UK makes this model unsuitable for UK-grown crops.

1.4.2. The Degree Growth Stage Model

The degree growth stage model, ^{o}GS, was developed by exposing *Cornus sericea* to temperatures between 5°C and 20°C and quantifying the physiology of dormancy development (Kobayashi and Fuchigami, 1983). The model was developed as a sine wave (Figure 1.4) and describes the annual cycle of plant bud development (Lang, 1989). Practically, however, a perfect sine wave is never achieved as the process is dependent on the plant's external environment. Five distinct stages have been identified – spring bud burst (0°GS), maturity induction (90°), onset of endodormancy (180°), deepest dormancy (270°GS), end of rest (315°GS) and spring bud burst (360°GS) (Fuchigami and Nee, 1987).

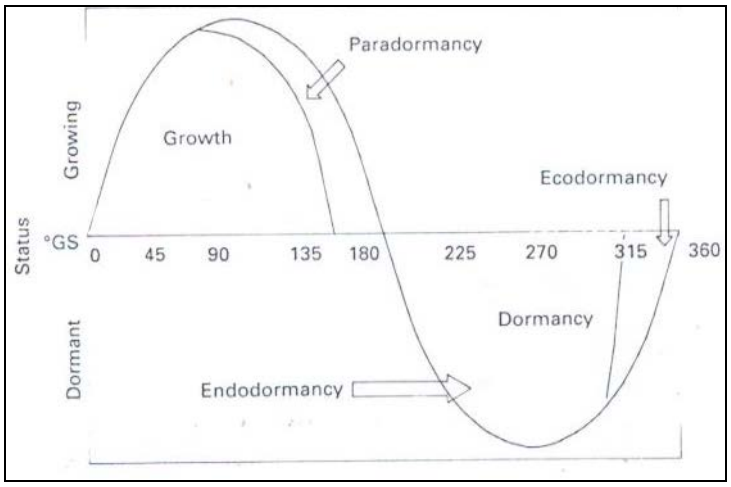


Figure 1.4. The sine wave depicting the Degree Growth Stage Model.

(after Lang, 1989)

The two-step model accurately predicted bud burst of *C. sericea* within three days of the actual date (Fuchigami and Nee, 1887) and it was successfully used to determine the effect of dormancy-breaking chemicals on *Prunus persica* (Siller-Cepeda *et al*., 1992). It is unclear, however, how the model can be practically and easily used in a commercial situation.

1.4.3. Temperature-Derived Models

Chilling models were developed originally to quantify the optimum chilling temperature required for subsequent bud burst and crop development. The majority of models were constructed by exposing plants/cuttings to specific chilling temperatures for specific durations and ranking the effectiveness of the temperature based on final bud burst. The original chill unit model, the Utah model, was developed for *Prunus persica* 'Redhaven' using this method (Richardson *et al*., 1974).

Developed by Richardson *et al*. (1974), the Utah model assumed that winter temperatures had different degrees of chilling effectiveness. A given temperature was equally effective, regardless of the duration of chilling received or dormancy stage. One hour at the optimum chilling temperature (6.1°C) was defined as one chilling unit, CU, higher/lower temperatures were deemed to contribute fractionally (Table 1.2).

The degree of weighting in this model is, however, limited, and similar models developed for *Prunus avium* 'Stella', 'Sunburst' and 'Summit' provided CU values for every °C (Mahmood *et al*., 2000a). Although 6.1ºC was determined to be the optimum chilling temperature, the Utah model assumed that temperatures between 2.5°C and 9.1°C were equally effective Richardson *et al*. (1974).

Table 1.2. Temperature and corresponding Utah Chill Units (after Richardson *et al*., 1974)

Temperature (°C)	Chill Units
<1.4	0.0
1.5 - 2.4	0.5
2.5 - 9.1	1.0
9.2 – 12.4	0.5
12.5 – 15.9	0.0
16.0 – 18.0	-0.5
>18.0	-1.0

Despite never claiming to be universally applicable, many authors attempted to apply the Utah model to different crop species, with mixed results. When compared against the North Carolina model, developed using *Malus domestica* 'Starkrimson Delicious', the optimum temperature was found to be slightly higher for the Utah model, 7^{o}C compared to 6.1^{o}C

(Shaltout and Unrath, 1983). When applied to *Prunus persica* 'Redhaven', Erez and Couvillon (1987) confirmed the optimum chilling temperature to be 6^{o}C. Weinberger (1956), however, reported that the optimum chilling temperatures for *P. persica* 'Hiley' and 'Elberta' were significantly higher, circa. 10°C, than reported by Richardson *et al* (1974) and Erez and Couvillon (1987). Using the Utah model, lower temperatures were found to contribute significantly less (Shaltout and Unrath, 1983; Erez and Couvillon, 1987). Similarly, the Utah model considered high temperatures to have less of a negative effect than the North Carolina model (Shaltout and Unrath, 1983). Based on this, Shaltout and Unrath (1983) concluded that their model was more accurate than the Utah model. Differences in the chilling requirement of cultivars, however, may explain the lack of correlation when the model was applied to other species (Plancher, 1984).

Using similar methods, species-specific models were developed for *Malus domestica* (Young and Werner, 1985; del Real-laborde *et al*., 1990), *Fragaria ananassa* 'Elsanta' (Tehrenifar *et al*., 1998), *F. ananassa* 'Glasa' and 'Tioga' (Kronenberg *et al*., 1976), *Prunus persica* 'Redhaven' (Scalabrellli and Couvillon, 1986; Erez and Couvillon, 1987), *Vaccinium vitis-idea* (Holloway *et al*., 1983), *Actinidia chinensis* 'Tomuri' (Guerriero *et al*., 1990) and *Prunus avium* 'Sunburst', 'Stella' and 'Summit' (Mahmood *et al*., 2000a).

Richardson *et al*. (1975) stated that after fulfillment of the chilling requirement, growth was dependant on exposure to temperatures above 4.5°C and refined the Utah model to include this post-chilling heat requirement. Measured in Growing Degree Hours (GDH's), one GDH is gained after exposure to one hour at a temperature 1°C higher than 4.5°C. GDH's are calculated by subtracting 4.5 from each hourly temperature that falls between 4.5°C and 25°C (Richardson *et al*., 1975). Increasing the post-chilling temperature of *Malus domestica* 'Red Delicious', 'Camuzat', 'Spatbluhender', '150', '226' and '337' from 10°C to 20°C decreased the time to first bud burst (Gianfagna and Mehlenbacher, 1985). After forcing at 10°C and 15°C, two distinct groups were visible. The first group, comprised of 'Red Delicious', '150' and '226', required less than five days to bud burst, regardless of the forcing temperature. The second group required 7-17 days to bud burst after forcing at 15°C and had not bud burst after 35 days of forcing at 10°C (Gianfagna and Mehlenbacher, 1985). It may be more appropriate, based on this evidence, to weight the post-chilling heat requirement, as the chill units are weighted, to accommodate the differing effectiveness of temperatures.

The relative importance of the chilling requirement compared to the post-chilling heat requirement has been the subject of debate for many years. Erez and Couvillon (1987) suggested that the chilling requirement was the most important factor, whereas Scalabrellli and Couvillon (1986), argue that GHD was more important. Chilling in excess of that required for 50% bud burst of *Malus domestica* 'Rome Beauty' 'Top Red Delicious' and 'McIntosh', *Prunus avium* 'Bing' and 'Lambert', *Prunus persica* 'Cardinal' and 'Coronet' and *Pyrus Communis* x *Pyrus pyrifolia* 'Kieffer' resulted in a decrease in GDH (Couvillon and Erez, 1985). The GDH of majority of the varieties responded in a curvilinear fashion, with GDH decreasing to a minimum as chilling increased until further chilling had no more of an effect (Swartz and Powell, 1981; Cannel and Smith, 1983; Couvillon and Erez, 1985; Scalabrelli and Couvillon, 1986).

Andersen (1992) modified the Utah model by lowering the GDH base temperature from 4.5°C to 2°C in an attempt to increase its accuracy. Using the original Utah model, the predicted date of chill satisfaction of *Betula* and *Alnus* was reported to be within 4.2 days and 5 days respectively of the actual date. Using the modified model, this was improved to 3.4 days and 2.8 days (Andersen, 1992). Ebert *et al*. (1986) improved on the accuracy of the Utah and North Carolina models stating that negation of chill units could only occur up to 96 hours after the last positive chill unit was registered.

The Utah model was utilised or modified to predict chilling requirements in cool temperate areas with some success but its use in warm temperate areas and the subtropics has proved less successful. The count of hours <7.2°C was compared with the Utah and North Carolina models for *Malus domestica* in three geographical areas (Sao Joaquim, Videira and Cacado) of Santa Catarina, Brazil (Ebert *et al*., 1986). In all areas in 1982, bud burst was poor, reflected in the low count of hours <7.2°C. The Utah and North Carolina models, however, only recorded low values in Cacador. The authors concluded that the North Carolina model was most suited to the Brazilian climate, and was marginally more accurate than the Utah model (Ebert *et al*., 1986). The effect of field chilling on *Prunus persica* 'Sunlite', 'Fantasia' and 'Flavortop' in six growing regions of Western Cape, South Africa, were compared using the Utah and Two-Step models (Linsley-Noakes and Allan, 1994). The two-step model was concluded to be superior.

1.4.4. *Ribes nigrum* Chill Unit models

To date, two researchers have investigated the effects of varying winter chilling on *Ribes nigrum*. Lantin (1973) and Plancher (1983a, 1983b, 1984) utilised cultivars that have been superceded by modern UK derived cultivars therefore the accuracy of the Lantin and Plancher models may be limited.

Lantin (1973) investigated the effects of winter chill on 59 *Ribes nigrum* and 28 *Ribes rubrum* cultivars. Two main experiments were carried out, the first looking at the effects of chilling on cut stems, the other looking at the effect of chilling on the whole plant. The author discovered significant differences between the chilling requirements of different blackcurrant cultivars.

At set periods of time, stem cuttings were taken from plants that had been exposed to natural field chilling. The cuttings were then placed in a growing chamber at 20°C and time to first bud burst as well as final bud burst was recorded. Lantin observed that when cultivars with a high chilling requirement did not receive sufficient chilling, greatly reduced and uneven bud burst occurred and therefore after mild winters, cultivars with a high chilling requirement had a significantly reduced yield compared with more severe winters. It was suggested that the cultivars could be placed into one of four groups (Figure 1.5) with Group 1 requiring little chilling and Group 4 requiring large amounts of chilling. The gradients of Graphs 1 to 3 are very similar but the increased gradient of Graph 4 is thought to be due to cultivars in this group requiring higher post-chilling temperatures.

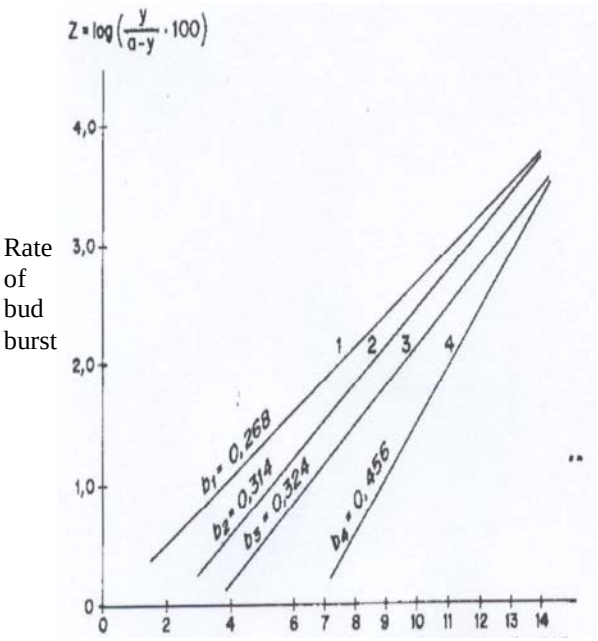


Figure 1.5. ***R. nigrum*** **- Lantin's groupings based on chilling requirement** (after Lantin, 1973)

After exposing whole plants to 2°C for different chilling durations, Lantin (1973) discovered that bud burst and the flower emergence increased with increased chilling duration. Plants exposed to less than 6 weeks chilling did not bud burst and after 6 to 10 weeks bud burst was uneven and limited and high rates of flower abortion were observed. Increasing the chilling duration to 14 weeks significantly increased bud burst and flower initiation progressed successfully.

Plancher (1983a, 1983b, 1984) conducted a range of dormancy experiments with *Ribes nigrum*. French cultivars were subjected to chilling temperatures ranging from -3°C to 12°C for a maximum of 12 hours. Differences due to cultivar were highlighted, with the lowest optimum chilling temperature (-3ºC) recorded for cultivars regarded as having the highest chilling requirement, and higher temperatures (3-6ºC) for cultivars with lower chilling requirements. Unlike research conducted on *Prunus avium* (Mahmood 1999), an interaction between chilling duration and chilling temperature was recorded. After chilling for 600 hours, one chill unit for *R. nigrum* 'Sewegard' was determined to be 0°C, however, as the chilling duration increased to 1200 hours, the optimum chilling temperature was considerably higher at 6°C. Again, the applicability of the research to modern-day cultivars is likely to be limited.

1.5. Climate Change and the implications for *Ribes nigrum* in the UK

Several commercial *Ribes nigrum* growers in the UK (Maynard, T.; Thompson, E., *Pers.comm*.) have noticed a changing behaviour in the crop, e.g. delayed and uneven bud burst and flower emergence, particularly for cultivars that bud burst late in the spring and are thought to have high chilling requirements. These symptoms result in uneven fruit production and ripening and may have severe consequences for the resulting quality and quantity of marketable berries. Climate change, in particular warmer winters, may have devastating implications for the UK blackcurrant industry.

1.5.1. Climate Change Scenarios

The United Kingdom Climate Impacts Programme (UKCIP) was established by the Department for Environment, Food and Rural Affairs (DEFRA) in April 1997, its remit being to co-ordinate research on the effects of climate change at regional and national levels. In 1998 and again in 2002, the UKCIP published reports (Hulme and Jenkins, 1998; Hulme *et al*., 2002) detailing four possible climate change scenarios. Based on the level of greenhouse gas emissions, the scenarios have been labeled as Low, Medium-low, Medium-high and High. The average carbon dioxide (CO_2) concentration between 1961 and 1990 was 334 parts per million (ppm). By 2080, the low and high climate change scenarios indicate this level may rise by 57% to 525ppm, and 143% to 810ppm respectively (Hulme *et al*., 2002). The remainder of this section will discuss the implications of climate change, but it should be stressed that the proposed scenarios are based on predictions which may or may not be accurate.

An annual temperature increase of 0.1ºC to 0.3ºC per decade will occur under the Low and High scenarios respectively. The warming is expected to demonstrate a southeast to northwest gradient, with the southeast consistently warming by several tenths of a °C more than the northwest, and warming will be greatest in winter. The occurrence of more extreme weather is predicted to increase (Table 1.3).

Table 1.3. The effect of different climate change scenarios on the UK climate

(after Hulme *et al*., 2002)

Scenario	Year	Temperature (ºC)	Precipitation (%)	Cloud cover (%)	R.H. (%)	Snowfall (%)	Soil Moisture Content (%)
Low	2020	+0.75	-5				
Low	2050	+1.75	-5				
Low	2080	+2.25	-5	-4.5	-4.5	-55	-5
Med-low	2020	+0.75	-5				
Med-low	2050	+1.75	-5				
Med-low	2080	+2.75	-5	-4.5	-4.5	-75	-5
Med-high	2020	+0.75	-5				
Med-high	2050	+2.25	-5				
Med-high	2080	+3.75	-5	-7.5	-7.5	-85	-15
High	2020	+1.25	-5				
High	2050	+2.25	-5				
High	2080	+4.25	-5	-7.5	-7.5	-95	-15

In 1997, the UK experienced its third warmest year: using the Medium-high and High scenarios, by 2020 more than half of the years will be warmer than 1997. By 2080, in all scenarios except the Low, most years will exceed the temperature of 1997 (Hulme *et al*., 2002).

The effect of climate change will be experienced world-wide, but the increase in global temperature is predicted to be similar to that reported for the UK. Cumming and Burton

(1996) modeled temperature data to Canadian forest regions. In three regions out of the eight studied, it was predicted that lack of winter chilling would dramatically alter the forests, at low, medium and high altitudes. Current native species are expected to die-back and be replaced by shrubs or non-native species that have lower chilling requirements.

1.5.2. The Effect of Climate Change on UK Production of *Ribes nigrum*

The Meteorological Office was commissioned to produce a report (Hough, 2002) detailing past temperature records and predicting future chilling in different blackcurrant-growing regions of the UK. Temperature was recorded in five regions – Leuchars (latitude; longitude 56.377;-2.861), Coltishall (52.728;1.365), Pershore (52.100;-2.059), Herstmonceux (50.889;0.322), Elmdon/Coleshill (52.500;-1.707), and Lyneham (51.881;-1.596) and this provided temperature data for East Scotland, Norfolk, South-West Midlands, East Sussex/Kent, South-West Midlands and East Somerset respectively. Chilling was recorded between October and March for the winters of 1996 - 2001 in all areas and data for the winters 1976-1977 and 1986-1987 were available for a number of sites.

The report used two methods to quantify chilling - chill units and chill hours. The first method assumed that temperatures between 2.5^{o}C and 9.1^{o}C were optimum for *Ribes nigrum*, based on the Utah model. Temperatures within this range were assigned a value of 1 and temperatures out-with this range were assumed to contribute less and were assigned fractional values (Table 1.3). Negative values indicate that temperatures above 16^{o}C can negate chilling units previously accumulated by the plants

Table 1.4. Temperatures that contribute to chill units. (after Hough, 2002)

Temperature (^{o}C)						
<1.5	1.5 - 2.4	2.5 - 9.1	9.2 - 12.4	12.5 – 15.9	16.0 - 18.0	>18.0
0	0.5	1.0	0.5	0	-0.5	-1

The second method assumed that temperatures between 0^{o}C and 7^{o}C contributed equally to chilling and one hour between this temperature range was assigned a value of 1. Temperatures out-with this range were assumed to have no effect and were not assigned a

value. As current commercial practice is to count the number of hours the field temperature falls between 0°C and 7°C, this method will be discussed.

As can be seen from Figure 1.6, there was considerable variation between years in the number of chill hours recorded in each region. The relatively low number of chill hours experienced in the winter of 1997-1998 was followed by late and uneven bud burst and hence reduced crop yields (Saunders, R., *Pers. comm.*)

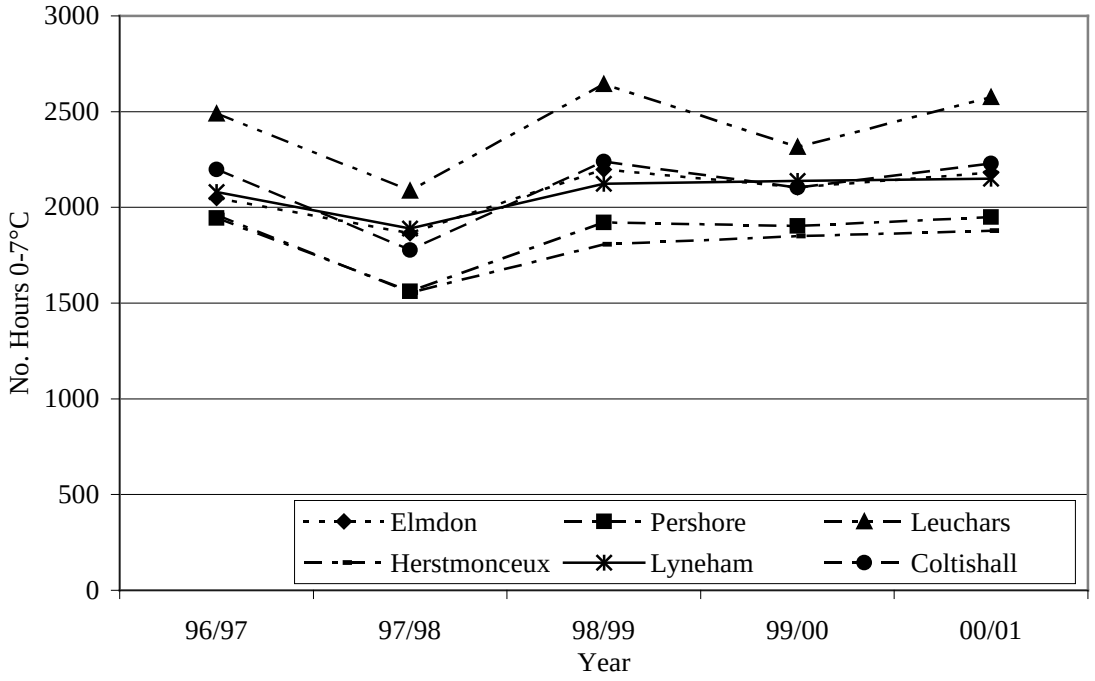


Figure 1.6. Chill hours experienced between 1996 and 2001 in different geographical locations. (after Hough, 2002)

Predicted chill accumulation, calculated based on the Medium-high climate change scenario which assumes that greenhouse gas emissions will increase annually by 1%, resulted in a marked decrease in chill hours (Figure 1.7).

The number of hours of chill are expected to decrease by 30 – 40% over the next 100 years and Atkinson *et al*. (2004) predicted that the number of hours <7.2°C will decrease by as much as 59% by 2080 under the High emission climate change scenario.

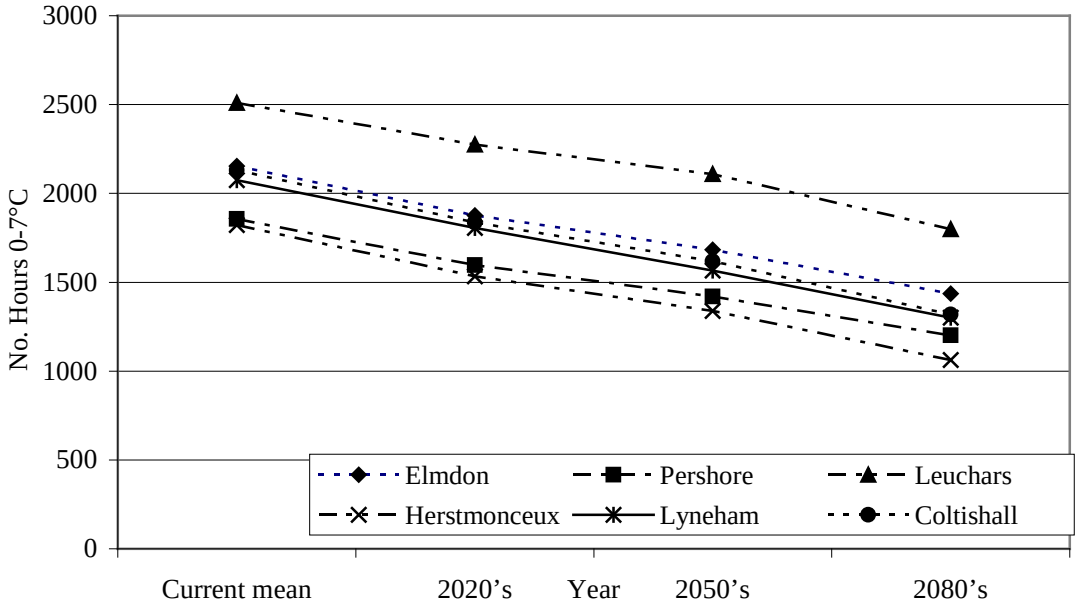


Figure 1.7. The predicted decline in chill hours. (after Hough, 2002)

Flower emergence is expected to become delayed, and yield decreased with symptoms being most severe in the South of England and yield increases in Tayside (Atkinson *et al*., 2004). This is because plants in this area receive excess chilling and which will be unaffected by the predicted temperature increases. Also, warmer spring temperatures were predicted to favour early bud burst and flower production, hence the fruiting ability of such plants could increase.

1.6. Forcing the Termination of Dormancy

Although chilling is required to terminate dormancy, and insufficient chilling is detrimental to subsequent growth and development, temperate-zone crops have been successfully cultivated in tropical countries. Crop management techniques have been manipulated, albeit with mixed results, in an attempt to grow such crops out-with their natural geographical range with exposure to minimum chilling.

1.6.1. Premature Defoliation

Assuming the theories of Tinklin and Schwabe (1970) and Mielke and Dennis (1978), if a growth inhibitor was synthesized and translocated from plant leaves to bud scales then into the bud, thus preventing bud burst, removing the source of the inhibitor would prevent accumulation. Subsequent growth, therefore, would be expected to be advanced.

In tropical countries, temperate-zone crops enter a shallow dormancy that is broken by exposure to stress, in the form of premature defoliation (Dennis, 1987). This technique has been employed to successfully cultivate *Prunus* spp. and *Malus domestica* in Thailand, Venezuela and India, where winter temperatures do not satisfy the chilling requirement (Dennis, 1987). Premature defoliation induced bud burst of *Prunus persica* 'Flordaprince' before any chilling had accumulated, based on the Utah and <7.2°C models, indicating that premature defoliation was the sole cause of bud burst (Lloyd and Firth, 1990). Defoliation of *P. persica* 'Flordagold' and 'Flordaprince' resulted in a decrease in dormancy, although it is unclear what measure of dormancy was utilised (Lloyd and Firth, 1990).

Premature defoliation, however, did not affect bud burst of *P. persica* 'Redhaven' (Crisosto *et al*., 1987), *Actinidia chinensis* 'Hayward' (Snelgar *et al*., 1997), *Olea europaea* 'Ascolano' and 'Manzanillo' (Rallo and Martin, 1991), *Ribes nigrum* 'White Bud' (Westmore, 2004), *R. nigrum* 'Vija' and Klone 8 (Plancher, 1983b). Treatment timing is an important factor however, and Crisosto *et al*. (1987), Westmore (2004) and Plancher, 1983b) defoliated the respective plants either just before the date of natural senescence, or when leaf fall had begun. Detrimental effects on *R. nigrum* 'Baldwin' flower production were observed after premature defoliation, and the earlier the treatment was applied, the more severe the reaction (Corke and Wilson, 1963). Contrastingly, however, defoliation increased the yield of *P. persica* 'Redhaven' (Crisosoto *et al*., 1987).

1.6.2. Nitrogen Application

Bud burst of *Malus domestica* 'Lord Lambourne' (Delap, 1966), 'Golden Delicious' (Terblanche *et al*., 1979) and 'Cox's Orange Pippin' (Delap, 1967) was advanced in tropical countries following nitrogen fertlisation. The rate of bud burst and final bud burst were unaffected by nitrogen application (Terblanche *et al*., 1979), however treated plants demonstrated higher fruit set and yields (Delap, 1966). The form of nitrogen may be important - urea and nitrate nitrogen were used in the above experiments, but application of nitram was detrimental to *Prunsus persica* 'Flordaprince' bud burst (George and Nissen, 1992). The timing of application may also be an important factor in the above discrepancies. Autumn application of nitrogen (0.016 $g.l.^{-1}$) advanced bud burst of *M. domestica* 'Cox's Orange Pippin' but spring and summer applications were ineffective (Delap, 1967).

1.6.3. Extended Photoperiodic Regimes

Extended photoperiods (24-hours) have been observed to substitute for insufficient chilling and promote growth and development (Campbell and Sugano, 1975), and have been successfully applied to promote premature bud burst of *Picea abies* (Heide, 1974a), *Ribes nigrum* 'Boskoop Giant' (Hoyle, 1960), *Acer saccharum* (Olmsted, 1961), *Picea sitchensis* (Cannell and Smith, 1983), *Fagus sylvatica, Larix decidua* and *Betula pubescens* (Wareing, 1954b). No effect of extended photoperiod was recorded for *Acer pseudoplatanus*, *Robina pseudoacacia* (Wareing, 1954b) and *Cornus alba* (Whalley and Cockshull, 1976), but this may be because the chilling requirements of these species were sufficiently satisfied (Heide, 1993a), or the chilling deficits may have been too large for treatment to overcome.

1.6.4. Application of Dormancy Breaking Chemicals

Dormancy breaking chemicals have been utilized to induce bud burst in several crop species e.g. *Malus domestica* grown in Yemen (Finetto, 1993) and *Prunus armeniaca* grown in Turkey (Kuden and Son, 1997). There are several commercially-available products that are rely either on oil, nutrient or chemical activity to force bud burst. The major drawback in using oils and chemicals, however, is the rigorous and expensive registration process that must be undertaken before application to the crop in the UK is permitted. Application has also been reported to have detrimental effects on the crop; e.g. hydrogen cyanamide can be toxic to flowers and DNOC-oil is phyto-toxic (Erez and Yablowitz, 1997).

GlaxoSmithKline identified the potential for the adjuvant Abacus (active ingredients alkylphenyl hydroxypolyoxyethylene, natural fatty acids and esterified rape seed oil) to be used as a dormancy breaking chemical. The mode of action of Abacus is unknown but it was suggested that when sprayed onto buds it formed a seal, preventing transpiration and resulting in a stress reaction (Mitchell, R., *Pers. Comm*.). Alkylphenyl hydroxypolyoxy-ethylene is a member of the nonylphenol ethoxylate family, and despite the equipment and technology available to chemists, the exact degradation process of this substance process has not been fully identified (Montgomery-Brown and Reinhard, 2003). The first step of degradation of this compound is the loss of ethoxylate (C_2H_40) side groups, which may further degrade to produce ethylene or a similar breakdown product, and may induce bud burst (Jonkers *et al*., 2001). Regardless of the lack of understanding in this subject area, initial field trials have proved promising.

1.7. Objectives

The primary objective of the work described in this thesis was to develop chill unit models, described in Chapter 4, for the commercially-important *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran'. Within this objective, the effects of varying winter chilling temperatures and durations of winter chilling on time to first bud burst and yield were investigated in Chapter 3, to establish to what extent chilling influences these factors.

The proposed effects of climate change scenarios have been hypothesised, yet practical research has not been undertaken to determine the effects on *Ribes nigrum* physiology. Elevated spring temperatures, as forecast under the climate change scenarios, were therefore simulated in Chapter 5 and effects on growth and development quantified. In addition, the ability of elevated temperatures to over-come chilling deficits was investigated.

Commercial *Ribes nigrum* cultivars are bred traditionally and although the breeders are well on their way to breeding low chill requirement into the crop in anticipation of the proposed climate change scenarios, such methods are not expected to produce results for several years. Growers have reported the symptoms of insufficient chilling for several years, including detrimental effects on flowering and yield. Short term solutions to this problem were therefore investigated in Chapter 6, aimed at manipulating the depth of dormancy to force premature bud burst in spring.

The physiology of modern-day *Ribes nigrum* has not been investigated, and as such no record of the timing and rate of *R. nigrum* floral initiation is available. Crop management practices aimed at reducing the depth of dormancy may be implemented at the beginning of flower initiation and hence may have a detrimental effect on this process. In addition, climate change is predicted to have a negative effect on flower production, but to understand these effects and overcome them, the timing of flower initiation and development were investigated in Chapter 7.

Chapter Two.
General Materials and Methods

2.1. Plant Material

Plant material was supplied by GlaxoSmithKline and unless otherwise stated, consisted of one-year old rooted softwood cuttings taken from certified disease-free stock by Steven Wickham (Harper's Farm, Goudhurst, Kent) and potted into peat. Plants were stored in Kent and hand watered until required. On delivery to the University of Reading's Experimental Field Site, the plants were arranged into double rows with 10cm between plants within a row and 80cm between rows. Black soft-walled, 16mm irrigation piping (Field Irrigation Ltd., Appledore Farm, Kent) was positioned in the middle of each double row and spaghetti drippers, supplying nutrients at a rate of $8lh^{-1}$, were added where required, with 2 drippers being placed in each 5l pot and one dripper placed in each 3l pot. In order to stabilise the plants, wooden stakes were placed at the end, a third of the way up and two thirds of the way up each double row. Horticultural wire was run up each double row and nailed to the stakes, 20cm above ground level. The plants were held onto the wire using plastic ties.

2.2. Irrigation System

Nutrients (Table 2.1) were supplied to the plants via a Dosatron (Dosatron International, Rue Pascal, B.P. 6, 33370 Tresses, Bordeaux, France). Irrigation timers were connected to the mains water supply to regulate the timing of the irrigation and a Dosatron was attached to the system after the timer to prevent the acid in the nutrient solution damaging the timer. The concentration of the stock nutrient solution was altered by turning the bottom of the Dosatron until the run-off irrigation solution reached an electrical conductivity (E.C.) of 1.5mS. In order to obtain a pH of 6-6.5, small volumes of nitric acid were added to the stock solution until the run-off irrigation reached the desired pH. The irrigation system was shut off at the end of October and the Dosatron and timer removed and placed inside to prevent frost damage. The irrigation system was left in a state such that if there was an unseasonably warm period and the pots began to dry out, water could be supplied to the plants.

Table 2.1. Composition of the stock nutrient solution

Nutrient	Chemical Symbol	Weight
Potassium Nitrate	KNO_3	2.5kg
Mono Potassium Phosphate	KH_2PO_4	0.9kg
Magnesium Sulphate	$MgSO_4$	1.0kg
Iron Ethylenediamine Tetraacetic Acid	FeEDTA	70.0g
Manganese Sulphate	$MnSO_4$	17.5g
Zinc Sulphate	$ZnSO_4$	15.0g
Disodium Octaborate Tetrahydrate	$Na_2B_8O_{13}.4H_20$	7.0g
Copper Sulphate	$CuSO_4$	5.0g
Sodium Molybdate	$NaMoO_4$	1.5g
Water	H_20	20L

2.3. Randomisation

Experiments were conducted in a polythene tunnel at the University of Reading's Experimental Field Site, Reading and in a glasshouse based in the School of Plant Science's Experimental Glasshouse Facility, Reading. Both structures were divided into positional blocks, in order to minimise differences in the growing environments within the structures as a result of orientation, ventilation and heating differentials.

The polythene tunnel was divided into five positional blocks, each being further sub-divided into 80 plots. When removed from the cold store, random number tables were utilized to assign a plot number (1-80) to each pot, ensuring one rep from each treatment was present in each of the five blocks.

The glasshouse was similarly divided into five positional blocks, each being further sub-divided into 100 plots. When removed from the cold store, random number tables were utilised to assign a plot number (1-100) to each pot, ensuring one rep from each treatment present in each of the five blocks.

2.4. Observations

Bud burst was taken as the point at which the bud had swollen, the bud scales fallen back and a new green leaf just started to emerge from the bud (Figure 2.1).

Plate 2.1. Point of bud burst of *R. nigrum* – bud has swollen, scales are peeling back and the new green leaf is just visible

Final bud burst was calculated by the equation:

Bud burst = (x/y) * 100

Where x – number of buds that burst

y – total number of buds

To determine the effect of apical dominance, the number of terminal buds that burst on each stem was counted. Discounting the terminal bud, stems were theoretically split into three separate sections, termed 'Top', 'Middle' and 'Bottom' and bud burst calculated using the above equation.

Time to anthesis was taken as the number of days, from the start of the experiment to the date of the first flower fully opening. The total number of flowers that fully opened and the total number of ripe, purple berries were counted over the whole of the plant. Shoot extension was measured from the previous season's hard wood to the tip of the present season's growth using a 30cm ruler.

2.5. Data Analysis

Statistical advice was regularly sought throughout the experiments from the University of Reading's Statistical Advice Centre. The majority of the experiments were of a completely randomized block design. For the chill unit model regression analysis, the data was transformed to radians using the equation:

$y = \sin^{-1}(\sqrt{p})$

where: y = radians

p = number of burst buds/total number of buds

Genstat's analysis of variance (ANOVA) was used to calculate the mean and probability based on 5% confidence intervals. When analysed, if significant interactions between the main treatments were discovered, the data were sub-divided and re-analysed. If no significant interactions were evident, data were pooled. Data are generally presented as mean values along with the associated least significant difference (LSD) and degrees of freedom values, generated from the ANOVA.

Chapter Three.
The Physiological Response of *Ribes nigrum* to Chilling

3.1. Introduction

The effect of chilling duration and chilling temperature on bud burst and whole plant behaviour has been studied in *Fragaria ananassa* (Bailey and Rossi, 1965; Tehranifar, 1998), *Ribes nigrum* (Plancher, 1983a), *Prunus persica* (Scalabrelli and Couvillon, 1986), *Prunus avium* (Mahmood *et al*., 2000a) and *Rubus idaeus* (Carew *et al*., 2001). The following review of literature discusses the effect of chilling duration and temperature on subsequent plant growth and development, after plants were removed from chilling and placed in forcing environments.

3.1.1. Differences due to Cultivar

As described in Chapter 1, the breeding histories of the three cultivars used in this experiment are very different - 'Ben Gairn' is reported to have a low chilling requirement, 'Ben Tirran' a high requirement and 'Ben Hope' is intermediate (Brennan, R. *Pers. Comm*.), which suggests that the cultivars' response to temperature differs. This has implications when constructing chill unit models – if the cultivars behave differently, separate models will have to be constructed, but if they behave similarly one model may be sufficient.

3.1.2. Effect of Chilling Duration

Many authors have reported the beneficial effects of prolonged chilling exposure on subsequent plant growth, bud burst and crop yield. Vegetative growth was stimulated when the chilling duration of *Fragaria ananassa* 'Elsanta' (Tehranifar *et al*., 1998), *F. ananassa* 'Catskill' (Bailey and Rossi, 1965), *Prunus avium* 'Stella' (Mahmood *et al*., 2000a), *Rubus idaeus* 'Autumn Bliss' (Carew *et al*., 2001) and *Rubus fructicosus* 'A-1836' and 'APF-13' (Lopez-Medina and Moore, 1999).

When the duration of chilling of *Malus domestica* was increased from 200 to 1200 units, final bud burst increased from 20% to 90% (del Real-Laborde *et al*., 1990). Similarly, as the chilling duration of *Ribes nigrum* increased from 1810 to 2950 chill units, bud burst increased from 30% to 80% (Plancher, 1983a). Increasing chilling duration of *Prunus persica* 'Redhaven' from 600 hours to 2040 hours had no effect on the bud burst behaviour of terminal buds, but an increase in percentage bud burst of lateral buds was achieved (Scalabrelli and Couvillon, 1986).

Time to first flower of *Fragaria ananassa* 'Elsanta' (Tehranifar *et al*., 1998), *Rubus idaeus* 'Autumn Bliss' (Carew *et al*., 2001), *Prunus avium* (Mahmood *et al*., 2000b) and *Rubus fructicosus* 'A-1836' and 'APF-13' (Lopez-Medina and Moore, 1999) was advanced as exposure to chilling temperatures increased. Flower bud production of *Prunus persica* 'Redhaven' was stimulated in plants exposed to 2040 hours of chilling compared to those exposed to 600 hours (Scalabrelli and Couvillon, 1986). As the chilling duration of *F. ananassa* 'Catskill' increased to 1000 hours, an increase in flower production was observed (Bailey and Rossi, 1965). Extending the chilling duration of *F. ananassa* 'Elsanta' by 2 weeks increased fruit production, although further extending the chilling duration had a detrimental effect (Tehranifar *et al*., 1998). Proportion of fruit set and average flower size of *P. avium* 'Stella' was improved as the duration of chilling increased from 360 hours to 1440 hours (Mahmood *et al*., 2000b).

3.1.3. Effects of Chilling Temperature

The effect of chilling temperature on plant growth and development has been widely reported, although the optimum temperature is very much dependant on species. Lantin (1973) predicted that dormancy was broken quicker as the chilling temperature of *Ribes nigrum* decreased from 7°C to –7°C. After 6 weeks of chilling *Fragaria ananassa* 'Elsanta', plants exposed to 3°C had longer petioles than those chilled at -2°C (Tehranifar *et al*., 1998). Compared to chilling at 15°C, exposure of *F. ananassa* 'Glasa' and 'Tioga' to 3°C resulted in rapid and vigorous plant growth (Kronenberg *et al*., 1976). While terminal bud burst of *Prunus avium* 'Redhaven' was unaffected by chilling temperature, lateral bud burst was increased as the chilling temperature increased from 2°C to 3°C to 7.2°C (Scalabrelli and Couvillon, 1986). Percentage bud burst of *Malus domestica* increased as the chilling temperature increase from -2°C to 6°C (del Real-Laborde *et al*., 1990). Chilling at 6°C, however, was more effective than 22°C. Richardson *et al*., (1974) deemed temperatures between 2.5°C and 9.1°C to be optimum for satisfying the chilling requirement of *Prunus persica* 'Redhaven' and temperatures above or below this contributed fractionally. Chilling *F. ananassa* 'Elsanta' at -2°C was as effective at promoting flower and fruit production as chilling at 3°C (Tehranifar *et al*., 1998). Exposing *P. persica* 'Redhaven' to 7.2°C had a detrimental effect on flower bud opening compared to chilling at 2°C (Scalabrelli and Couvillon, 1986). Yield of *F. ananassa* 'Elsanta' was increased after exposure to 4°C compared to 8°C, 18°C or 20°C (Lieten, 1992).

3.1.4. Budsticks v. Whole Plants

There has been much debate concerning the material used in experiments aimed at quantifying the effects of chilling regimes on endodormancy. Snelgar *et al*. (1997) used *Actinidia chinensis* budsticks (unrooted cuttings), initially stating that there was no difference in the behaviour of budsticks compared to whole plants. The results of the experiment, however were contradictory. Compared to whole plants, budsticks taken in May burst bud earlier, over a prolonged period of time, had a lower final percentage bud burst and produced less flowering shoots and inflorescences (Snelgar *et al*., 1997) . When budsticks were cut in June, however, the only difference was a reduction in the number of inflorescences. Previous research on *A. chinensis* concluded that budsticks required significantly less chilling to promote bud burst compared to whole plants (Guerriero *et al*., 1990). Bud burst discrepancies between whole plants and budsticks were also reported for *Ribes nigrum* where apical buds burst bud first on whole plants, whereas basal buds were the first to burst on budsticks (Plancher, 1983b). Plancher (1983b) hypothesised that the chilling requirement of budsticks was lower than that of whole plants, a view supported by Lantin (1973). It was suggested that the injury caused by removing a branch from a plant interfered with the chilling requirement (Lantin, 1973). Mahmood *et al*. (2000a) reported that budsticks of *Prunus avium* 'Stella' had a lower final percentage bud burst compared to whole plants, but that the responses of both materials to varying chilling regimes were directly comparable. Although Nishimoto and Funisaki (1995) reported no difference in the chilling requirement of whole *Prunus persica* 'Chiyohime' plants compared to budsticks, large variations in chilling hours (below 7°C) were discovered between years. In 1987, 1988 and 1989, 820 hours, 1085 hours and 964 hours respectively were required to satisfy chilling of budsticks, but whole plants required just 974 and 930 hours. On average, however, 956 hours were required for budsticks and 952 hours for whole plants, and it was concluded the chilling requirement of budsticks was comparable to that of whole plants (Nishimoto and Funisaki, 1995).

The first objective of the experiment described in this Chapter was to determine the extent to which the physiological response of *Ribes nigrum* cultivars 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' to identical chilling regimes differed. The ability of chilling temperature and duration to overcome endodormancy and not only advance the time of first bud burst but also to increase crop yield was examined. Additionally, to increase the number of experiments

conducted by substituting whole plants for budsticks, the final objective was to determine if budsticks responded to chilling in a similar manner to whole plants.

3.2. Materials and Methods

3.2.1. Experiment 1. The Effect of Chilling Temperature and Duration

In addition to providing data to facilitate the construction of chill unit models, this experiment was designed to determine the effects of varying chilling temperatures and durations on time to first bud burst and crop yield.

Plant Material

One year old potted plants of the cultivars 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' were utilised in this experiment, as described in Chapter 2. Cuttings were taken in spring 2002 and placed into 3l ('Ben Gairn') and 5l ('Ben Hope' and 'Ben Tirran') pots of peat. 'Ben Hope' cuttings did not root and one year old bushes were transplanted from the field (Old Shield's Farm, Arleigh, Essex) on 29 November into 5l pots filled with peat. 'Ben Tirran' and 'Ben Gairn' plants were stored outside in Goudhurst, Kent, and 'Ben Hope' plants in Essex, being hand-watered when required. The plants were delivered to the University of Reading's Field Unit on 13 December 2002 where they were placed in double rows and tied into supporting wire.

Cold Storage

Originally, five temperature treatments were chosen: -3.4°C, 0.1°C, 1.5°C, 3.4°C and 8.9°C (see Table 3.1). Temperature data loggers (Gemini Data Loggers, Chichester, UK) were placed in the walk-in cold stores to monitor temperature over 48 hours and ensure continuity. Twenty five plants of each cultivar were chosen at random and placed in the designated cold stores on 20 December 2002. Black polythene was wrapped around the stems to prevent the cold air desiccating the buds.

Table 3.1. Average two-weekly temperature readings from the cold stores and polytunnel

Temp/ Week	-3.4 ± 0.7°C	0.1 ± 0.7°C	1.5 ± 0.6°C	3.4 ± 0.6°C	8.9 ± 0.6°C	17.1±6°C
2	-3.40 ± 0.7°C	0.02 ± 0.7°C	1.08 ± 0.6°C	3.85 ± 0.6°C	8.66 ± 0.5°C	17.16 ± 3°C
4	-3.39 ± 0.7°C	-0.17 ± 0.6°C	1.77 ± 0.6°C	3.70 ± 0.3°C	8.93 ± 0.6°C	16.72 ± 4°C
6	-3.49 ± 0.6°C	-0.22 ± 0.6°C	1.41 ± 0.7°C	3.73 ± 0.2°C	8.99 ± 0.5°C	17.13 ± 3°C
8	-3.43 ± 0.6°C	-0.05 ± 0.6°C	1.49 ± 0.5°C	3.72 ± 0.2°C	9.01 ± 0.5°C	17.54 ± 6°C
10	-3.43 ± 0.6°C	-0.08 ± 0.4°C	1.96 ± 0.5°C	3.78 ± 0.6°C	8.96 ± 0.5°C	17.16 ± 5°C

Five plants of each cultivar were placed in the polytunnel, set at 17.1±6°C (Table 3.1) to act as a control on the same date. Plants were watered by hand when required.

Randomisation

Five plants of each cultivar were removed from each cold store and placed in the polytunnel at two-week intervals commencing 3 January 2003. Using random number tables, one plant from each treatment was placed in each of the five blocks, as described in Chapter 2. Plants were checked daily and time to first bud burst recorded. Thereafter, the number of buds that had burst was recorded three times a week. On 16 July 2003, plants were harvested by hand and number of berries produced by each plant was recorded.

Nutrition

Nutrients were supplied as detailed in Chapter 2. In order to aid pollination, a box of bees were ordered from XXXX and placed in the polytunnel on XXXX.

Statistical Analysis

The data were analysed using Analysis of Variance with Genstat VI (Lawes Agricultural Trust) statistical package. Mean values are presented and significance indicated using the 95% confidence interval. The fruit production data for all three cultivars were not normally distributed hence data were transformed and re-analysed.

3.2.2. Experiment 2. Budsticks v. Whole Plants

This experiment was designed to compare the bud burst behaviour of budsticks (unrooted cuttings) with that of whole plants.

Plant Material

One year old potted plants of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' (as described in Chapter 2) were utilised in this experiment. Cuttings of all three cultivars were taken in the spring of 2003 and delivered to the University of Reading's Experimental Field Unit on 2 July 2003. On 22 December 2003, 120 cuttings were taken from the plants using sharpened secateurs. Cuttings from each cultivar were divided into two labeled bundles and each bundle placed into a black polythene bag with a water-saturated sponge to prevent the cuttings desiccating. One bag was placed in a cold store maintained at 2.1°C cold store, the other in a -4.2°C cold store (Table 3.2). At the same time, 20 whole plants of each cultivar were placed in the cold stores, wrapping the stems in black plastic to prevent desiccation. Fifteen cuttings of each cultivar were placed into beakers containing 100ml of water and set in the polytunnel as controls, five plants of each cultivar were also used as controls.

At two-week intervals from 5 January 2004, 15 cuttings and five plants from each cultivar were removed from the cold stores and randomly placed in one of the five polytunnel blocks. Cuttings were placed into beakers containing 100ml of water and twice a week the beakers were emptied, rinsed out and re-filled with fresh water. Every week, the beakers were randomly moved to the next polytunnel block. When the irrigation system was turned on, the water in the beakers was replaced with the diluted nutrient solution the whole plants were provided with (see Chapter 2).

Statistical Analysis

As advised by the University of Reading's Statistical Advice Centre, the bud burst data collected for the budsticks were averaged to provide five sets of bud burst data for comparison with whole plant behaviour.

3.3. Results

3.3.1. Experiment 1. The Effect of Chilling Temperature and Duration

Results were analysed and significant ($P<0.001$) interactions between cultivar, chilling temperature and chilling duration were noted. As a result, data were further divided into individual cultivars and re-analysed.

'Ben Gairn'

Time to First Bud Burst

Control plants took significantly longer (20.2 days, data not shown) to bud burst compared to plants that were chilled. As a result, control data were eliminated from further statistical analysis to allow direct comparison of the effects of chilling duration and chilling temperature.

Bud burst was highly dependant on chilling temperature ($P=0.021$) and duration ($P<0.001$), but the interaction between these factors was not significant ($P=0.5$). As the chilling temperature increased from -3.4ºC to 3.4ºC, bud burst was delayed, but further increasing the temperature advanced bud burst (Figure 3.1). As the chilling duration increased from 2 to 8 weeks, bud burst was advanced (Figure 3.2). Further increasing the duration had a slightly detrimental effect, but this was not significant.

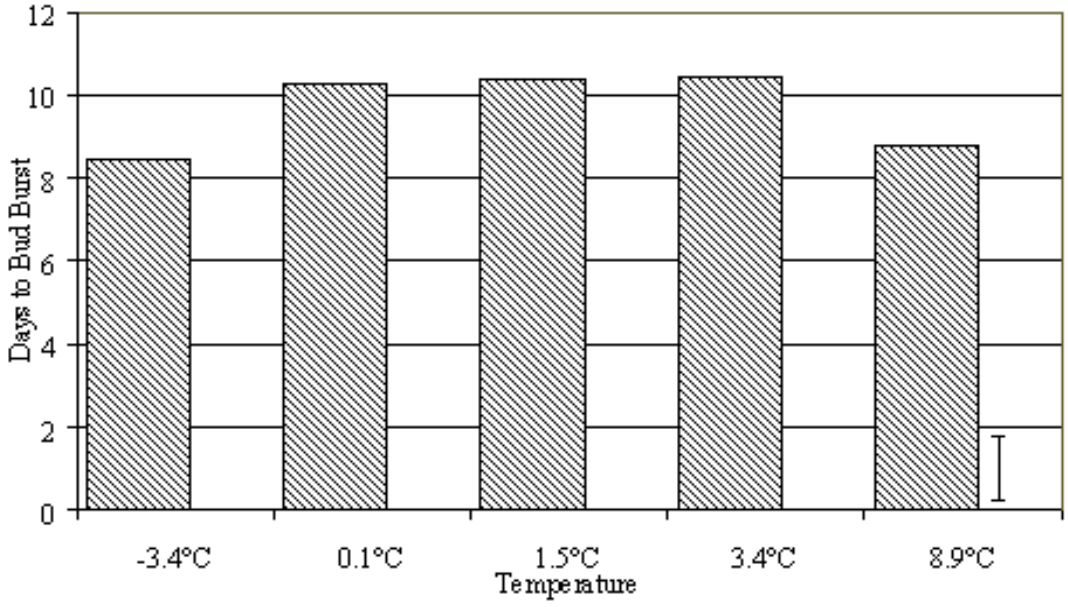


Figure 3.1. *R. nigrum* 'Ben Gairn'. Effect of chilling temperature on time to first bud burst. Error bar represents L.S.D. (P=0.05), d.f. = 89.

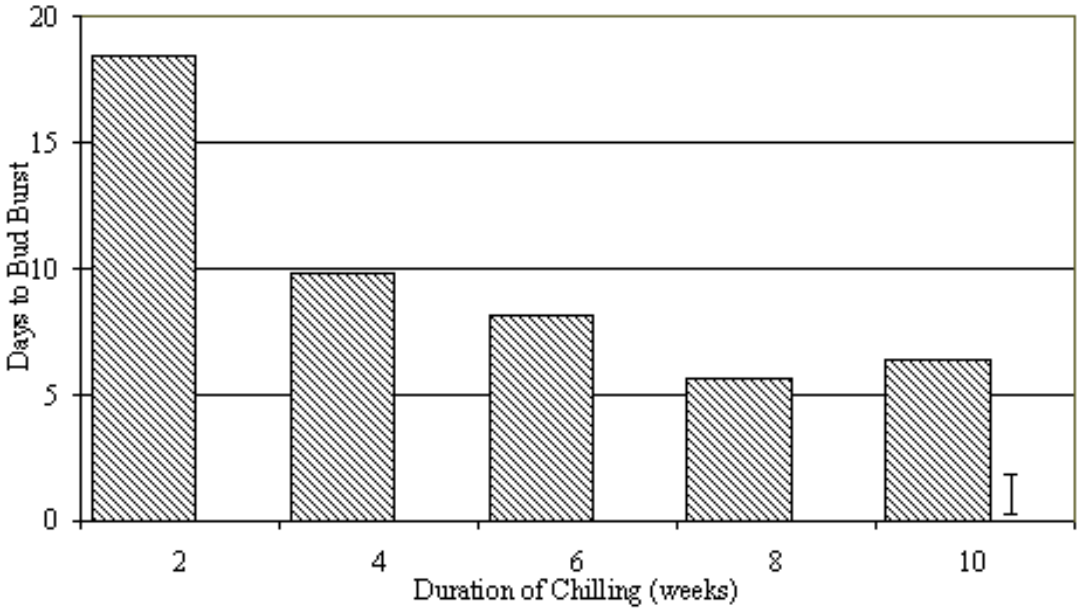


Figure 3.2. *R. nigrum* 'Ben Gairn'. Effect of chilling duration on time to first bud burst. Error bar represents L.S.D. (P=0.05), d.f. = 89.

Fruit Production

Control plants produced significantly (P=0.01) less fruit (0.35, data not shown). To compare the effect of chilling temperature and duration on berry production, control data were eliminated from the statistical analysis. Both chilling temperature and chilling duration had significant (P=0.04; P=0.001 respectively) effects on fruit production, although there was no significant (P=0.218) interaction between chilling temperature and duration. Increased production was associated with temperatures of -3.4°C and 0.1°C (Figure 3.2). As the chilling temperature further increased to 8.9°C, higher temperatures were detrimental and the number of fruit produced significantly decreased. As the chilling duration increased from 8 to 10 weeks the number of berries produced significantly increased.

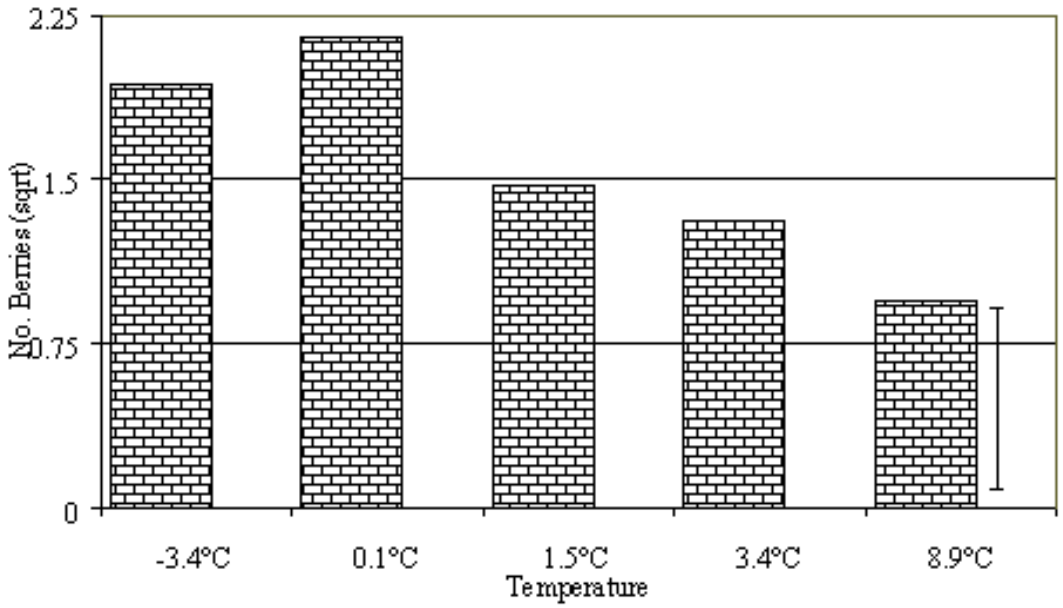


Figure 3.2. ***R. nigrum*** **'Ben Gairn' – effect of chilling temperature on berry production. Error bar represents L.S.D. (P=0.05), d.f. = 88.**

'Ben Hope'

Time to First Bud Burst

Control plants took significantly (P<0.001) longer (22 days, data not shown) to bud burst compared to plants that were exposed to artificial chilling. When analysed without control data, both chilling temperature and chilling duration had significant (P<0.001) effects on time to first bud burst, although the interaction was insignificant (P=0.066).

Chilling temperature had a significant effect only when plants were chilled at 8.9°C, where plants in this treatment bud burst significantly faster than those exposed to lower temperatures.

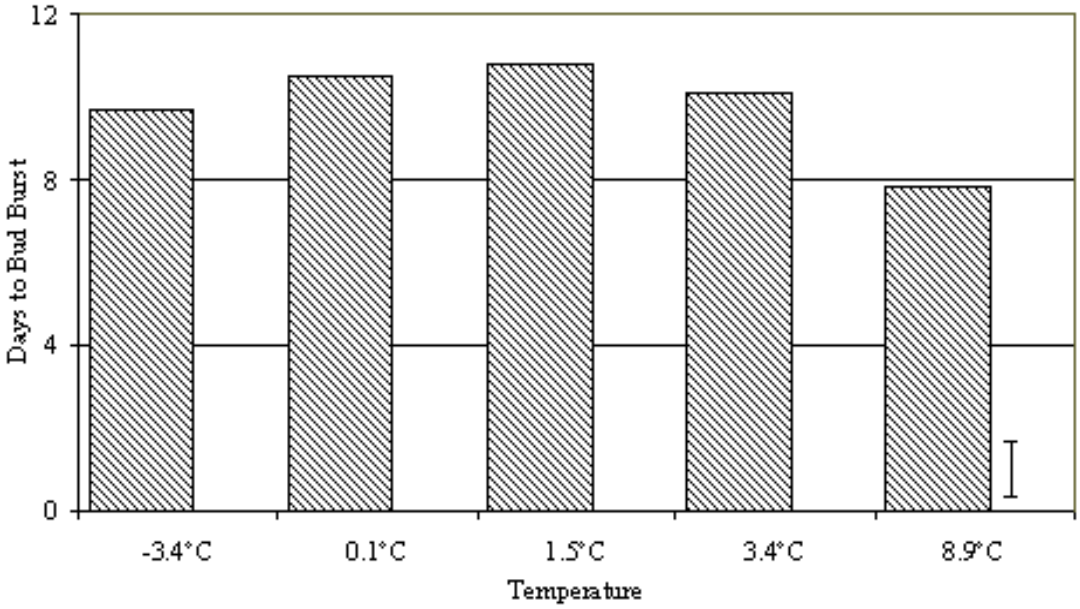


Figure 3.3. *R. nigrum* 'Ben Hope'. Effect of chilling temperature on time to first bud burst. Error bar represents L.S.D. (P=0.05), d.f. = 88.

Faster bud burst was found to be directly related to longer chilling durations (Figure 3.4).

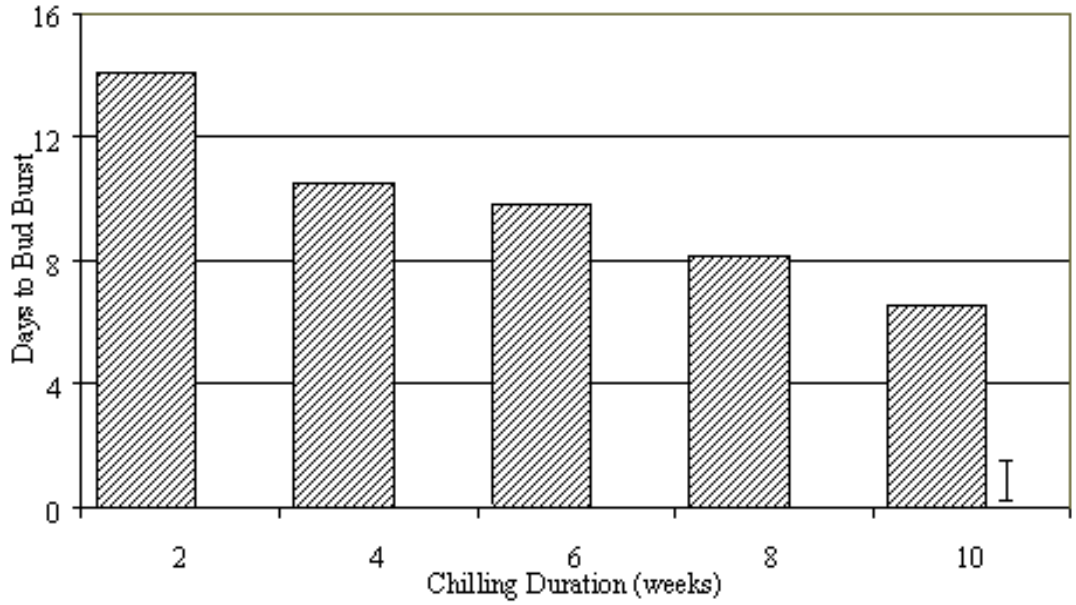


Figure 3.4. *R. nigrum* 'Ben Hope'. Effect of chilling duration on time to first bud burst. Error bar represents L.S.D. (P=0.05), d.f. = 88.

Fruit Production

Chilling temperature, chilling duration and the interaction had significant effects on fruit production (Figure 3.5). As a result, data were divided into individual treatments (chilling temperature and duration) and re-analysed. Except for the 0.1°C treatment, plants chilled for 2 weeks did not produce any berries.

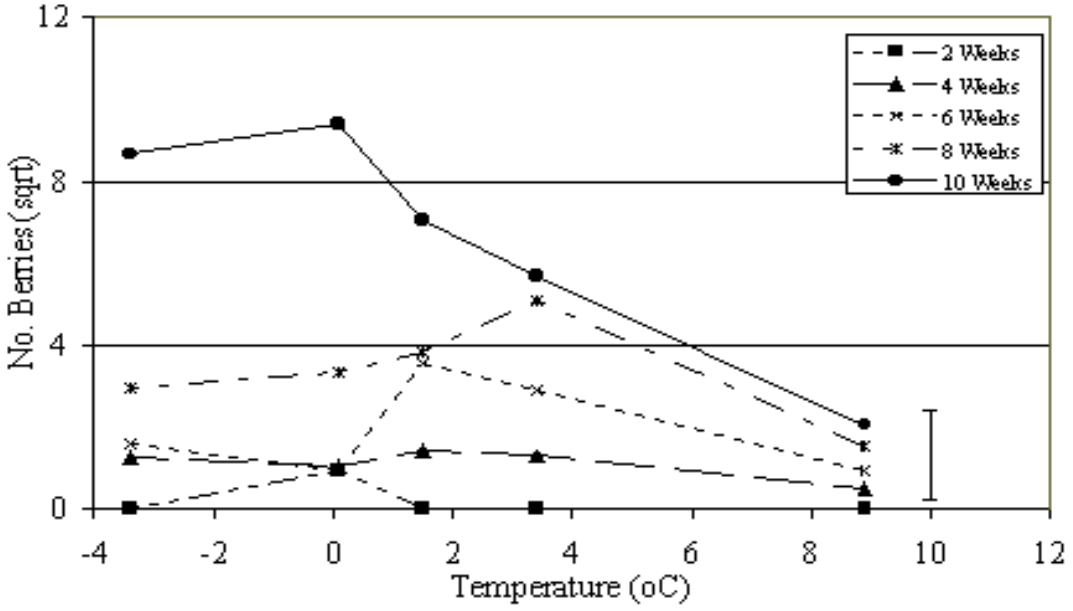


Figure 3.5. *R. nigrum* 'Ben Hope' – effect of chilling temperature and duration on berry production. Error bar represents L.S.D. (P=0.05), d.f. = 90.

Except after chilling for 10 weeks, there was no significant difference in fruit production between plants chilled at -3.4ºC and 8.9ºC. After chilling for 2, 4, 6, 8 and 10 weeks, as the chilling temperature increased to 0.1ºC, 1.5ºC, 1.5ºC, 3.4ºC, and 0.1ºC respectively, fruit production increased. Exposure to temperatures exceeding this was detrimental.

When plants were chilled for up to 6 weeks, the optimum temperature for advancing bud burst was 3.4°C. At longer chilling durations (8 to 10 weeks), temperatures below 1.5°C were associated with significantly ($P<0.001$) increased berry production There was no significant ($P=0.193$) effect of chilling duration when plants were chilled at 8.9°C.

'Ben Tirran'

Time to First Bud Burst

Control plants took significantly (P<0.001) longer (74.2days, data not shown) to bud burst compared to plants that were artificially chilled. Chilling temperature, chilling duration and the interaction (Figure 3.6) between chilling temperature and chilling duration had significant (P<0.001) effects on time to first bud burst. Data were further divided into individual treatments and re-analysed.

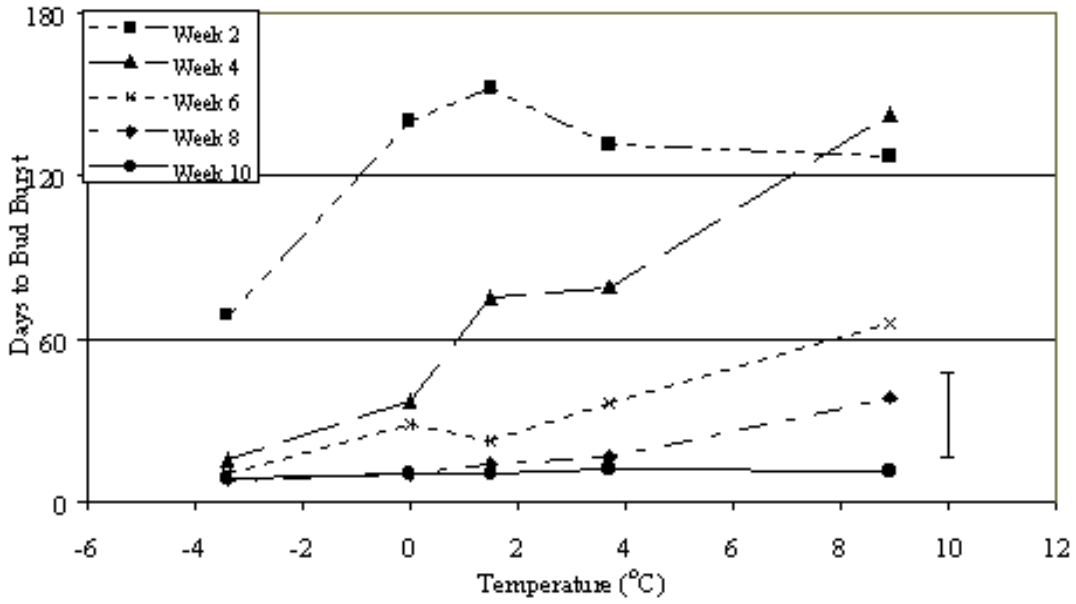


Figure 3.6. *R. nigrum* 'Ben Tirran'. Effect of chilling temperature and chilling duration on time to first bud burst. Error bar represents L.S.D. (P=0.05), d.f.= 94.

As the chilling temperature increased from -3.4ºC to 8.9ºC, increased chilling duration was associated with advanced bud burst. After exposure to -3.4ºC and 0.1ºC, plants chilled for 2 weeks burst bud significantly later than those chilled for longer durations. As the chilling temperature increased from 0.1ºC to 8.9ºC, differences between chilling durations became more apparent. There was no effect of temperature when plants were chilled for 10 weeks.

Fruit Production

Control plants produced significantly (P<0.001) less berries (2.0) than those chilled for more than 4 weeks. As a result, control data were omitted from analysis and differences between plants exposed to artificial chilling were analysed. The effects of chilling temperature, chilling duration and the interaction (Figure 3.7) between chilling temperature and chilling duration were highly significant (P<0.001) so data were further sub-divided and analysed per treatment.

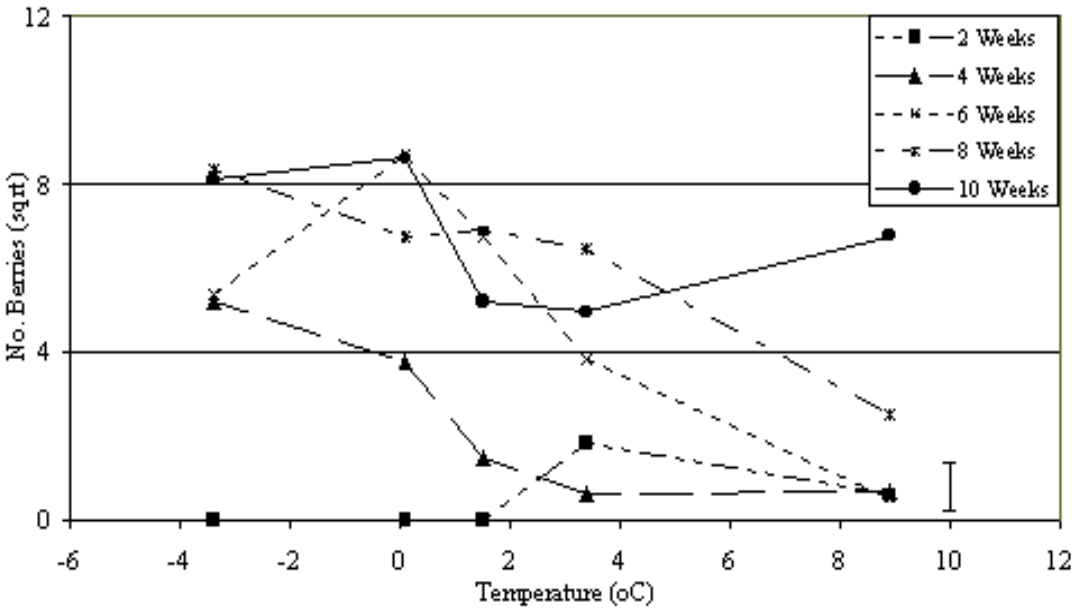


Figure 3.7. *R. nigrum* 'Ben Tirran' – effect of chilling temperature and duration on berry production. Error bar indicates L.S.D. (P=0.05), d.f. = 87.

Although there was slight variation within each chilling duration, increased fruit production was evident in plants chilled for 4, 6, 8 and 10 weeks as the chilling temperature decreased. There were two exceptions to this, however. After receiving 2 weeks of chilling, fruit production decreased as the temperature decreased from 3.4ºC to 1.5ºC, and no fruit were produced when the temperature fell below this. Fruit production initially decreased, in plants that had received 10 weeks of chilling, as the temperature decreased from 8.9ºC to 3.4ºC, but then increased upon further chilling

3.3.2. Experiment 2. Budsticks v. Whole Plants

Initial analysis determined a significant (P<0.001) difference between cultivars and a significant interaction between cultivar and material and cultivar and chilling duration. As a result, data were split into individual cultivars and re-analysed.

'Ben Gairn'

For both temperature treatments, final bud burst of whole plants was significantly (P<0.001) higher compared to budsticks (Figure 3.11).

For the budsticks, there was no significant effect of chilling temperature (P=0.889) or chilling duration (P=0.324) but there was significant (P<0.001) interaction between these factors. After 2 weeks of artificial chilling, bud burst was promoted by chilling at 2.1°C. In contrast, after 10 weeks of chilling, budsticks exposed to -4.2°C had a significantly more buds burst.

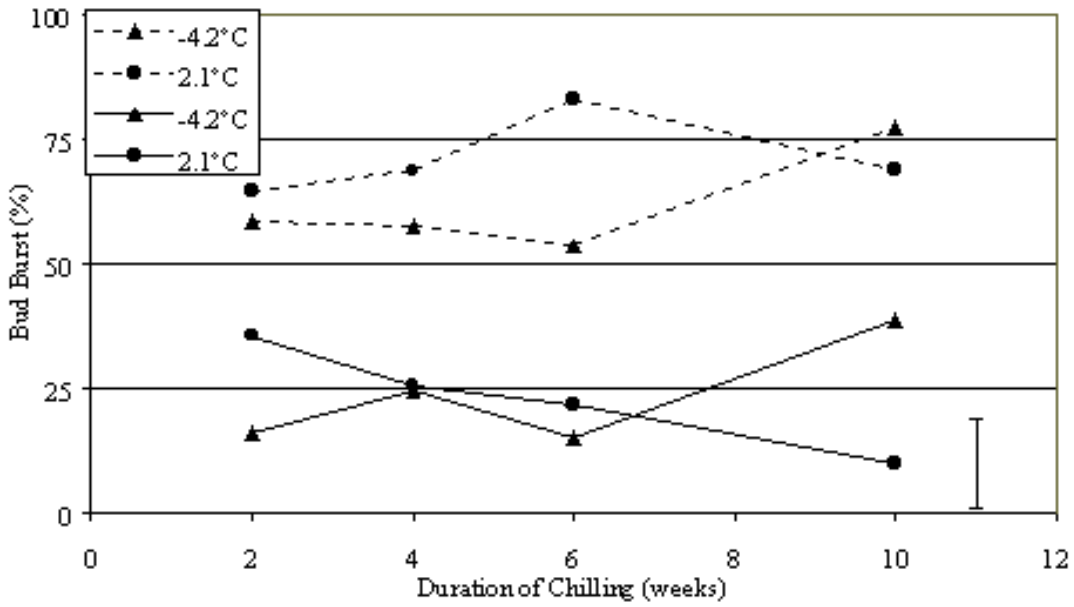


Figure 3.11. ***R. nigrum*** **'Ben Gairn' – comparison of bud burst of whole plants and budsticks. Broken line represents whole plants, unbroken line represents budsticks. Error bar represents L.S.D (P=0.05), d.f. = 64.**

For whole plants, there was no significant effect of chilling temperature (P=0.09), chilling duration (P=0.437) and no interaction between chilling temperature and chilling duration (P=0.125).

'Ben Hope'

Bud burst was significantly (P<0.001) higher for whole plants compared to budsticks (Figure 3.12). In addition, the trends with regards to treatment were different.

Chilling temperature and chilling duration had significant (P=0.047; P=0.016 respectively) effects on final bud burst of budsticks but there was no significant (P=0.105) interaction between these two factors. After chilling for 10 weeks, increased bud burst was related to lower storage temperatures. As the chilling duration increased to 10 weeks, final bud burst significantly increased.

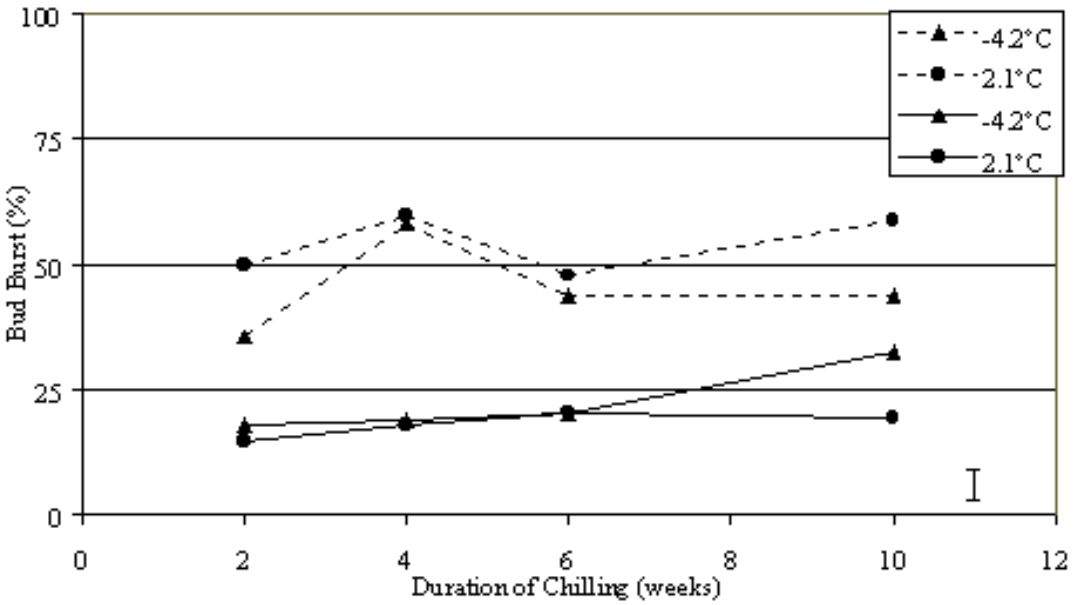


Figure 3.12. ***R. nigrum*** **'Ben Hope' - comparison of bud burst of whole plants and budsticks. Broken line represents whole plants, unbroken line represents cuttings. Error bar represents L.S.D (P=0.05), d.f.=59.**

For whole plants, chilling temperature, chilling duration and the interaction were not significant (P=0.153; P=0.249; P=0.8 respectively).

'Ben Tirran'

Final bud burst of whole plants was significantly (P<0.001) higher than that of budsticks (Figure 3.13). For both types of plant material, chilling duration was significant (P<0.001) but there was no significant effect of chilling temperature (P=0.533 (budsticks); P=0.971 (whole plants)) and no significant (P=0.437; P=0.111 respectively) interaction between chilling temperature and chilling duration. Bud burst was promoted by increasing the chilling duration from 6 to 10 weeks (budsticks) or 4 to 10 weeks (whole plants).

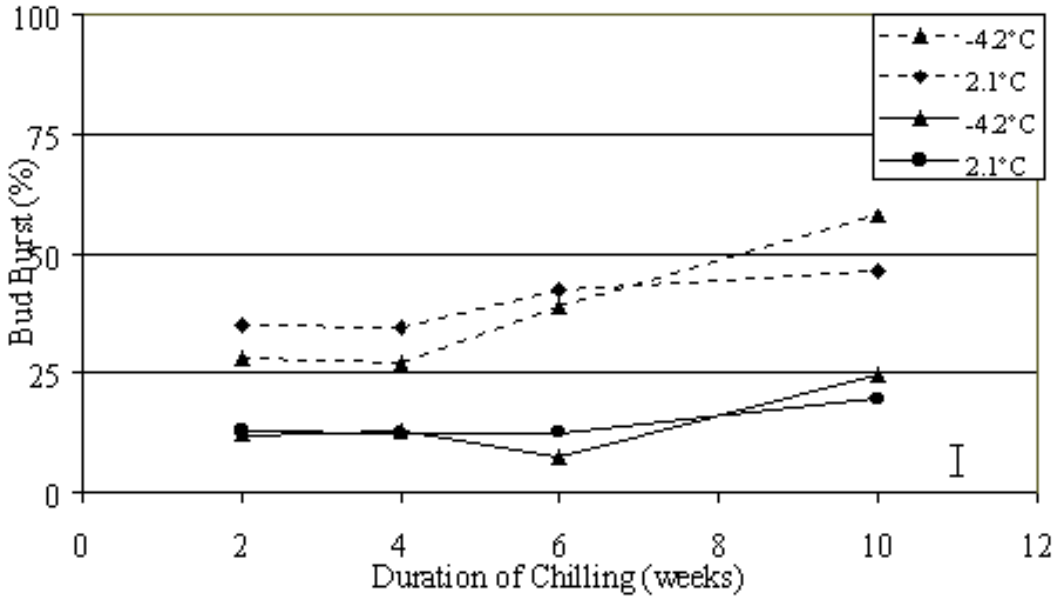


Figure 3.13. ***R. nigrum*** **'Ben Tirran' - comparison of bud burst of whole plants and budsticks. Broken line represents whole plants, unbroken line represents cuttings. Error bar represents L.S.D (P=0.05), d.f.=65.**

Discussion

3.4.1. Differences due to Cultivar

In both experiments, highly significant differences between cultivars, and significant interactions between cultivars and the main treatments were detected.

Regardless of chilling temperature, increasing the chilling duration of 'Ben Gairn' had no effect on time to bud burst, yet was beneficial for 'Ben Hope' and 'Ben Tirran'. As the chilling temperature of 'Ben Gairn' increased from -3.4ºC to 3.4ºC, bud burst was delayed, but further increasing the temperature to 8.9ºC advanced bud burst. Increasing the chilling temperature of 'Ben Hope' to 1.5°C delayed bud burst, but further increases advanced bud burst. Bud burst of 'Ben Tirran' was advanced as chilling temperature increased. Previous authors have reported differences between cultivars of the same species, particularly for *Ribes nigrum* (Plancher, 1984), *Malus domestica* (Couvillon and Erez, 1985; Young and Werner, 1985), *Malus sylvestrus* (Swartz and Powell, 1981), *Pyrus* spp. Speigel-Roy and Alston, 1979) and *Fragaria ananassa* (Kronenberg *et al*., 1976). However, *Prunus avium* 'Stella', 'Sunburst' and 'Summit' responded similarly when exposed to chilling regimes and the optimum chilling temperatures for satisfying endodormancy were determined to be extremely similar - 3.2°C, 3.7°C and 3.2°C respectively (Mahmood *et al*., 2000a).

The degree of variation between cultivars was found to vary. Speigel-Roy and Alston (1979) reported bud burst differences of up to 3 months for *Pyrus serrulata*. Bud burst of *Malus domestica* was highly dependant on rootstock, with 'MM106' requiring an additional 670 chill units compared to 'M7a' (Young and Werner, 1985). Similarly, *Malus sylvestrus* 'Spatbluhender' required 1650 additional chill units compared to 'Subtropical Apple' (Swartz and Powell, 1981). These differences, however, may reflect the fact that the optimum chilling treatments are cultivar-specific. Time to bud burst of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' in Experiment 1 after receiving 6 weeks of chilling at 8.9ºC, was 7.6 days, 7.2 days and 65.6 days. After being exposed to the optimum treatment, however, differences between cultivars were relatively insignificant – 5.4, 5.2 and 9 days for 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' respectively.

It was unclear whether the later bud burst reported for 'Ben Tirran' (9 days) compared to 'Ben Gairn' (5.4 days) and 'Ben Hope' (5.2 days) is simply a reflection of cultivar differences, or if

additional chilling of 'Ben Tirran' would further reduce the time to first bud burst. The optimum chilling duration for advancing 'Ben Tirran's bud burst was 10 weeks and this was also the maximum duration plants were chilled for, so perhaps further increasing the chilling duration would advance bud burst. Lantin (1973) did not report significant differences in the time to bud burst between *Ribes nigrum* cultivars. After field chilling of *Malus domestica* until 13 March, 'Spatbluhender' and 'Red Delicious' burst bud after 23 days and 6 days respectively (Gianfagna and Mehlenbacher, 1985). As the chilling requirement was increasingly fulfilled, 'Spatbluhender' burst bud after 21 days and 'Red Delicious' after 1 day, hence the differences between the cultivars was not significantly reduced by increasing the exposure to chilling.

The differences between cultivars discovered in this experiment should not be wholly unexpected. The breeding histories (see Chapter 1) of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' are extremely complex. As well as being crossed with several *Ribes nigrum* cultivars, including British, Swedish and Russian cultivars, 'Ben Gairn' contains germplasm from *Ribes dikuscha*, 'Ben Hope' from *Ribes grossularia* and 'Ben Tirran' from *Ribes rubrum*. As discussed in Chapter 1, the chilling requirement of a cultivar is genetically pre-determined, and the complex crosses used to produce 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' would be expected to have an effect on each cultivar's chilling requirement. Even within *R. nigrum*, it is likely, due to extremely harsh winters, that Russian cultivars have a higher chilling requirement than British cultivars.

This experiment utilised the extreme range of available cultivars – 'Ben Gairn' is one of the earliest flowering and 'Ben Tirran' is one of the latest flowering cultivars, with 'Ben Hope' intermediate. It is possible that, disregarding the breeding histories, the behaviour of cultivars with similar flowering dates and similar chilling requirements may be comparable, however this hypothesis cannot be tested using only these three cultivars. In order to establish if there is a similarity between *Ribes nigrum* cultivars, this experiment would have to be extended to include a much wider range of cultivars. Such in-depth experimentation, however, is out-with the scope of this research and would require much larger facilities than are currently available. Until this hypothesis can be tested, future experiments should not rely on one *R. nigrum* cultivar to explain the behaviour of *R. nigrum* cultivars as a whole. For continuity, and

because they represent the three extremes of the crop, 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' will continue to be used in the remainder of the experiments.

3.4.2. Effect of Chilling Temperature

Control plants of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' bud burst significantly later than chilled plants. This is slightly contradictory to previous research where several species have completely failed to bud burst or develop following exposure to limited or increased winter temperatures, including *Oleo europaea* 'Ascolano' and 'Manzanillo' (Rallo and Martin, 1991), *Prunus persica* 'Redskin' and 'Redhaven' (Erez *et al*., 1979) and *Prunus avium* 'Stella', 'Sunburst' and 'Summit' (Mahmood *et al*., 2000a). In this experiment, however, *Ribes nigrum* control plants were subjected to natural chilling temperatures until late December, thus a proportion of the chilling requirement would have been fulfilled which would enable a number of buds to burst, although at a reduced rate.

Time to first bud burst of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' was highly dependant on chilling temperature. Bud burst of 'Ben Gairn' and 'Ben Tirran' was advanced by exposure to decreased chilling temperature but the opposite was true for 'Ben Hope'. Delayed bud burst in response to increasing chilling temperature has been reported for a number of species, including *Rubus ideaus* 'Autumn Bliss' (Carew *et al*., 2001), *Fragaria ananassa* 'Glasa' and 'Tioga' (Kronenberg *et al*., 1976), *Prunus persica* 'Redskin' and 'Redhaven' (Erez *et al*., 1979), *Prunus avium* 'Stella', 'Sunburst' and 'Summit' (Mahmood *et al*., 2000a) and *Rubus. fructicosus* 'A-1836', 'APF-13' and 'NC194' (Lopez-Medina and Moore, 1999). Time to bud burst of *Actinidia chinensis* 'Hayward' was advanced as the chilling temperature decreased and it was concluded that for every 1°C the temperature fell below 18°C, bud burst was advanced by 2.4 days (Snelgar *et al*., 1997). However, previous results from McPherson *et al*. (1995) did not support this theory. Bud burst of *A. chinensis* 'Hayward was advanced by 14 and 28 days as the chilling temperature decreased from 13°C to 10°C and 13°C to 7°C respectively (McPherson *et al*., 1995). Based on Snelgar *et al*.'s (1997) theory, bud burst is advanced by 7.9 days for each 1°C decrease in temperature.

It is unclear why decreasing temperature had a detrimental effect on 'Ben Hope's bud burst, as this is contrary to previous research. Differences between cultivars may explain the discrepancy, but there have been no other reports detailing this level of cultivar specificity.

Recent research has indicated that dormancy may be controlled by the gene PsDRM1 (Stafstrom, 2002) and/or closing of plasmodesmata (Rinne and Schoot, 2004), but both theories rely on exposure to cold temperatures overcoming dormancy, which is the opposite of 'Ben Hope's reaction.

Specific temperature responses, whereby an optimum temperature has a beneficial effect and deviations from this are detrimental (represented by a bell-shaped curve), have been noted in previous research. The optimum chilling temperature for *Prunus persica* 'Redhaven' was determined to be 6°C and temperatures below 1.4°C or above 12.5°C did not contribute to the chilling satisfaction (Richardson *et al*., 1974). A similar result was obtained by Erez and Couvillon (1987) who reported the optimum chilling temperature of *P. persica* 'Redhaven' to be 8°C, with temperatures below 0°C and above 14°C having no effect. The response of *Cornus sericea* to increasing temperatures from 5°C to 20°C was bell-shaped, with bud burst increasing as temperature increased from 0°C and decreased from 20°C (Kobayashi *et al*., 1982). The time to bud burst graph of 'Ben Gairn' was the opposite of this – as the chilling temperature deviated from 3.4ºC, bud burst was advanced.

As the chilling temperatures of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' decreased from 8.9ºC to –3.4°C, a positive effect on fruit production was observed. As the lowest temperature utilized in this experiment was -3.4ºC, the fact that this was the optimum treatment suggested that the chilling requirement for flower/fruit production for 'Ben Gairn' and 'Ben Tirran' may not have been satisfied. It is unknown, however, whether the reduction in fruit number with increasing temperature was a result of reduced flower number or low fruit set, since these parameters were not recorded. Previous research suggested that exposure to warmer temperatures inhibited anthesis as opposed to reducing fruit set. In support of this theory, *Rubus ideaus* 'Autumn Bliss' failed to produce flowers when exposed to insufficient chilling (Carew *et al*., 2001) and only 2.5% of *Prunus persica* 'Redhaven's' unchilled flower buds burst (Scalabrelli and Couvillon, 1986). The optimum temperature for fruit production of all cultivars was found to be -3.4ºC, and as this was the lowest temperature the plants were exposed to, further decreasing the temperature may prove to be as or more beneficial.

In general, however, the number of fruit produced, particularly by 'Ben Gairn' was considerably lower than would be expected under field conditions, which may be a reflection

of poor pollination. Although boxes of bees were placed in the polytunnel to pollinate the plants, the activity of the bees appeared to be low, and very few bees were actually observed leaving the box. Care must be taken, therefore, in interpreting the results, and further experimentation is required.

The maximum temperature plants were exposed to in this experiment was 8.9°C and compared to control plants, exposure to this treatment significantly advanced bud burst and increased fruit production. This is contrary to previous research that observed similar temperatures having very little or no effect on bud burst (Kronenberg *et al*., 1976; Frisby and Seeley, 1993; Mahmood *et al*., 2000a). Exposure to 8.9ºC was found to be less effective for 'Ben Tirran', which is likely to be a result of recent breeding objectives. 'Ben Tirran' was one of the first releases of SCRI's high chilling requirement project, aimed at delaying bud burst in an attempt to avoid the late frost damage that affects earlier bursting cultivars. It is not surprising, therefore, that this cultivar is less reactive to higher temperatures.

3.4.3. Effect of Chilling Duration

For all cultivars, a positive relationship between chilling duration and time to bud burst was observed. A similar result has been reported in all dormancy experiments, covering a wide range of species, including *Actinidia chinensis* 'Tomi' (Guerriero *et al*., 1990), *Prunus avium* 'Stella', 'Sunburst' and 'Summit' (Mahmood *et al*., 2000a), *Picea abies*, *Pseudotsuga menziesii* and *Malus domestica* (Cannell and Smith, 1983). Such a result was expected, as increasing the duration of chilling exposure would increasingly satisfy the chilling requirement.

For 'Ben Gairn' and 'Ben Hope', increasing the duration of chilling had no significant effect on fruit production until the duration increased from 8 to 10 weeks, after which time fruit production significantly increased. Increasing the duration of chilling 'Ben Tirran' from 4 to 8 weeks also increased the number of berries produced. Again, this result has been widely supported by previous research (Lantin, 1973; Guerriero *et al*., 1990). Although initial research conducted on *Fragaria ananassa* 'Elsanta' observed no effects of increasing chilling duration on fruit production, a subsequent experiment reported an increase in fruit production as the duration of chilling at –3°C increased from 0 to 4 weeks (Tehranifar, 1998). As discussed above, however, the results of this experiment must be interpreted with caution, as

low fruit set may be attributed to poor pollination, and this result therefore may not be wholly representative.

This experiment firmly established that increasing the duration of chilling of *Ribes nigrum* had beneficial effects on time to bud burst and potentially beneficial effects on fruit production. For 'Ben Hope' and 'Ben Tirran', chilling for 10 weeks advanced bud burst. Similarly, the optimum chilling treatment for maximizing 'Ben Gairn' and 'Ben Hope' fruit production was 10 weeks. As this was the maximum duration plants were chilled for, it is possible that extending the period of chilling beyond 10 weeks would result in further beneficial effects.

The effects of chilling temperature and duration were found to be inter-dependent. Particularly for 'Ben Tirran', when chilling temperatures were low, short durations of exposure were sufficient to bud burst or promote fruit production. At higher temperatures, however, longer durations were required in order to satisfy the chilling.

3.4.4. Practical Implications

The proposed climate change scenarios suggest an annual temperature increase of 0.75°C to 3.75°C, depending on the severity of the scenario (Hulme *et al*., 2002). The results of this experiment demonstrate the promotive effect of low temperature and increased chilling durations on time to bud burst and fruit production of *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran'. Based on the climate change scenarios, however, the potential for the cultivars to be exposed to the required temperatures will be significantly reduced. The implications for *R. nigrum* production under the most severe climate change scenario may simply be academic, as new cultivars with reduced chilling requirements are in the breeding process, and the likelihood that 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' will be utilised in 2080 is extremely low. However, growers have been reporting the symptoms of insufficient chilling over the past couple of years, the severity of which is likely to increase with time. New cultivars with lower chilling requirements may not be available until 2015, therefore short-term solutions to this problem are required.

3.4.5. Comparison of the Bud Burst Behaviour of Whole Plants and Budsticks

This experiment first of all compared the final bud burst of budsticks with whole plants and secondly compared the behaviour of the different plant materials after exposure to different chilling regimes.

For 'Ben Gairn' and 'Ben Hope', significantly more buds burst on whole plants compared to budsticks and after 10 weeks of chilling at -4.2°C, bud burst was 200% increased on whole plants. This agrees with work conducted on *Prunus avium* 'Sunburst' and 'Stella' (Mahmood, 1999) and *Actinidia chinensis* (Snelgar *et al*., 1997). However, contradictory results have also been reported. The chilling requirements of *Pyrus pyrifolia* 'Kosiu' budsticks were comparable to that of whole plants (Nishimoto and Funisaki, 1995) and bud burst of *A. chinensis* 'Tomuri' (Guerriero *et al*., 1990) and *Vitis vinifera* 'Kyoho' budsticks exceeded that of whole plants (Nishimoto and Funisaki, 1995). This is contradictory to the suggestion that the chilling requirement of *Ribes nigrum* cuttings was lower than that of whole plants and that for any given chilling regime bud burst would be greatest for budsticks (Lantin, 1973; Plancher, 1983b).

After further research, discrepancies into the cutting material used in each experiment were discovered. The authors who reported no correlation between budstick and whole plant behaviour (Guerriero *et al*., 1990, Lantin, 1973; Plancher, 1983b) utilised single-node cuttings. The inhibitory effect of ABA (discussed in Chapter 1) is removed in single-node cuttings, which may explain the increased bud burst levels compared to traditional budsticks (Guerriero *et al*., 1990). Additionally, after apical bud removal, the concentration of the growth promoting auxins and cytokinins (responsible for cell elongation and cell division respectively) increased which could promote bud burst (Salisbury and Ross, 1992; Campbell, 1996). Cutting material, therefore, appears to be an important aspect of this study. It was hypothesised that the cutting injury, incurred as a result of the budstick being removed from the mother plant, was the driving factor behind the elevated final bud burst readings of single-node cuttings (Lantin, 1973; Plancher, 1983b). Similarly, Chandler and Tufts (1993) reported that cutting a twig and placing the resultant budstick into forcing temperatures was not representative of the chilling requirement being fulfilled as the cutting action reduced the rest influence. However, other factors must be involved - if cutting injury was the sole cause of

premature and successful bud burst of single-node cuttings, a similar effect should have been evident in whole budsticks.

Although Mahmood *et al*. (2000a) reported that bud burst of *Prunus avium* 'Sunburst' and 'Stella' budsticks was lower than that of whole plants, the behaviour of the budsticks in response to chilling duration and temperature was similar and a similar result was observed for 'Ben Tirran'. The delayed bud burst and general lack of response of 'Ben Tirran' whole plants, however, suggests that the chilling requirement of this cultivar had not been adequately fulfilled therefore the result may not be representative. This theory is supported by the results of 'Ben Gairn' and 'Ben Hope'. For 'Ben Gairn' budsticks and whole plants, there was no significant effect of chilling temperature or chilling duration, but the interaction between chilling temperature and duration was significant for the budsticks. Chilling temperature and duration had significant effects on final bud burst of 'Ben Hope' budsticks, yet there was no such effect for whole plants.

Unlike previous research, the budsticks utilised in this experiment were taken from 2-year old plants and as a result were small, thin and most probably had significantly reduced carbohydrate reserves compared to larger cuttings or whole plants. Indeed, Plancher (1983b) warned that budsticks should only be used for chilling experiments if they were long enough, suggesting that the small budsticks used in this investigation were inadequate. The reduced energy reserves available in the small cuttings may have affected the ability of the budsticks to bud burst, and hence to respond to temperature regimes. The over-all bud burst behaviour of the budsticks, therefore, may have been compromised by their immaturity.

By the end of the experiment, all of the budsticks had produced a number of roots which may have affected the bud burst potential. Energy that would otherwise have been utilised to promote growth and development of buds was employed for root growth. Devoid of a root system, which transports water, nutrients and cytokinins to the shoot, the bud burst potential of the budsticks may have been compromised. Over-production of cytokinin resulted in a decrease the inhibitory effect of apical dominance (Taiz and Zeiger, 2002) and it is possible therefore, that lack of cytokinin could increase the inhibitory effect of apical dominance, resulting in the low bud burst recorded in this experiment.

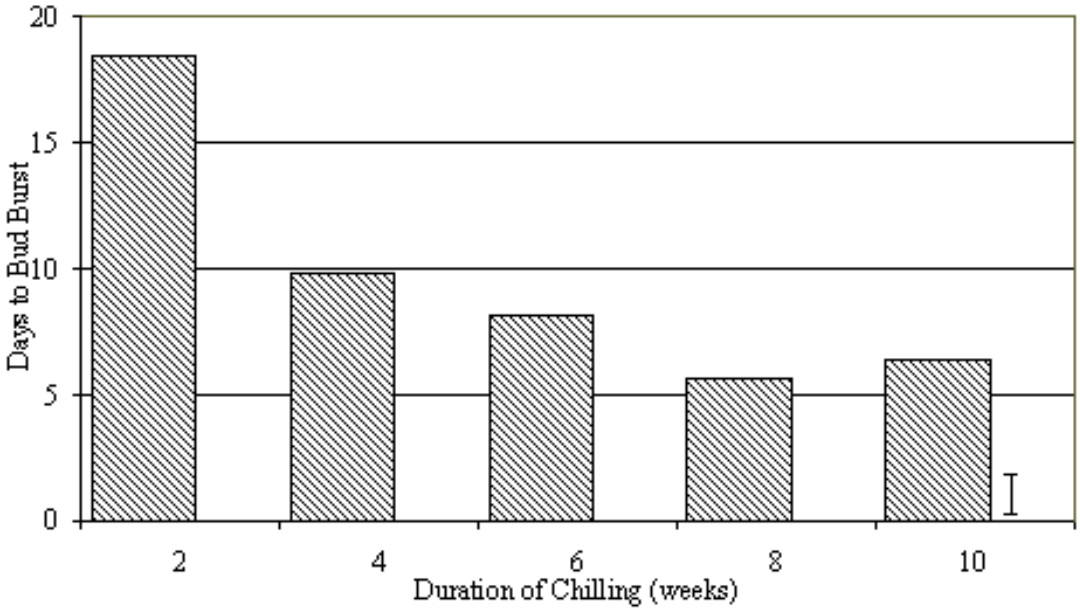
Due to the reduced space required for storage, and hence the increased replications potentially available, the use of budsticks as opposed to whole plants is highly desirable. The results of this experiment, however, suggest that 2-year old *Ribes nigrum* budsticks of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' do not respond in a similar manner to whole plants after exposure to identical chilling regimes. The use of such material in experiments should therefore be avoided. The ability of larger, more mature cutting material has been reported to mirror that of whole plants (Mahmood *et al*., 2000a) hence future research should concentrate on relating the behaviour of this material to that of whole plants.

3.5. Conclusions

The response of *Ribes nigrum* to chilling temperatures and chilling durations was found to be cultivar-dependant. This has implications for *R. nigrum* research in general, in that experiments conducted using one cultivar cannot be used to explain the reaction of other cultivars. In the long-term, quantifying the relationship between *R. nigrum* cultivars, in terms of bud burst behaviour, would be invaluable. In the meantime, experiments must utilise a range of cultivars in order to determine a range of responses.

Chilling temperature and duration were found to significantly affect the time to first bud burst and fruit production of all three cultivars. For 'Ben Gairn' and 'Ben Tirran', as the chilling temperature decreased from 8.9°C and the chilling duration increased, time to bud burst was advanced and fruit production increased. Particularly for 'Ben Tirran', at lower temperatures, chilling duration was relatively insignificant. However, as the chilling temperature increased, exposure to longer chilling durations was necessary in order to satisfy the chilling requirement, promote bud burst and maximise fruit production. The potential decrease in the average UK temperature as a result of climate change may dramatically delay bud burst and reduce *R. nigrum* fruit production, as a result of lack of winter chilling. Further work must be conducted to quantify the optimum chilling treatment for maximising bud burst and determine the effects of climate change scenarios on the availability of chilling.

Plant material was found to have a significant effect, not only on final bud burst but also on the response to chilling temperature and duration. This experiment discovered that the bud burst behavior of 2-year old budsticks was not comparable to the bud burst behaviour of whole plants. Previous research, however, has suggested that older, more mature budsticks may be more representative, hence future research should concentrate on this material. In the meantime, experiments will be conducted using whole plants.



Chapter Four.
Ribes nigrum Chill Unit Model Construction and Validation

4.1. Introduction

Temperate-zone crops require a period of low winter temperature to satisfy their chilling requirement and terminate dormancy. If exposure to the appropriate temperature is not achieved, delayed and uneven bud burst occurs the following spring, and detrimental effects on flower emergence can significantly reduce crop quality and yield. The chilling requirement is species-dependant and as a result, chill unit models have been developed for a range of plants.

4.1.1. Fluctuating Temperature Models

Such models consider the interruption of chilling temperatures with warm temperatures to be more effective at overcoming endodormancy than exposure to chilling temperatures alone (Lang, 1989). Chilling temperatures are considered to be between 0°C and 13°C, whereas warm temperatures are 13°C to 15°C (Erez and Couvillon, 1987). This model has been found most effective in countries that experience warm daytime temperatures, but very cold night time temperatures, e.g. South Africa. It has proved successful for predicting bud burst of *Prunus persica* 'Redhaven' (Erez and Couvillon, 1987), 'Starcrest' and 'Baby Gold 9' (Barbal and Melo-Aberu, 2002). However, this model would be unsuitable for the UK climate, given the lack of temperature differential between day and night.

4.1.2. Physiological Models

The changing physiology of the crop over a growing season is considered in the Degree Growth Stage model, and interpreted as a sine wave with five stages (Fuchigami and Nee, 1987). Successful bud burst prediction was achieved using this model with *Cornus sericea* (Kobayashi *et al*., 1982) and *Prunus persica* 'Redhaven' (Siller-Cepeda *et al*., 1992). Application of this model, however, appears to be highly complex, and as a result very little research has relied on it.

4.1.3. Weighted Temperature Models

By far the most popular and successful, such models are generally constructed by exposing plants/budsticks to a range of chilling temperatures and monitoring effects on subsequent growth and development. One hour's exposure to the optimum chilling temperature is denoted as 1 chill unit, and temperatures above/below this are allocated fractional values. Of all the chill unit models available, weighted models have been found to be not only species specific, but cultivar-specific (Weinberger, 1956; Lantin, 1973; Plancher, 1983). Weighted chill units have been constructed for *Prunus persica* 'Redhaven' (Richardson *et al*., 1974), *Prunus cerasus* (Anderson *et al*., 1986), *Fragaria ananassa* 'Elsanta' (Tehranifar, 1997) and *Prunus avium* 'Stella', 'Sunburst' and 'Summit' (Mahmood *et al*., 2000).

The effect of chilling temperatures on *Ribes nigrum* have been studied (Plancher, 1983a,b; Plancher 1984; Lantin 1973), but the research was conducted on French and UK cultivars that are no longer commercially acceptable. Additionally, breeding objectives have changed since the original work was conducted, especially in respect to the chilling requirement. Therefore, no research has been conducted on current, commercially-important *R. nigrum* cultivars.

4.2. Materials and Methods

4.2.1. Chill Unit Model Construction

Chill Unit Model 1

Final bud burst data, obtained from Experiment 1 (described in Chapter 3) were converted to radians as advised by the University of Reading's Statistical Advisory Service using the equation:

$$\text{radian} = \sin^{-1}(\sqrt{p})$$

where p = proportion = number of burst buds/total number of buds

Multiple regression analyses were performed to determine the relationship between bud burst and D, T, D^2, T^2 and D*T, where D = chilling duration and T = chilling temperature, as described by Mahmood *et al*. (2000a). The regression analyses were first of all conducted to determine a relationship between bud burst and chilling duration, then the other factors were added (built-up) one at a time. If one factor was

deemed to be insignificant (P>0.05), it was removed from the analysis. As a precaution, the regression analysis was then performed with all the data and the insignificant factors were removed (built-down) according to the highest significance. The same result was obtained when both regressions were conducted for all cultivars. If no relationship between chilling duration and chilling temperature was observed (P>0.05), the duration data was pooled to provide one bud burst data point for each temperature. This data was plotted firstly on a two-dimensional graph. The bud burst data is transformed into chill units by dividing each data point by the value that gave the highest bud burst data.

4.2.2. Chill Unit Model Validation

Tiny Talk temperature data loggers (Gemini DataLoggers, Chichester, UK) were placed in commercial *Ribes nigrum* fields and hourly temperatures were recorded from October until April. From 1 January, budsticks were cut from selected bushes at 4-day intervals and placed in buckets of water in a warm forcing environment. When 75% bud burst was achieved, the chilling requirement was concluded to have been satisfied. Data was collected by John Atwood (ADAS) in Norfolk in 2002/2003 and 2003/2004 and by Edward Thompson (GSK grower) in Gloucester 2003/2004. The derived chill unit models for 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' were applied to the temperature data and based on the date of chill satisfaction, the number of accumulated chill units up to the date when chill was reported to have been satisfied were calculated. Accumulated chill units for all three data points (Norfolk 2002/2003, Norfolk 2003/2004 and Gloucester 2003/2004) were averaged to produce a mean value of chill satisfaction. Deviations from the mean were used to predict the accuracy of the models. In order to be robust and accurate, consistent values of chill accumulation should be apparent for the model, regardless of geographical location or year. Chill unit accumulation of the <7°C, 0-7°C, Lantin and Utah chill unit models were calculated, to compare the predictive capacity.

4.3. Results

4.3.1. Chill Unit Model Construction

The GSK/Fraser Chill Unit Model

'Ben Gairn'

As the chilling temperature decreased (Plate 4.1) and the chilling duration increased (Plate 4.2), a positive effect on bud burst was recorded (Figure 4.1).

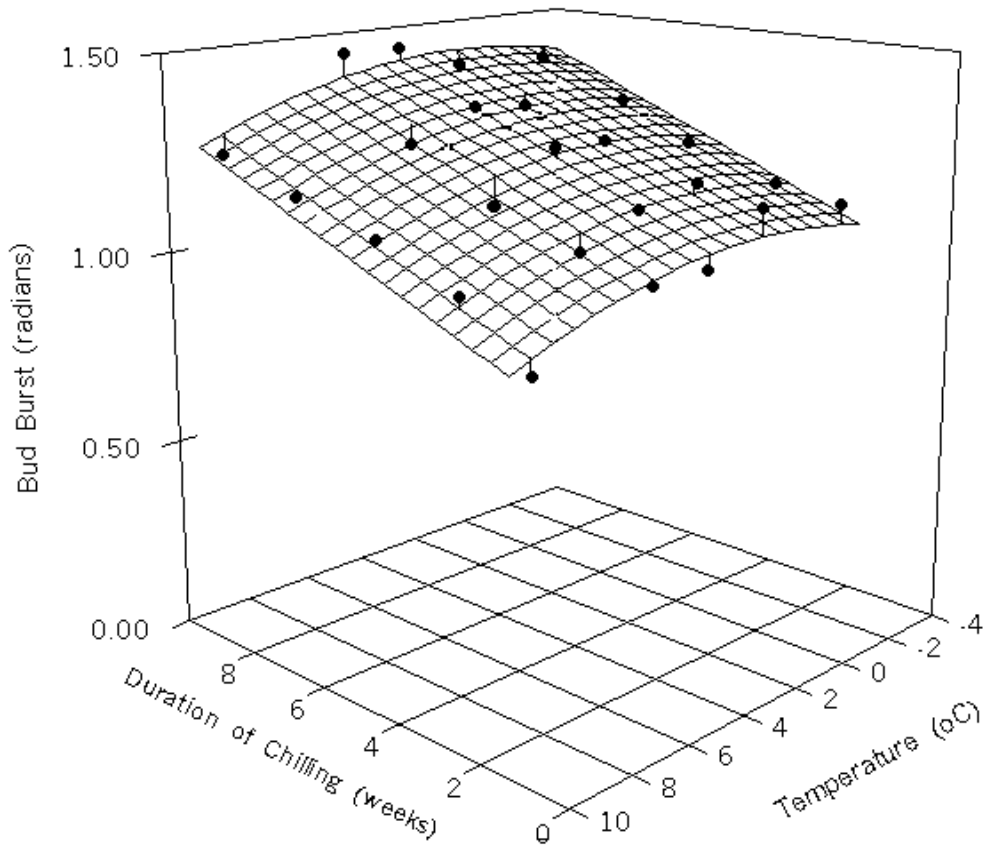


Figure 4.1. ***R. nigrum*** **'Ben Gairn' – the effect of chilling temperature and duration on bud burst. The plane was fitted by multiple regression analysis where: bud burst (radians) = 0.045583D - 0.00206T^2 + 0.962354 (standard errors 0.009, 0.0009 and 0.009 respectively)**

Plate 4.1. *R. nigrum* 'Ben Gairn' – effect of chilling temperature on bud burst
L-R: control, -3.4°C, 0.1°C, 3.4°C, 8.9°C

Plate 4.2. *R. nigrum* 'Ben Gairn' – effect of chilling duration on bud burst
L-R: Control, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks

Regression analysis determined no significant interaction between chilling temperature and chilling duration (P=0.712), hence chilling duration data were pooled to provide one data point for each chilling temperature (Figure 4.2).

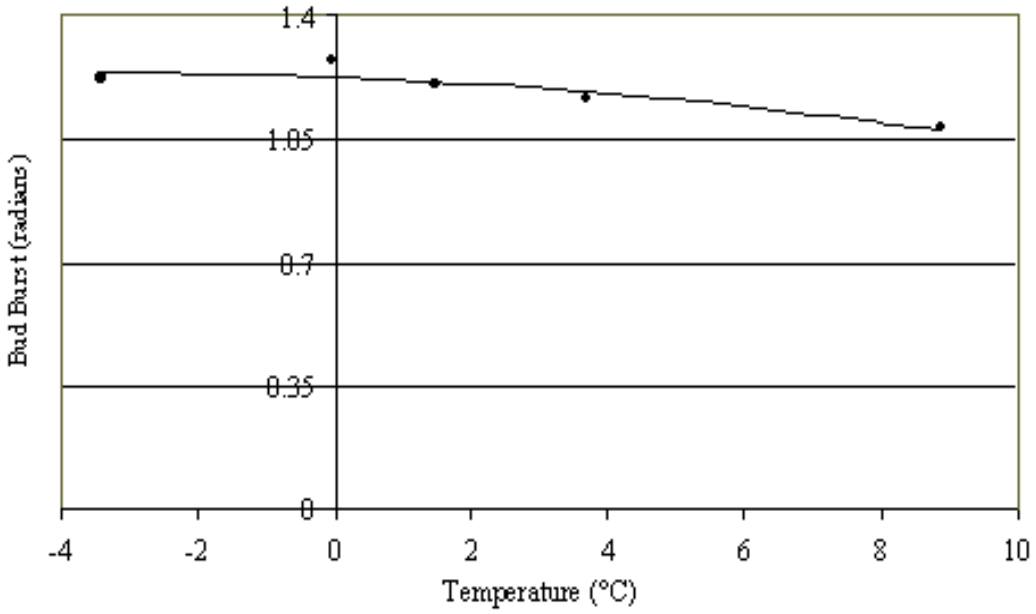


Figure 4.2. ***R. nigrum*** **'Ben Gairn' – relationship between chilling temperature and bud burst. Data pooled across chilling durations. Bud burst = $-0.0012T^2 - 0.0067T + 1.2252$, $R^2 = 0.8518$.**

According to the relationship described in Figure 4.2., the greatest bud burst occurred after exposure to -3.4°C. One chill unit, therefore, is one hour's exposure to -3.4°C, and higher temperatures contribute fractionally (Figure 4.3).

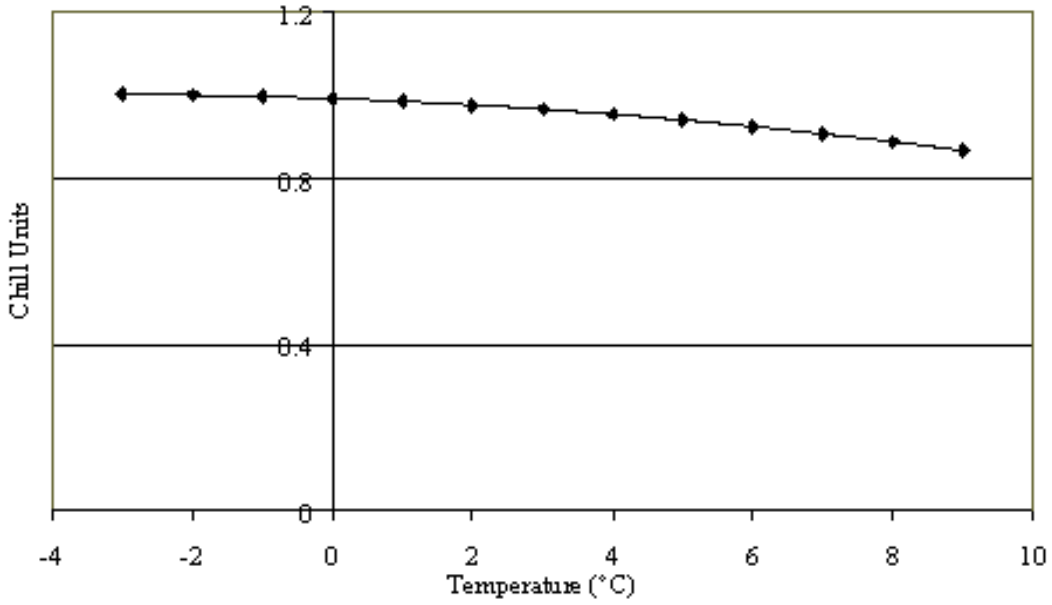


Figure 4.3. ***R. nigrum*** **'Ben Gairn' – relationship between chilling temperature and chill unit. Chill units = $-0.0009T^2 - 0.0059T + 0.9903$.**

'Ben Hope'

As the chilling temperature decreased from 8.9°C (Plate 4.3) and the chilling duration increased (Plate 4.4), positive effects on bud burst were recorded (Figure 4.4). Regression analysis demonstrated no significant (P=0.31) interaction between chilling temperature and chilling duration hence the bud burst data for each chilling duration were pooled to provide data for each chilling temperature (Figure 4.5).

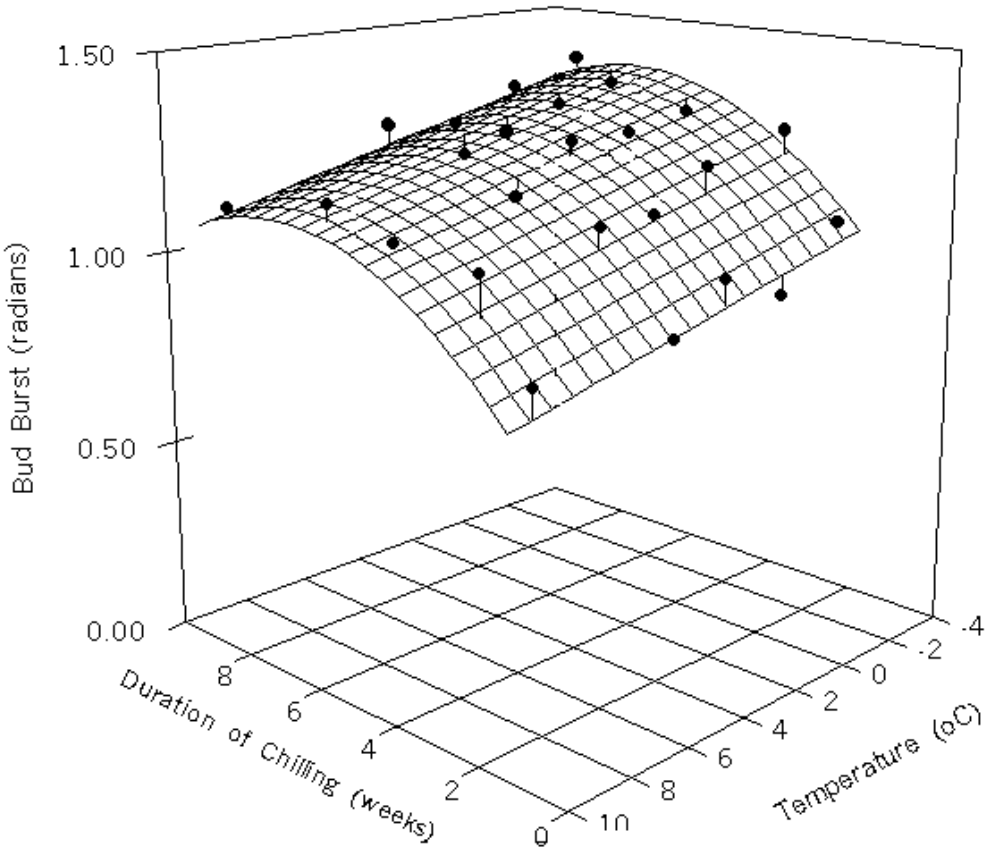


Figure 4.4. ***R. nigrum*** **'Ben Hope' – the effect of chilling temperature and duration on bud burst. The plane was fitted by multiple regression analysis where: bud burst (radians) = -0.0169D + 0.01135D^2 - 0.01907T + 0.6334 (standard errors 0.0352, 0.0029, 0.0052 and 0.093 respectively)**

Plate 4.3. *R. nigrum* 'Ben Hope' – effect of chilling temperature on bud burst
L-R: control, -3.4°C, 0.1°C, 3.4°C, 8.9°C

Plate 4.4. *R. nigrum* 'Ben Hope' – effect of chilling duration on bud burst
L-R: Control, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks

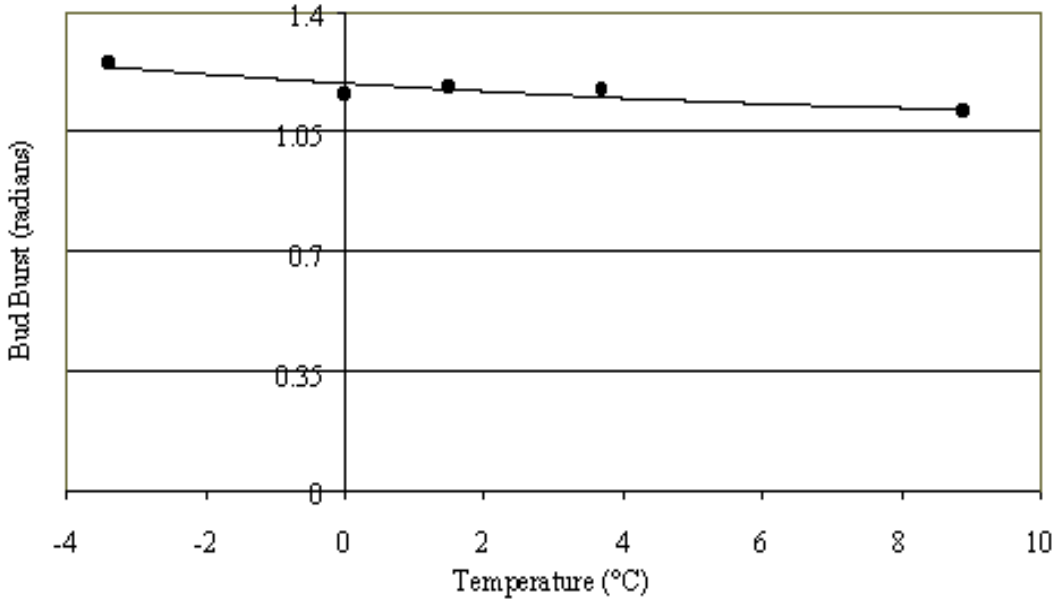


Figure 4.5. *R. nigrum* 'Ben Hope' – relationship between chilling temperature and bud burst. Data pooled across chilling durations. Bud burst = $0.0005T^2 - 0.126T + 1.1913$, $R^2 = 0.8484$.

According to the relationship described in Figure 4.5, the greatest bud burst occurred when plants were exposed to -3.4°C. Therefore, one chill unit is one hour's exposure to -3.4°C, and higher temperatures contribute fractionally (Figure 4.6).

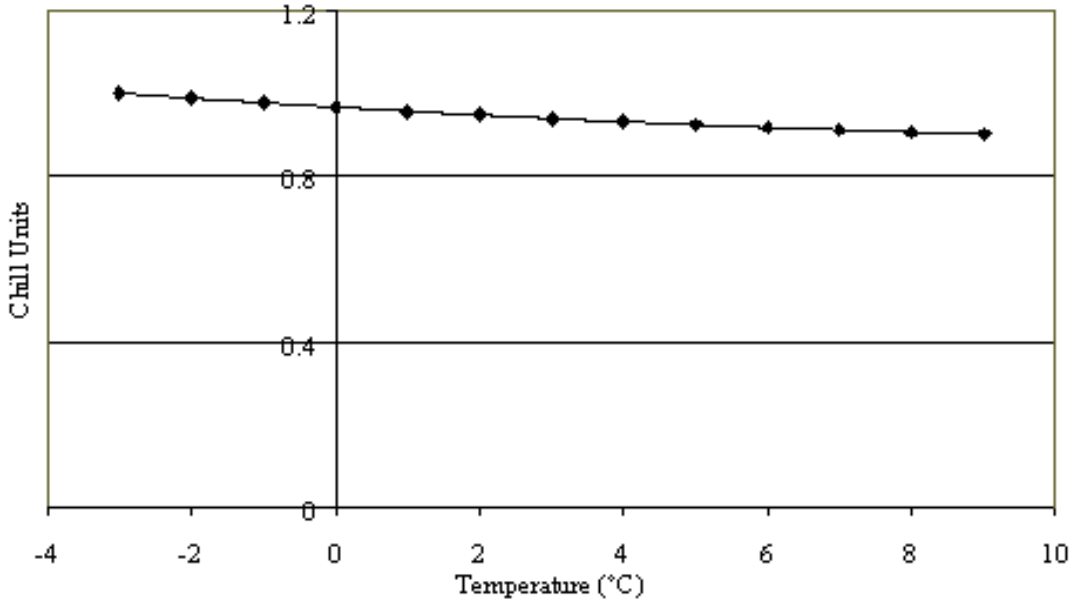


Figure 4.6. *R. nigrum* 'Ben Hope' – relationship between chilling temperature and chill unit. Chill units = $-0.0003T^2 - 0.0101T + 0.996$.

'Ben Tirran'

As the chilling temperature decreased (Plate 4.5) and the chilling duration increased (Plate 4.6), an increase in bud burst was observed (Figure 4.7). Increasing the duration of chilling to 10 weeks significantly increased bud burst. The relationship between chilling temperature and chilling duration was insignificant (P=0.215) therefore data were pooled across durations (Figure 4.8).

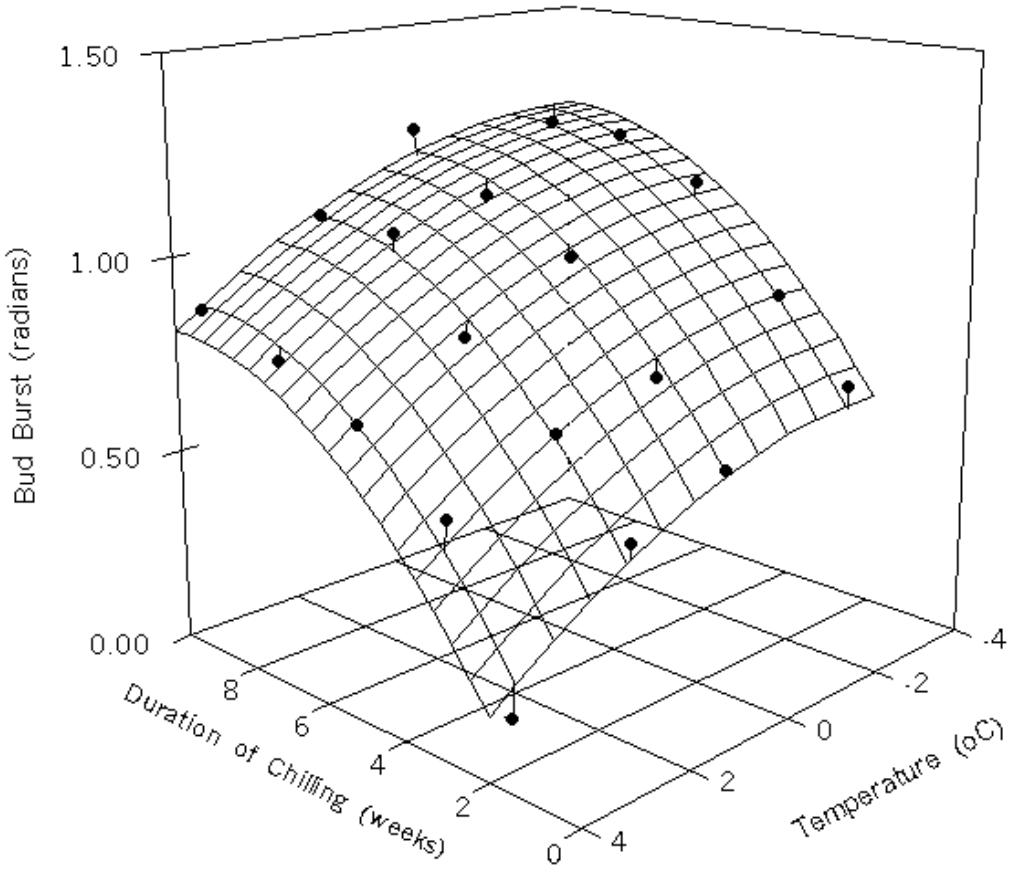


Figure 4.7. ***R. nigrum*** **'Ben Tirran' – the effect of chilling temperature and duration on bud burst. The plane was fitted by multiple regression analysis where: bud burst (radians) = 0.2096D – 0.0107D^2 – 0.05918T – 0.0063T^2 + 0.0993 (standard errors 0.061, 0.005, 0.013, 0.0017 and 0.001 respectively)**

Plate 4.5. *R. nigrum* 'Ben Tirran' – effect of chilling temperature on bud burst
L-R: control, -3.4°C, 0.1°C, 3.4°C, 8.9°C

Plate 4.6. *R. nigrum* 'Ben Hope' – effect of chilling duration on bud burst
L-R: Control, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks

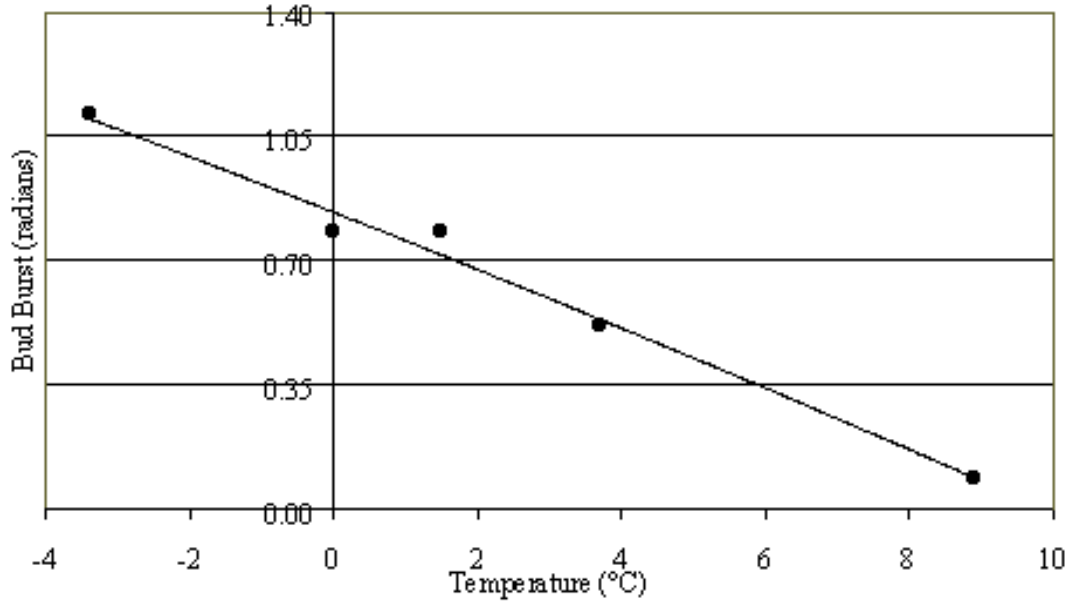


Figure 4.8. *R. nigrum* 'Ben Tirran' – relationship between chilling temperature and bud burst. Bud burst = $-0.0005T^2 - 0.0793T + 0.8363$, $R^2 = 0.9867$

According to the relationship described in Figure 4.8., the greatest bud burst occurred after exposure to -3.4°C. One chill unit, therefore, is one hour's exposure to 3.4°C, and higher temperatures contribute fractionally (Figure 4.9).

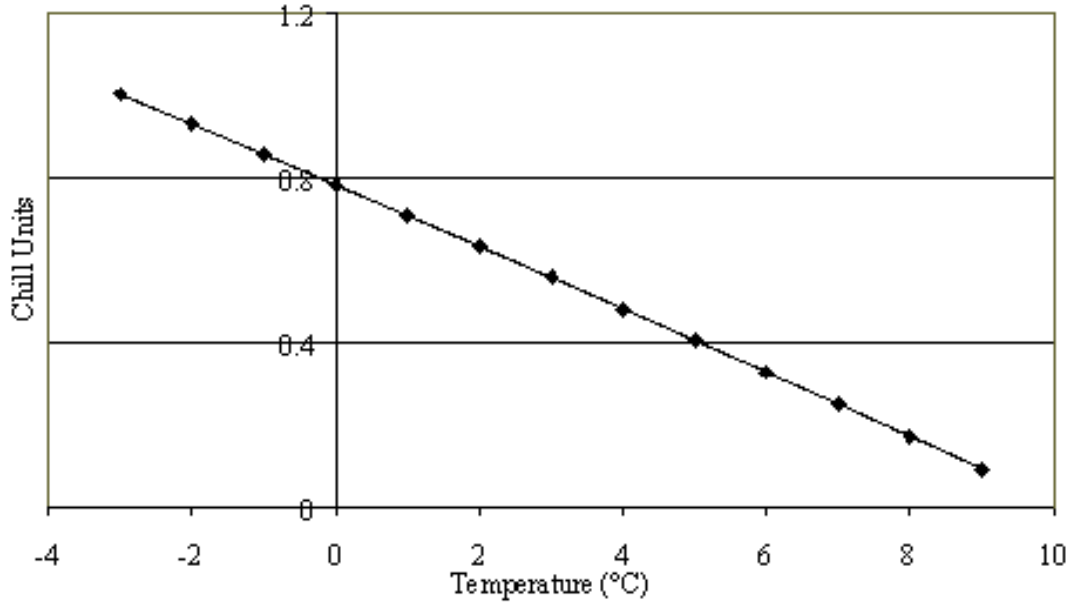


Figure 4.9. *R. nigrum* 'Ben Tirran' – relationship between chilling temperature and chill unit. Chill units = $-0.0003T^2 – 0.074T + 0.7804$.

4.3.2. Chill Unit Model Validation

'Ben Gairn'

The accuracy of GSK/Fraser, <7°C, 0-7°C, Lantin and Utah models at predicting date of 'Ben Gairn's' date of chilling satisfaction is shown in Figure 4.10. To be of use to growers, models must be able to accurately predict chill satisfaction in a range of geographical areas and over several years.

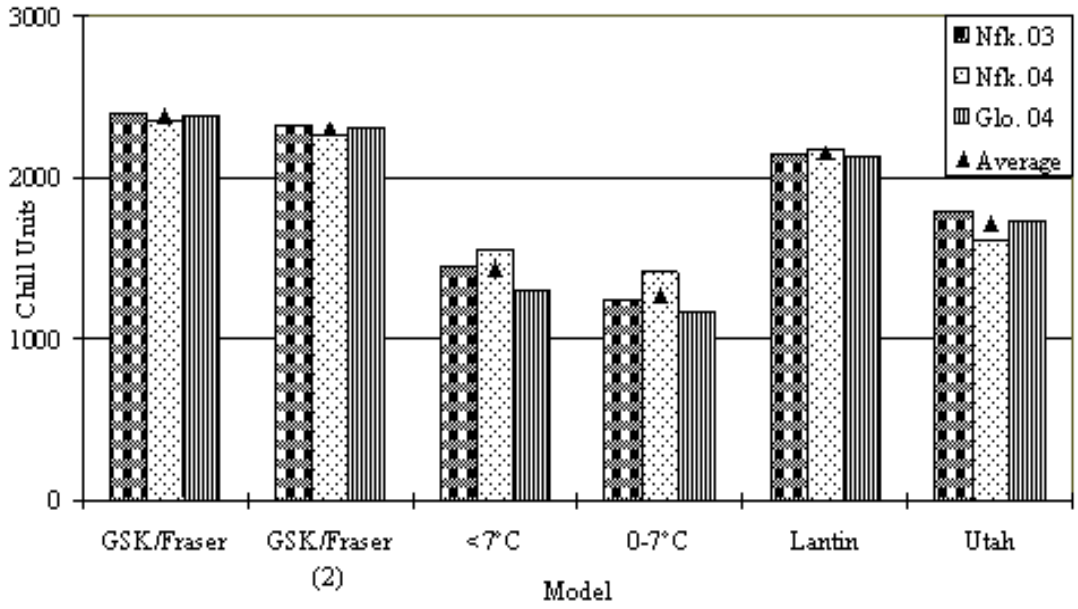


Figure 4.10. ***Ribes nigrum*** **'Ben Gairn' – comparison of chill unit model predictions**

The <7°C did not accurately predict the date of chilling satisfaction using the available data. For two out of the three years, the 0-7°C and Utah models predicted chill satisfaction within 79 and 52 hours of a two year average, however these models could not accurately predict chill satisfaction in Norfolk 2003/2004. Using the three data points, GSK/Fraser predicted chilling satisfaction within 22, 24 and 2 hours when compared to a three year average. Similarly, the Lantin model predicted chill satisfaction within 2, 21 and 12 hours. For 'Ben Gairn', therefore the GSK/Fraser and Lantin models were the most accurate.

'Ben Hope'

The <7°C, 0-7°C, Lantin and Utah models did not accurately predict chilling satisfaction using data from two locations and two years (Figure 4.11).

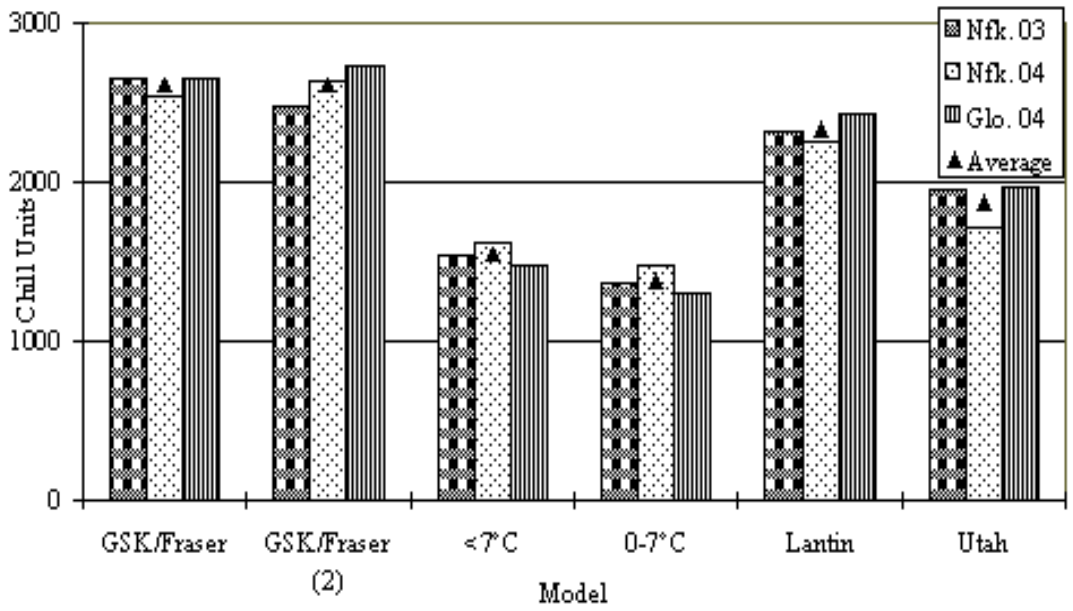


Figure 4.11. ***Ribes nigrum*** **'Ben Hope' – comparison of chill unit model predictions**

The original GSK/Fraser model, however, predicted chilling satisfaction within 40, 74 and 34 hours of the 3-year average and was the most accurate of the tested models.

'Ben Tirran'

The 0-7°C, Lantin and Utah models could not accurately predict chilling satisfaction of 'Ben Tirran' (Figure 4.12). The <7°C model however, predicted chilling satisfaction within 61, 4 and 42 hours of a 3-year average and GSK/Fraser within 59, 13 and 72 hours, making these models the most accurate.

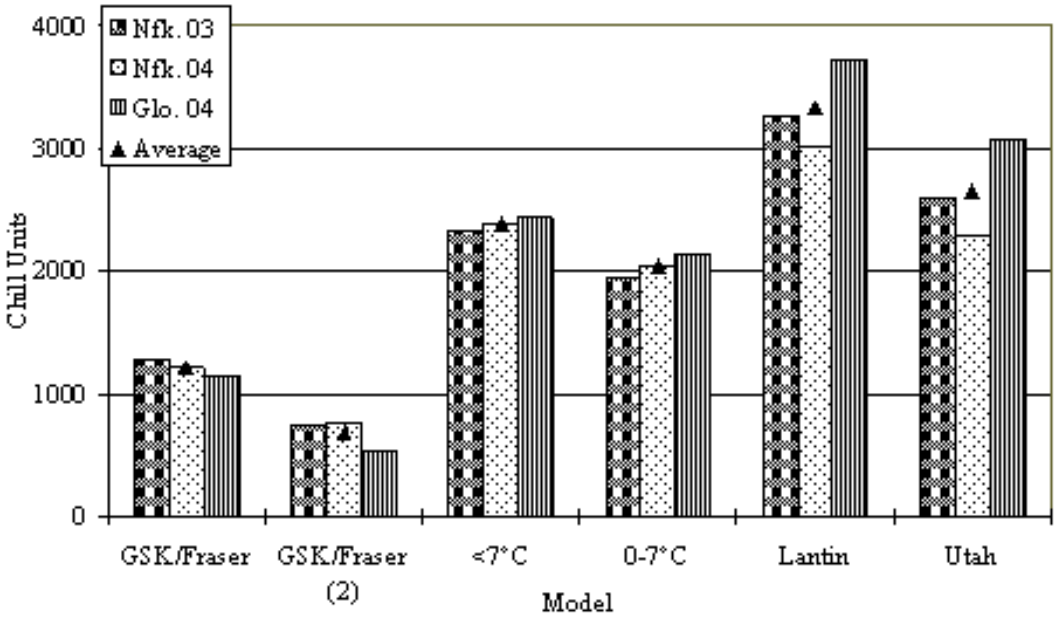


Figure 4.12. ***Ribes nigrum*** **'Ben Tirran' – comparison of chill unit model predictions**

The optimum chilling temperature for 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' was found to be -3.4ºC, which was the lowest temperature utilized in this experiment. This suggests that temperatures below -3.4ºC may be as or more effective. Based on this hypothesis, it was decided to repeat the experiment in the subsequent year and lower the temperature range.

4.2.3. Materials and Methods

Experiment 3.

Plant Material

One year old potted plants of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' were utilised in this experiment. Cuttings of all three cultivars were taken in the spring of 2003 and delivered to the University of Reading's Field Unit in August 2003 where they were tied into the supporting wire and connected to the irrigation system, as described in Chapter 2. Plants were irrigated four times a day with Avoncrop's Soft Fruit Mix 2 for a total of one hour, and the system was shut down on 29 September 2003. The plants suffered an attack of *Puccinia* and were successfully treated with a chemical spray of Nimrod T (active ingredient triforine) at a rate of 30ml in 10L of water on 17 September 2003.

Cold Storage

Twenty plants each of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' were placed in cold stores maintained at 2.1°C, -4.2C and -10.1°C°C on 22 December 2004 and wrapped in black plastic to prevent the stems dessicating. Average two-weekly temperature values are shown in Table 4.1. The -4.2°C cold store failed to maintain a constant temperature and fluctuations of ±2.3°C were recorded, which may impact on the chill unit models.

Table 4.1. Average two-weekly temperature readings from the cold stores and polytunnel

Temp/ Week	-10.1 ± 0.5°C	-4.2 ± 2.3°C	2.1 ± 0.5°C	17.1 ± 6°C
2	-10.10 ±0.4°C	-4.13 ± 2.3 °C	2.29 ± 0.5°C	16.98 ± 4°C
4	-10.1 ± 0.5°C	-4.40 ± 0.5°C	1.9 ± 0.4°C	17.39 ± 6°C
6	-10.16 ± 0.5°C	-3.90 ± 1.6°C	2.09 ± 0.4°C	16.99 ± 4°C
10	-10.17 ± 0.4°C	-3.98 ± 0.9°C	2.03 ± 0.3°C	17.41 ± 6°C

Five plants of each cultivar were removed from each cold store after receiving 2, 4, 6 or 10 weeks of chilling and randomly placed in a polytunnel set at 17.1°C. Five plants of each cultivar were placed in the polytunnel on 22 December to act as control plants.

Randomisation

This followed the protocol described for Experiment 1. The first sub-set of plants were removed from the cold store on 5 January 2004 and the plants were harvested on 15 June.

Nutrition

Nutrients were supplied as detailed in Chapter 2.

Chill Unit Model Construction

Data from this experiment were combined with data from Experiment 1, and chill unit models were constructed as described earlier.

4.3.3. Results

The GSK/Fraser (2) Chill Unit Model

'Ben Gairn'

Modification of the original GSK/Fraser chill unit model altered the bud burst response of the plants (Figure 4.13).

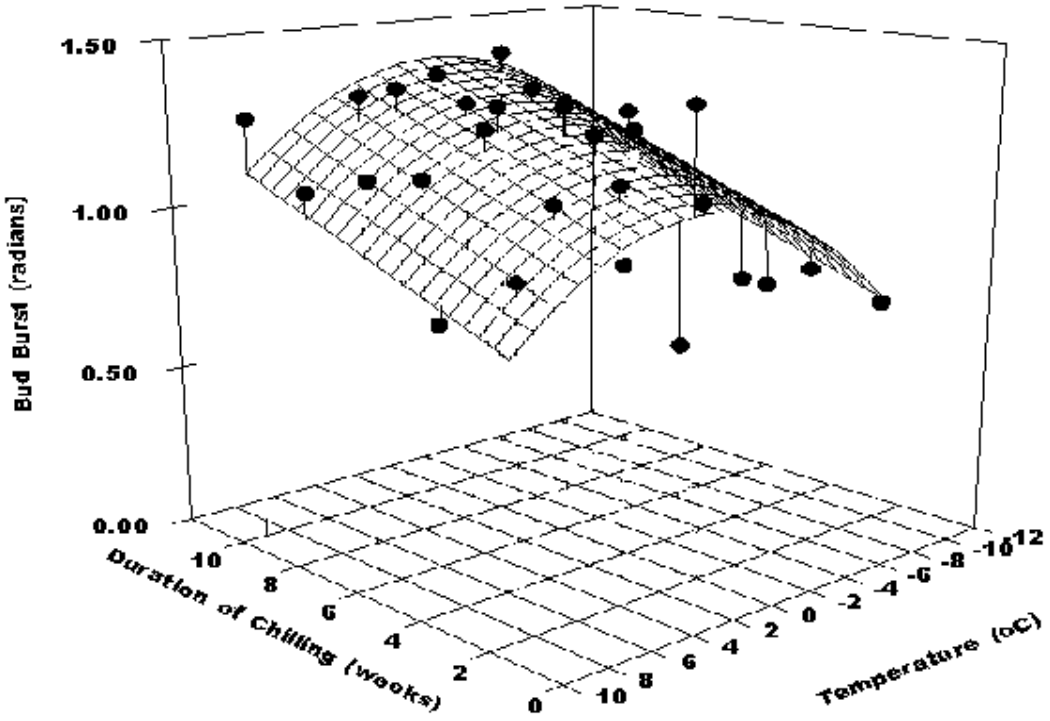


Figure 4.13. *R. nigrum* 'Ben Gairn' – the effect of chilling temperature and duration on bud burst. The plane was fitted by multiple regression analysis where: bud burst (radians) = 0.03639D -0.00308T^2 + 0.989559 (standard errors 0.007, 0.001 and 0.065 respectively)

No significant interaction (P=0.563) between chilling temperature and chilling duration were demonstrated, hence chilling duration data were pooled to provide one data point for each chilling temperature (Figure 4.14).

Data obtained from the -4.2°C cold store was significantly lower than that obtained the previous year in the -3.4°C. As mentioned previously, this cold store failed to maintain a constant chilling temperature and the data may be suspect. When the data point was omitted and the data re-analysed (Figure 4.15), results were better correlated (R^2 = 0.9106 compared to R^2 = 0.8173) without the suspect data.

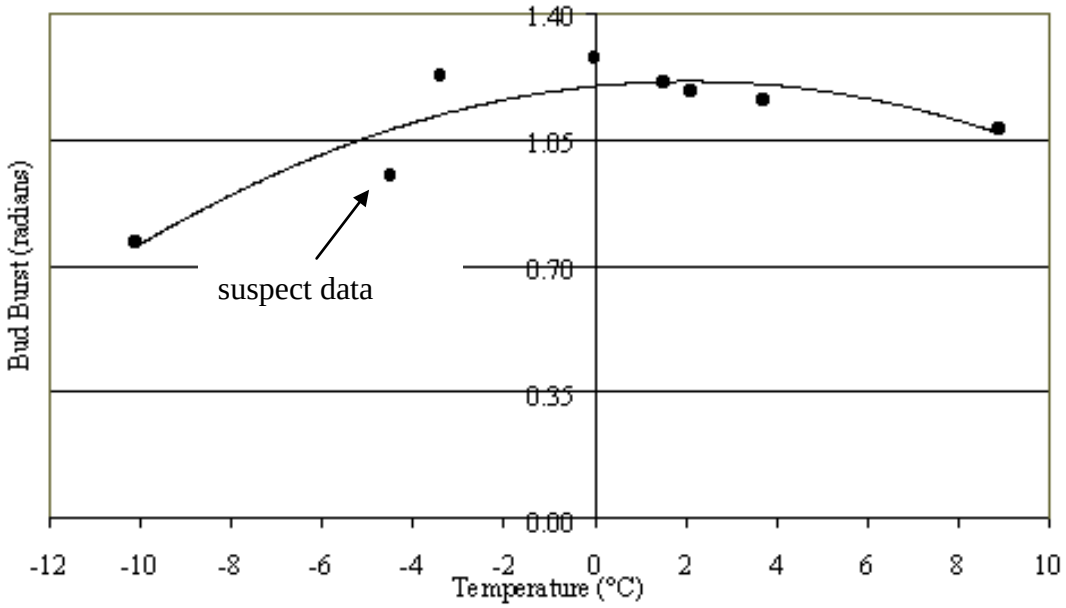


Figure 4.14. ***R. nigrum*** **'Ben Gairn' – relationship between chilling temperature and bud burst. Bud burst = -0.0031T2 + 0.0128T + 1.1964, R^2 = 0.8173.**

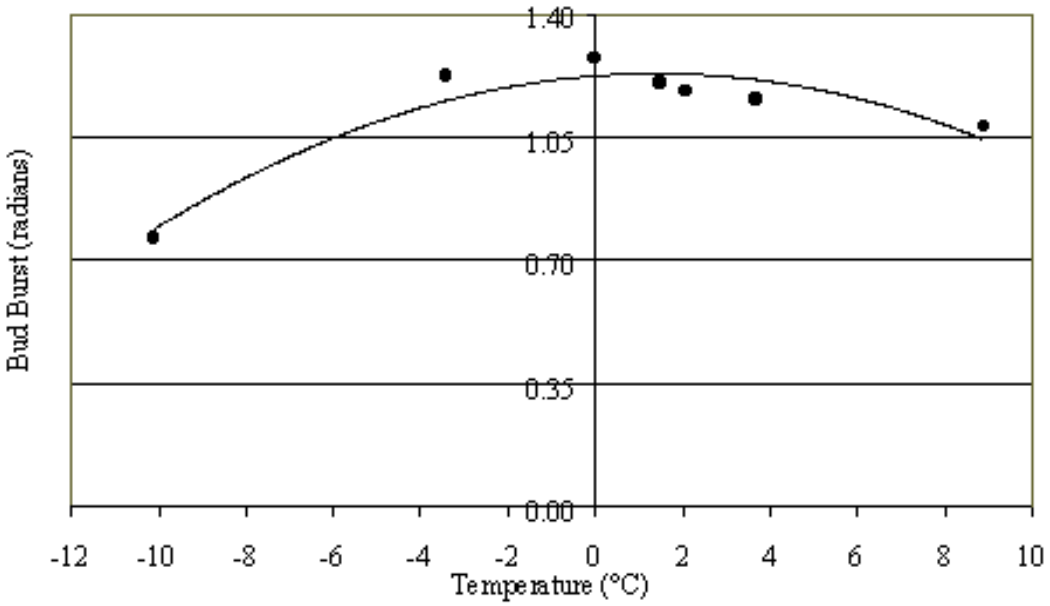


Figure 4.15. ***R. nigrum*** **'Ben Gairn' – relationship between chilling temperature and bud burst. Bud burst = -0.0033T^2 + 0.0094T + 1.2237, R^2 = 0.9106.**

According to the relationship described in Figure 4.12, the optimum temperature for maximising bud burst was 1°C and higher/lower temperatures contributed fractionally. The model was extended to predict the effects of temperatures out-with the experimental range (Figure 4.16).

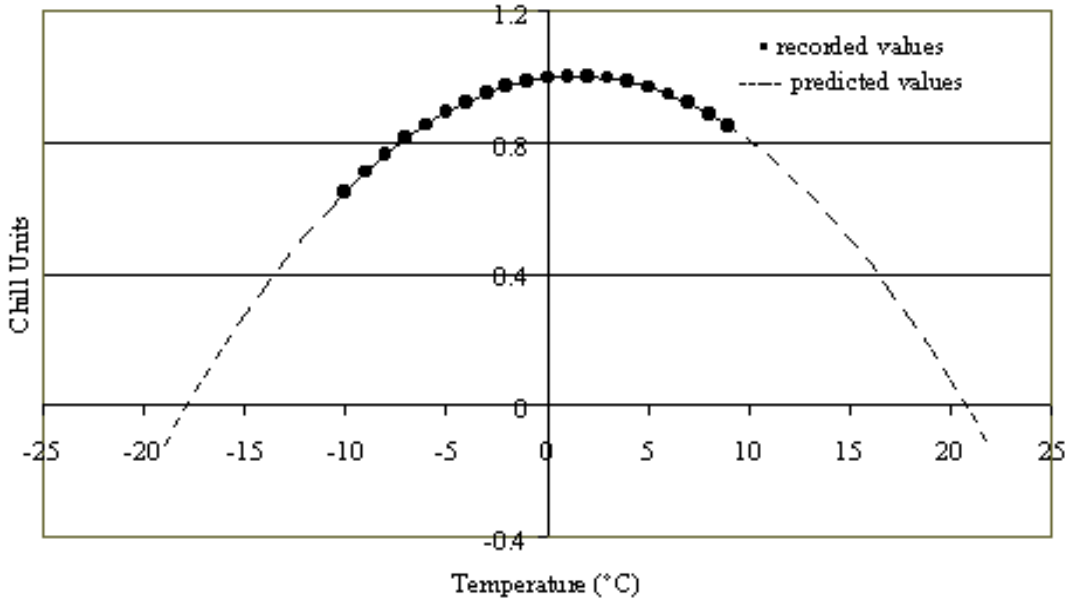


Figure 4.16. ***R. nigrum*** **'Ben Gairn' – relationship between chilling temperature and chill units. Chill unit = -0.0027T^2 + 0.0076T + 0.995**

According to the extended model, temperatures below –17.9°C or above 20.8°C negate the effects of chill accumulation.

'Ben Hope'

The bud burst response to chilling temperature and chilling duration, encompassing data from Experiment 1 and Experiment 3, is shown in Figure 4.17.

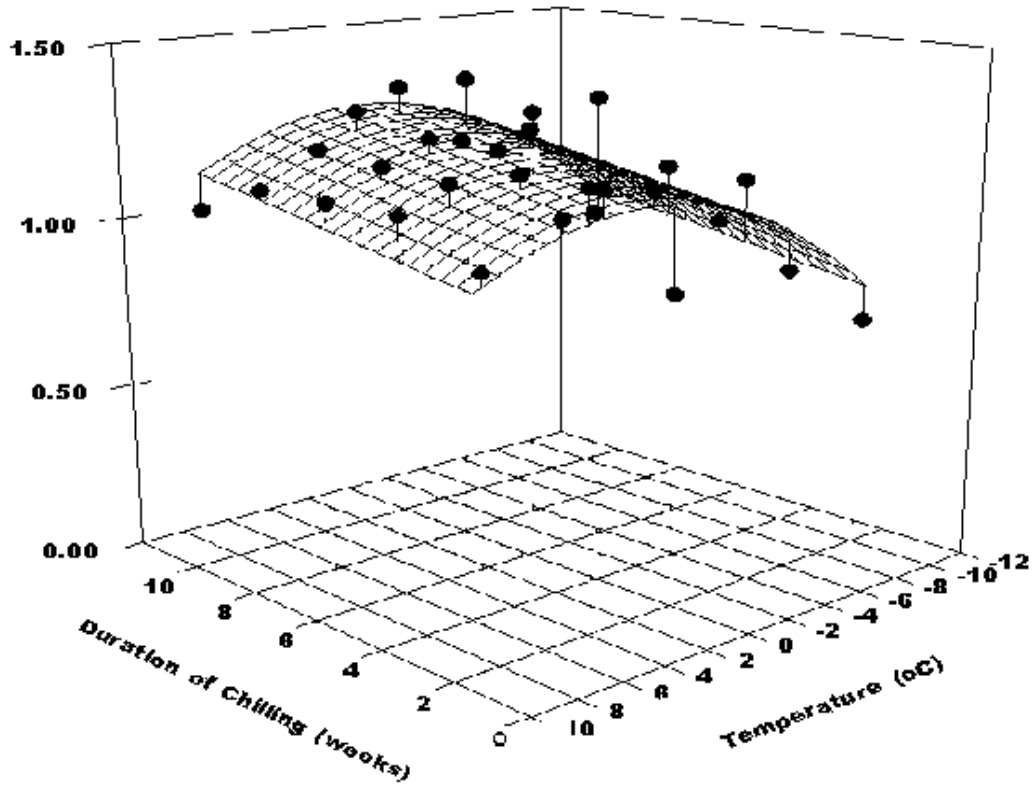


Figure 4.17. ***R. nigrum*** **'Ben Hope' – the effect of chilling temperature and duration on bud burst. Plane was fitted by multiple regression analysis where: bud burst (radians) = 0.02091D – 0.0019T^2 + 1.028994 (standard errors 0.009, 0.001 and 0.066 respectively).**

Regression analysis demonstrated no significant (P=0.31) interaction between chilling temperature and chilling duration hence the bud burst data for each chilling duration were pooled (Figure 4.18). Again, data from the -4.2°C cold store appeared to be suspect, and removal of the data (Figure 4.19) increased the accuracy of the model.

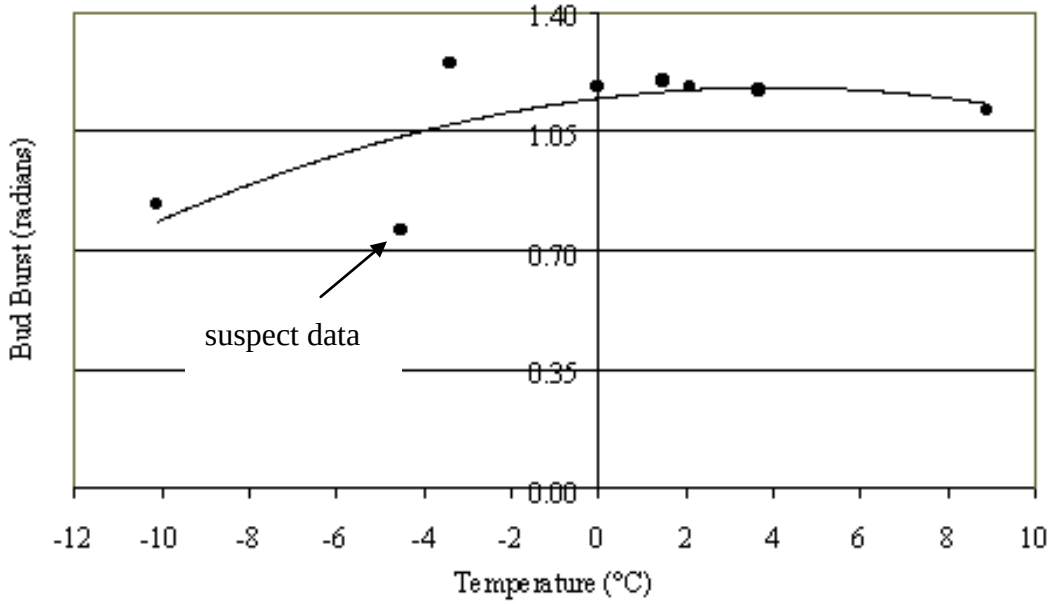


Figure 4.18. ***R. nigrum*** **'Ben Hope' – relationship between chilling temperature and bud burst. Bud burst = -0.002T^2 + 0.158T + 1.455, R^2 = 0.5163.**

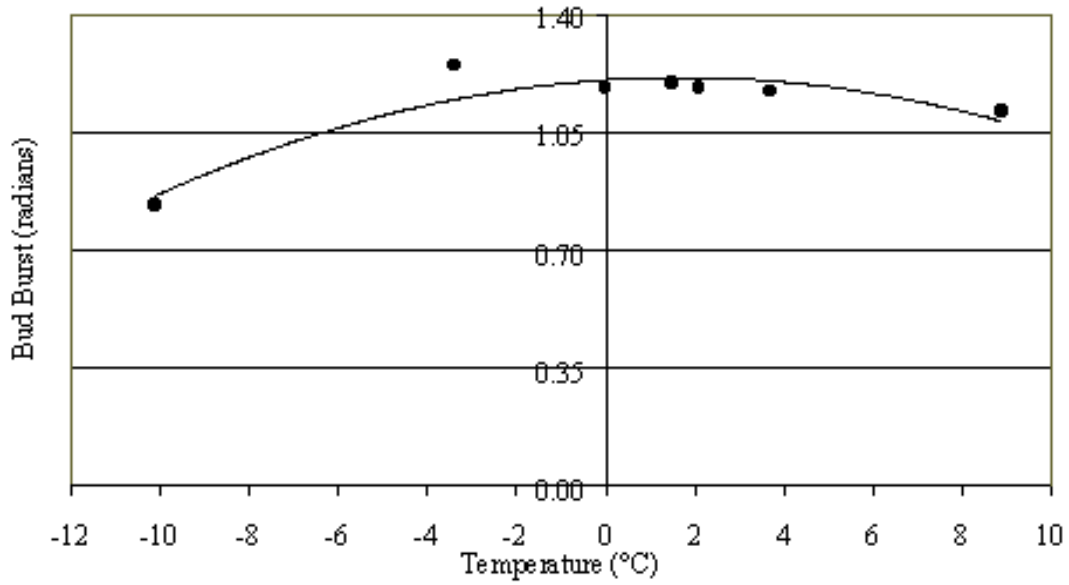


Figure 4.19. ***R. nigrum*** **'Ben Hope' – relationship between chilling temperature and bud burst. Bud burst = -0.0025T^2 + 0.0085T + 1.2041, R^2 = 0.8705.**

According to the relationship depicted in Figure 4.19, one chill unit is equivalent to one hour of chilling at 1.7°C and higher or lower temperatures contribute fractionally (Figure 4.20). The model was extended to predict the range of temperatures that contribute to chilling (Figure 4.20) and temperatures below –20.4°C or above 23.7°C negate the effects of chill accumulation.

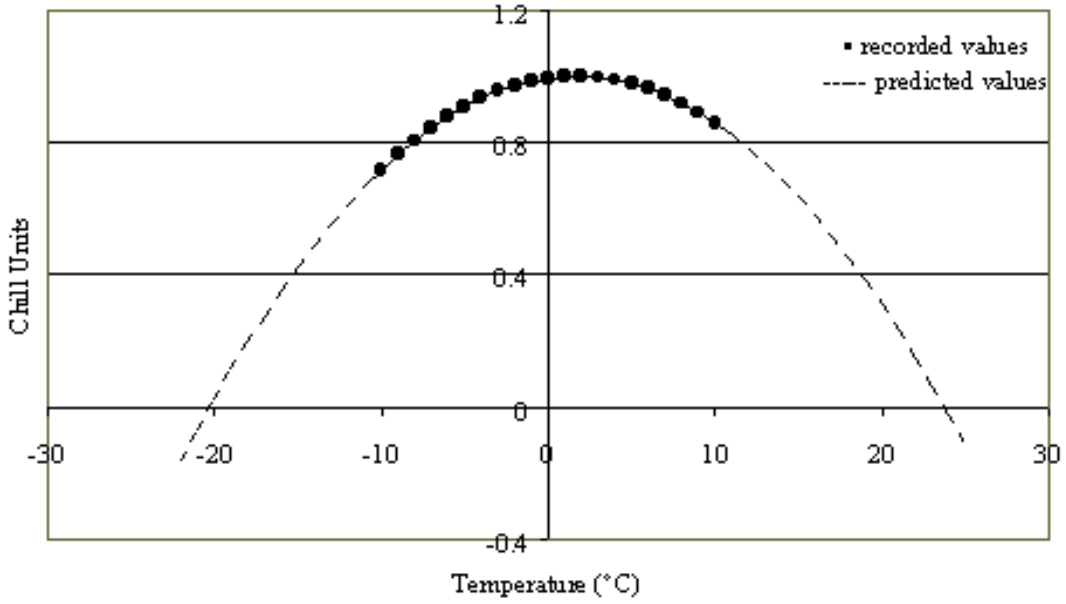


Figure 4.20. ***R. nigrum*** **'Ben Hope' - relationship between chilling temperature and chill units. Chill units = $-0.0021T^2 + 0.007T + 0.994$**

'Ben Tirran'

Bud burst response to chilling temperature and chilling duration, encompassing data from the first and second year's experiments, is shown in Figure 4.21.

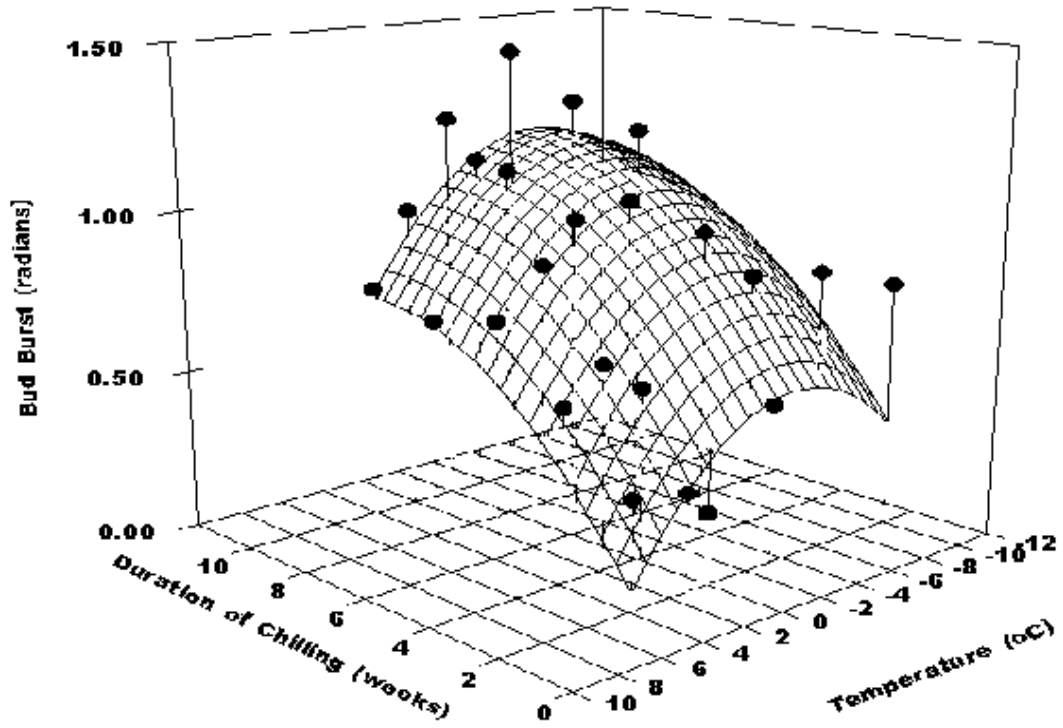


Figure 4.21. ***R. nigrum*** **'Ben Tirran' - the effect of chilling temperature and duration on bud burst. The plane was fitted by multiple regression analysis where: bud burst (radians) = 0.06709D – 0.005668T – 0.00632T^2 + 0.492852 (standard errors 0.01, 0.02, 0.002 and 0.09 respectively)**

As with the above models, all temperature data was initially analysed (Figure 4.22), but the accuracy of the model was improved when data from the -4.2°C cold store were omitted (Figure 4.23).

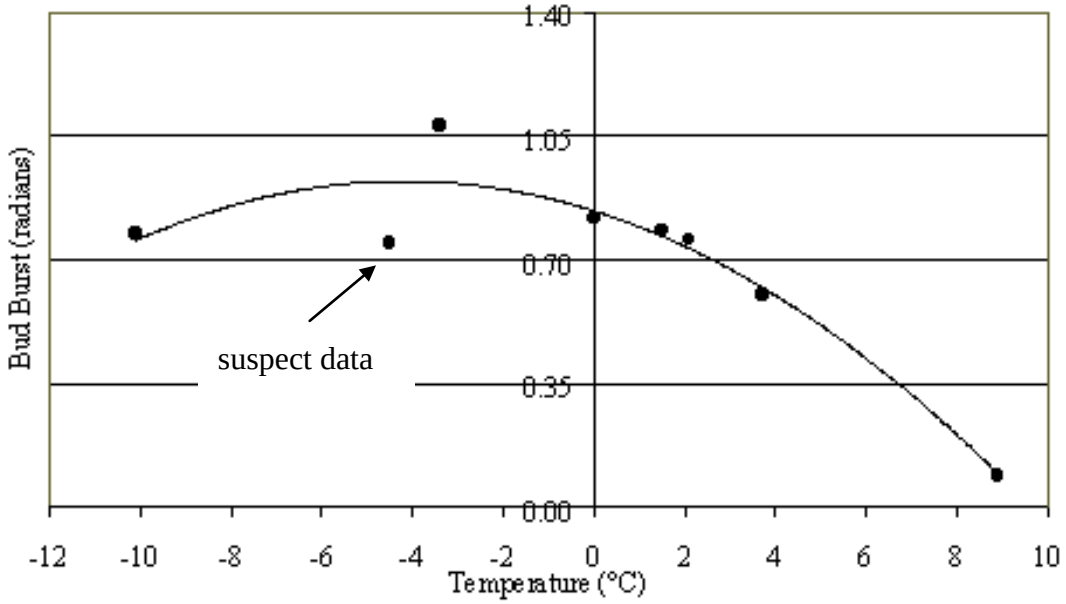


Figure 4.22. ***R. nigrum*** **'Ben Tirran' – relationship between chilling temperature and bud burst. Bud burst = -0.0048T^2 - 0.0405T + 0.8374, R^2 = 0.8957**

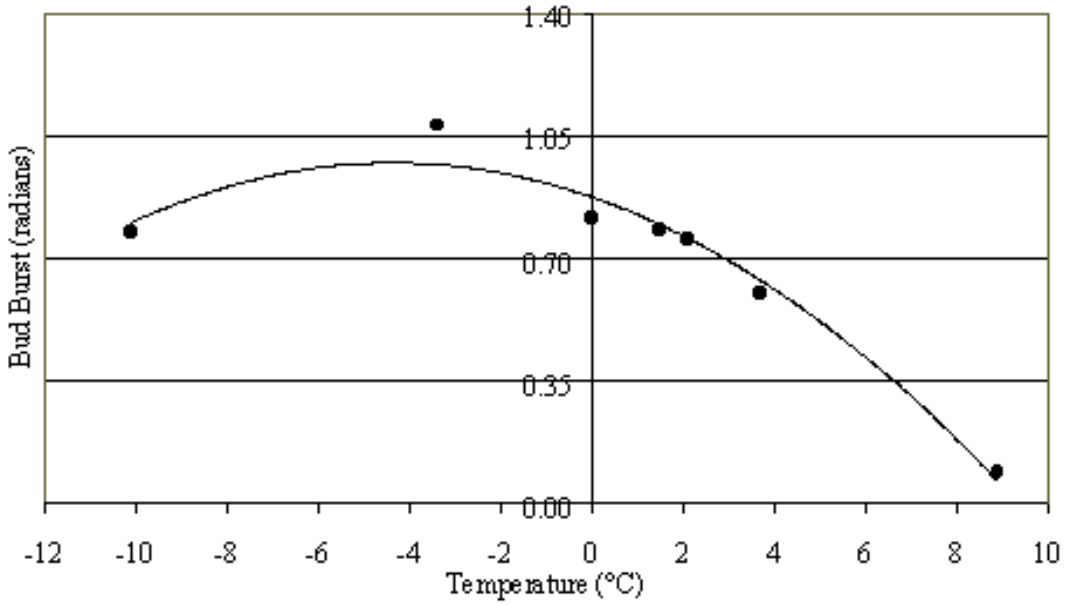


Figure 4.23. ***R. nigrum*** **'Ben Tirran' – relationship between chilling temperature and bud burst. Bud burst = -0.0052T^2 - 0.0451T + 0.8743, R^2 = 0.9661**

According to the relationship described in Figure 4.23, a chill unit model was constructed (Figure 4.24). One chill unit is equivalent to one hour of chilling at –4.3°C and higher or lower temperatures contribute fractionally. Temperatures below –18.1°C or above 9.4°C negate the effects of chill accumulation.

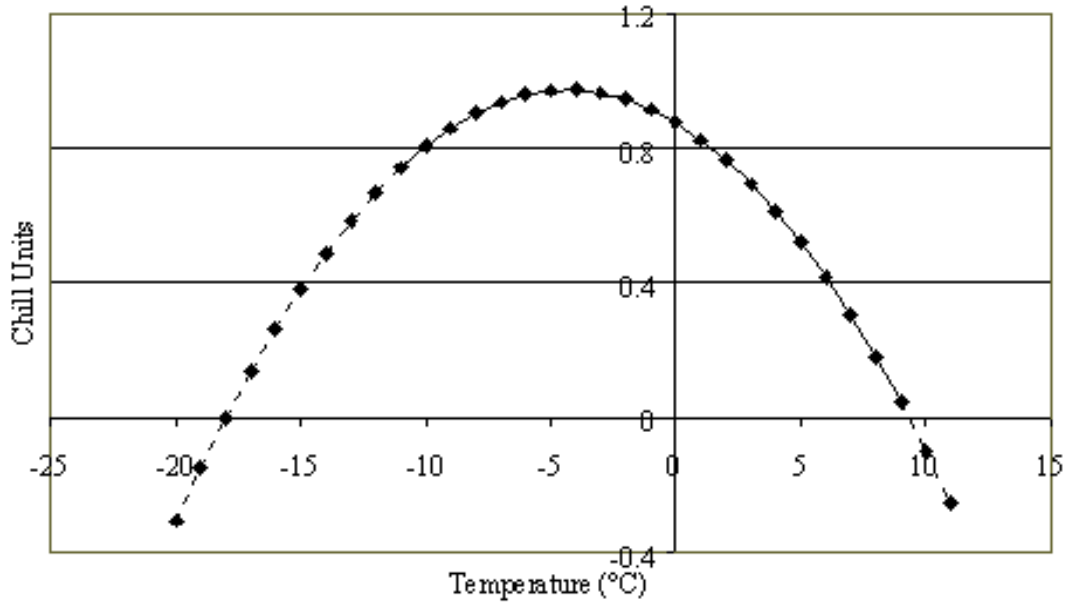


Figure 4.24. ***R. nigrum*** **'Ben Tirran' – relationship between chilling temperature and chill units. Chill units = -0.0068T^2 – 0.0583T + 0.8759**

4.3.4. Chill Unit Model Validation

'Ben Gairn'

The accuracy of the GSK/Fraser (2) compared to the original GSK/Fraser, <7°C, 0-7°C, Lantin and Utah models is shown in Figure 4.25.

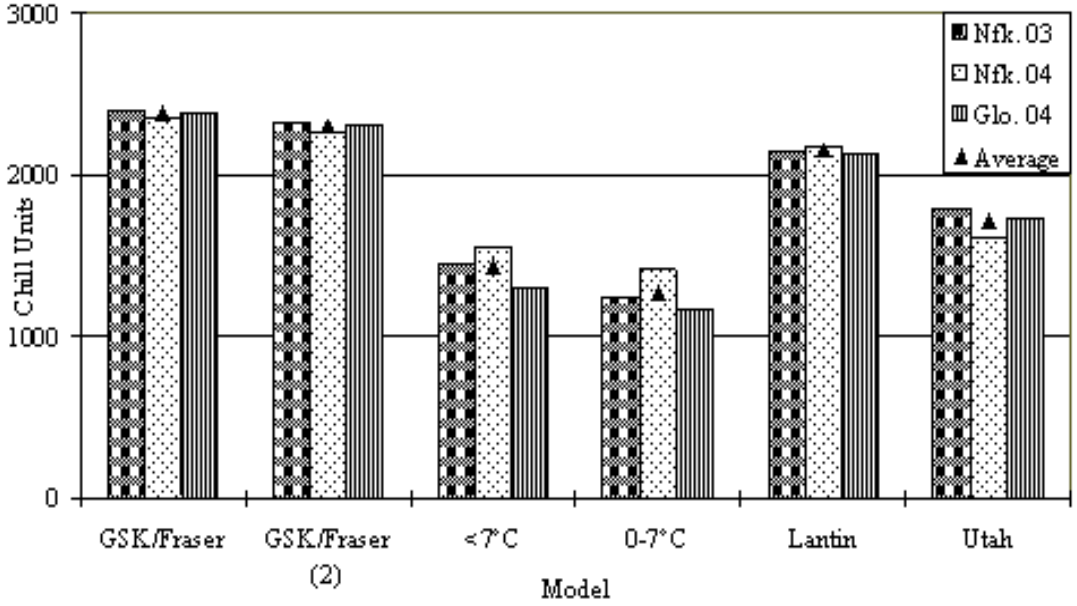


Figure 4.25. ***Ribes nigrum*** **'Ben Gairn' – comparison of chill unit model predictions**

When compared to a three year average, the GSK/Fraser (2) predicted chilling satisfaction within 22, 34 and 14 hours. This was comparable to the accuracy of the original GSK/Fraser model and the Lantin model. As discussed previously, the <7ºC, 0-7ºC and Utah models did not accurately predict the date of chilling satisfaction. For 'Ben Gairn', therefore, GSK/Fraser, GSK/Fraser (2) and the Lantin models were most accurate.

'Ben Hope'

Compared to the models tested, the GSK/Fraser (2) model was the least accurate, with wide variations between years and geographical locations (Figure 4.26).

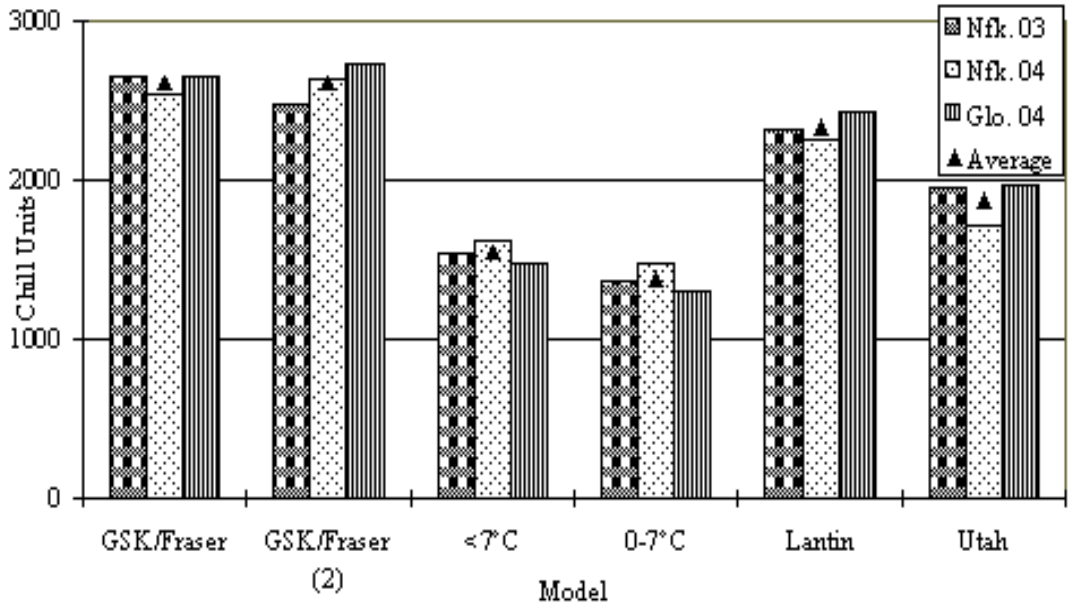


Figure 4.26. ***Ribes nigrum*** **'Ben Hope' – comparison of chill unit model predictions**

The <7ºC, 0-7ºC, Lantin and Utah models failed to accurately predict the date of chilling satisfaction. The original GSK/Fraser chill unit model, therefore was found to be the most accurate.

'Ben Tirran'

Of the models tested, the GSK/Fraser (2) model gave the lowest predictions and only accurately predicted chill satisfaction in two years (Figure 4.27). The original GSK/Fraser model and the <7°C model remained the most accurate.

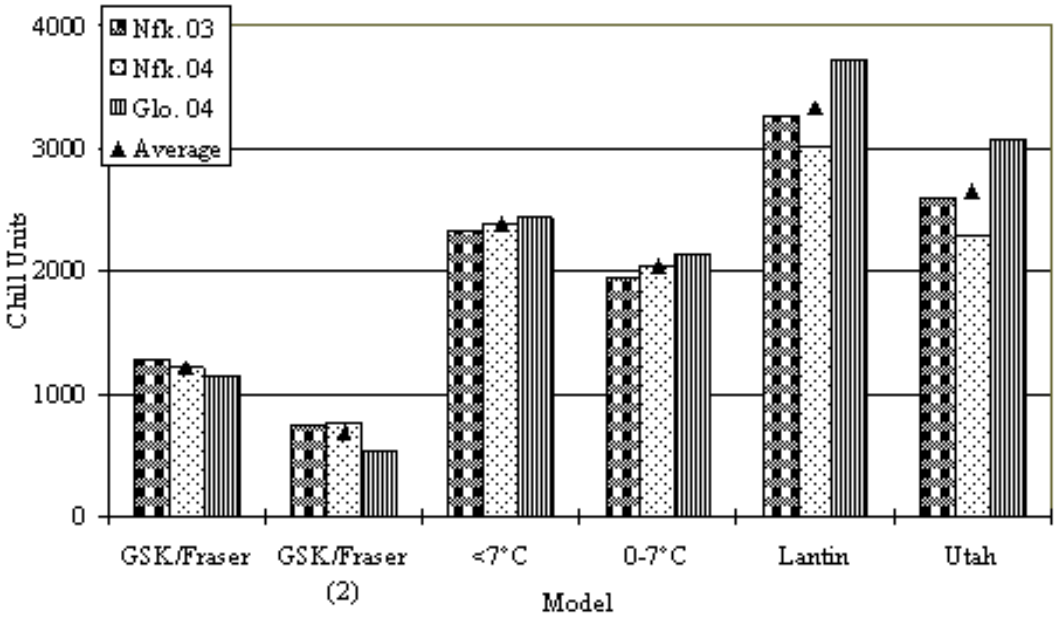


Figure 4.27. ***Ribes nigrum*** **'Ben Tirran' – comparison of chill unit model predictions**

4.4. Discussion

Initially, chill unit models were constructed for *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' based on a temperature range of -3.4°C to 8.9°C. It became apparent, however, that temperatures out-with this range may be as effective, if not more so, at satisfying the chilling requirement. The experiment was modified in the subsequent year, using plant material obtained from the same source which had been maintained under as near identical conditions as possible to the original plants. In addition, a sub-group of plants was placed in the 1.5°C cold store used in the original experiment, to determine differences between the years. Unfortunately the cold store maintained a slightly higher running temperature of 2.1°C, making direct comparisons between the two sets of data less accurate. Incorporation of the second year's data into the original GSK/Fraser model dramatically changed the model. Both models were validated using field data encompassing different geographical regions and different years.

4.4.1. Temperature Response

The 'typical' shape of the chill unit response to temperature is bell-shaped, with the effectiveness of temperature increasing to a maximum and decreasing thereafter (Mahmood, 1999). Using the original GSK/Fraser model, however, a more linear response was obtained for 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', suggesting that the optimum temperature was equal to or lower than -3.4°C. This is considerably lower than the optimum chilling temperatures of other temperate-zone fruit crops e.g. 2°C was optimum for *Fragaria ananassa* (Tehranifar, 1997) and 3.2°C, 3.7°C and 3.2°C for *Prunus avium* 'Stella', 'Sunburst' and 'Summit' respectively (Mahmood *et al.* 2000a). When the subsequent year's data were incorporated into the original model, the shape of the chill unit models altered dramatically, and the beginnings of a 'typical' bell-shaped curve were evident for all cultivars. Using the modified GSK/Fraser (2) model, the optimum chilling temperature of 'Ben Gairn' and 'Ben Hope' increased to 1°C and 1.7°C respectively, although 'Ben Tirran's optimum temperature decreased to -4.3°C. The *Ribes nigrum* breeding programme has involved germplasm exchange with Scandanavia and New Zealand (Brennan, 1996). These countries have harsher winters than the UK, and it stands to reason that *R. nigrum* cultivars from these countries can withstand and even thrive in low to sub-zero temperatures. Additionally, one of the primary *R. nigrum* breeding objectives in the early 1990's was to increase the chilling requirement and delay vegetative bud burst to prevent the extensive frost damage suffered by early-bursting cultivars (Brennan, 1996). It is not surprising, therefore, that the optimum chilling temperatures of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' are lower than other crops.

4.4.2. The Role of Temperature in Over-coming Endodormancy

Plants enter an endodormant state as a defence mechanism to ensure survival during cold winters. During release from dormancy, water is transported into the bud and a reversal in respiratory mechanism from an energy-consuming to an energy-producing system is observed (Faust and Wang, 1993). Production of DNA, RNA and proteins increases and the bud starch concentration decreases as a result of conversion to sugars for energy (Faust and Wang, 1993; Lang, 1989). Despite the extensive research conducted on this topic, the mechanisms behind dormancy induction and release are still relatively unknown. The role of hormones is a hotly disputed subject,

with initial workers suggesting a definite hormonal control, others disputing this (Mielke and Dennis, 1975; Powell, 1987; Masai *et al*., 1993). Dormancy release has been associated with decreased levels of ABA and increased levels of gibberellins, cytokinins and indole acetic acid (IAA) (Edwards, 1985; Olsen *et al*., 1997; Bondock *et al*., 1995; Piola *et al*., 1998; Masai *et al*., 1993). Exogenous application of indole acetic acid (IAA), gibberellic acid (GA_3), zeatin (Edwards, 1985; Nadel *et al*., 1991; Taylor *et al*., 1995; Gu and Read, 2004) has resulted in release of dormancy, and ABA application has delayed bud burst (Taylor *et al*., 1995; Baldwin *et al*., 2000). The fate of applied hormones, however, is unknown, and any correlation between hormone application and bud bust must be made with caution.

Alternatively, bud burst may be regulated by genetic control and hormone concentration may be a result of, rather the cause of, bud burst. The concentration of KNAP2 was reported to reach a maximum prior to bud burst and then decrease rapidly, followed by an increase in GA (Brunel *et al*., 2002; Horvath *et al*., 2003). Gibberellic acid concentration was found to be negatively correlated with the concentration of KNAP2 (Horvath *et al*., 2003).

4.4.3. Differences due to Cultivar

Using the GSK/Fraser models, ‘Ben Gairn’ and ‘Ben Hope’ responded to chilling in a similar manner – the optimum temperature for both cultivars was -3.4°C using the original model and 1°C and 1.7°C respectively using GSK/Fraser (2). The effectiveness of temperature was also similar with 8.9°C contributing 0.86 and 0.90 chill units for ‘Ben Gairn’ and ‘Ben Hope’ respectively. This is perhaps not surprising, as ‘Ben Hope’ bursts bud circa. seven days after ‘Ben Gairn’ (Atwood, 2004). The temperature response of *Prunus avium* ‘Stella’, ‘Sunburst’ and ‘Summit’ was also found to be near-identical (Mahmood, 1999). Despite requiring 50% less chill units, the date of chilling satisfaction of ‘Ben Tirran’ is circa. six weeks later than ‘Ben Gairn’ and ‘Ben Hope’. The temperature response of ‘Ben Tirran’, however, was more pronounced than the other cultivars and exposure to 8.9°C contributed only 0.09 chill units. If the GSK/Fraser and GSK/Fraser (2) models are extended to predict the effects of higher temperatures, temperatures greater than 10.4°C and 9.4°C respectively may negate chilling. Previous authors have reported

vast differences in the chilling response of cultivars within a species (Weinberger, 1956; Lantin, 1973; Plancher, 1983b).

4.4.4. Comparison of Models

GSK/Fraser and GSK/Fraser (2) were the only models constructed using individual cultivar data and the other tested models (<7ºC, 0-7ºC, Utah and Lantin) assumed that all cultivars behaved in a similar manner in response to temperature. As discussed, although the temperature responses of 'Ben Gairn' and 'Ben Hope' were similar, 'Ben Tirran' behaved differently, and this calls into question the validity of using one model to represent all cultivars.

The <7°C did not accurately predict the date of chilling satisfaction of 'Ben Gairn' or 'Ben Hope', but was one of the most accurate for 'Ben Tirran', whereas the 0-7°C model failed to accurately predict the date of chilling satisfaction of any of the cultivars. The lack of correlation between the count of hours <7°C and chilling satisfaction of 'Ben Gairn' and 'Ben Hope' as reported in this experiment was unexpected. According to the GSK/Fraser and GSK/Fraser (2) models, chilling at 8.9°C was nearly as effective as chilling at -3.4°C, and the <7°C assumed that temperatures below 7°C contributed to chilling equally. A closer correlation between these cultivars and the <7°C was therefore predicted. Contrastingly, higher temperatures were predicted to contribute significantly less to 'Ben Tirran', yet the <7°C was one of the most accurate models. A count of hours was found to be an unreliable indicator of chilling satisfaction of *Fragaria ananassa* (Tehranifar, 1997) a view supported by Richardson *et al*. (1974) who concluded that a count of hours below a base temperature was ineffective at predicting chilling satisfaction and no correlation between years was observed.

The Utah model failed to accurately predict the date of chilling satisfaction of any of the cultivars, which was not completely unexpected. The robustness of the model, in relation to its application to crop species, has been challenged previously (Shaltout and Unrath, 1983; Erez and Couvillon, 1987). As discussed in Chapter 3, the response of *Ribes nigrum* to chilling temperature and duration was cultivar-

dependant. It is unreasonable, therefore, to expect a model developed for *Prunus persica* to accurately predict the date of chilling satisfaction of other species.

Although the Lantin model was one of the most accurate for 'Ben Gairn' it was less so for 'Ben Hope' and 'Ben Tirran'. The model was constructed over 30 years ago using historical temperature and bud burst data and was validated by the author for a number of *Ribes nigrum* cultivars. This model is based on the theory that that as the chilling temperature decreases, the effectiveness at overcoming dormancy increases. This is supported by the GSK/Fraser model, and in theory extending this model out-with the experimental temperature range would further support this theory. However, the validity of utilising historical data, as opposed to experimental data, to predict the chilling response is questionable.

4.4.5. Differences Between GSK/Fraser and GSK/Fraser (2)

Given the unexpected beneficial effect of -3.4°C on bud burst, the experiment was extended the following year to include a -10.1°C treatment. Although every attempt was made to use identical plant material and to maintain the plants in near-identical environments, differences between similar treatments over the two years were apparent. In particular, although the original model suggested temperatures lower than -3.4°C were effective at overcoming dormancy, the subsequent experiment did not uphold this for 'Ben Gairn' and 'Ben Hope'.

Based on field data, the average number of chill units required for satisfaction of 'Ben Gairn's' chilling requirement was predicted to be 2304 and 2384 using GSK/Fraser and GSK/Fraser (2) respectively, and both models accurately predicted the date of chilling satisfaction. Although there was no difference in the average number of chill units required for 'Ben Hope' using GSK/Fraser (2604) and GSK/Fraser (2) (2603), the latter model failed to accurately predict the date of chilling satisfaction over the data set, whereas the GSK/Fraser model was one of the most accurate, suggesting that this model may be the more robust and commercially applicable of the two models. The average number of chill units required for satisfaction of 'Ben Tirran's chilling requirement was 1211 and 683 using GSK/Fraser and GSK/Fraser (2) respectively. Again, the original GSK/Fraser model was one of the most accurate for this cultivar,

and although GSK/Fraser (2) was accurate using 2 years data, it was not robust enough to predict chill satisfaction over the three years. These discrepancies question the validity of using data from two consecutive years.

There were several key differences between the two models, as discussed, and this may be due to a number of factors. Pre-treatment conditions have been reported to affect the chilling response (Plancher, 1984; Erez and Couvillon, 1987). More importantly. however, the plants utilised in Experiment 3 suffered an outbreak of *Puccinia*, and although this was successfully controled by chemical application, the infection may have affected the chilling response of the plants. The results of this experiment, and therefore the reliability of the GSK/Fraser (2) models, may have been compromised by the *Puccinia* infection and should be interpreted with caution. The hypothesis that Experiment 3's data is unreliable is further supported by the reduced accuracy of the GSK/Fraser (2) models for 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' compared to that of the original GSK/Fraser models. Based on this evidence, the results of Experiment 3 were deemed to be unreliable, and therefore the original GSK/Fraser model should be used preferentially.

The accuracy of the GSK/Fraser models were comparable with previous research, with the chilling satisfaction of 'Ben Gairn' being predicted within 24 hours (±1 day), 'Ben Hope' within 74 hours (±3 days) and 'Ben Tirran' within 72 hours (±3 days). In comparison, the chilling requirement of *Prunus persica* 'Redhaven' was predicted within 2-8 days (Richardson *et al*., 1974); *Alnus* and *Betula* were predicted within 2.5 days (Andersen, 1992), *Prunus cerasus* 'Montmorency' ±4 days (Anderson *et al*., 1986), *Picea sitchensis*, ±2.6 days (Cannell and Smith, 1983) and *Cornus sericea* ±2 days (Kobayashi *et al*., 1982).

4.4.6. Date of Chill Accumulation

When validating the GSK/Fraser and GSK/Fraser (2) models, chill unit accumulation was recorded from 1 October. The date of chilling accumulation is a contentious subject, with no clear agreement between researchers. In terms of practical application, a calendar date on which chill accumulation recordings begin is preferential and several researchers have taken this view point. Starting dates ranging

from 1 September (Anderson *et al*., 1986) to 1 November (Tehranifar, 1997) and 10 November (de la Rosa and Rallo, 2000) have been suggested in the Northern Hemisphere, and 1 February (Tisne-Agostini *et al*., 1992) to 1 April in the Southern Hemisphere (Ebert *et al*., 1986).

It was suggested that chill accumulation began when the first chill unit was recorded (Richardson *et al*., 1974), however the maximum temperature at which *R. nigrum* chilling accumulated was not established in this experiment and Tehranifar (1997) found no correlation between this date and chill accumulation of *Fragaria ananassa*. Previous authors have extended chill unit models out-with the experimental range to determine this temperature, however this method is purely speculative and not scientific. Nevertheless, this method was applied to GSK/Fraser and according to Richardson *et al*. (1974), chill unit accumulation of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' should begin when the ambient temperature falls below 20.8°C, 23.7°C and 9.4°C respectively. Presumably this would occur for 'Ben Gairn' and 'Ben Hope' before 1 October, hence further experimentation is required to clarify this.

4.4.7. Commercial Application

To be commercially acceptable, a chill unit model must be relatively simple to use and must also accurately calculate chilling hours over a range of geographical locations and years. Although limited data was available, every attempt was made to validate several chill unit models using these criteria. Prior to 2002, a count of hours between 0-7°C was used by a limited number of growers to quantify the chilling requirement of *Ribes nigrum*. Based partly on the results of the GSK/Fraser model, this was revised and current commercial practice is to count the number of hours whereby the temperature falls below 7°C. Although the Lantin model was constructed for *R. nigrum*, it is questionable whether the growers are aware of the existence of this model, at least one grower contacted had not heard of it. The original model was published in French, and is reasonably dated. The correlation between French *R. nigrum* cultivars may not have been directly apparent, or, more than likely, the results were lost in translation. Had the model been known however, its application would most likely have been limited due its complex nature, whereby mathematical equations are utilised to allocate the effectiveness of a given

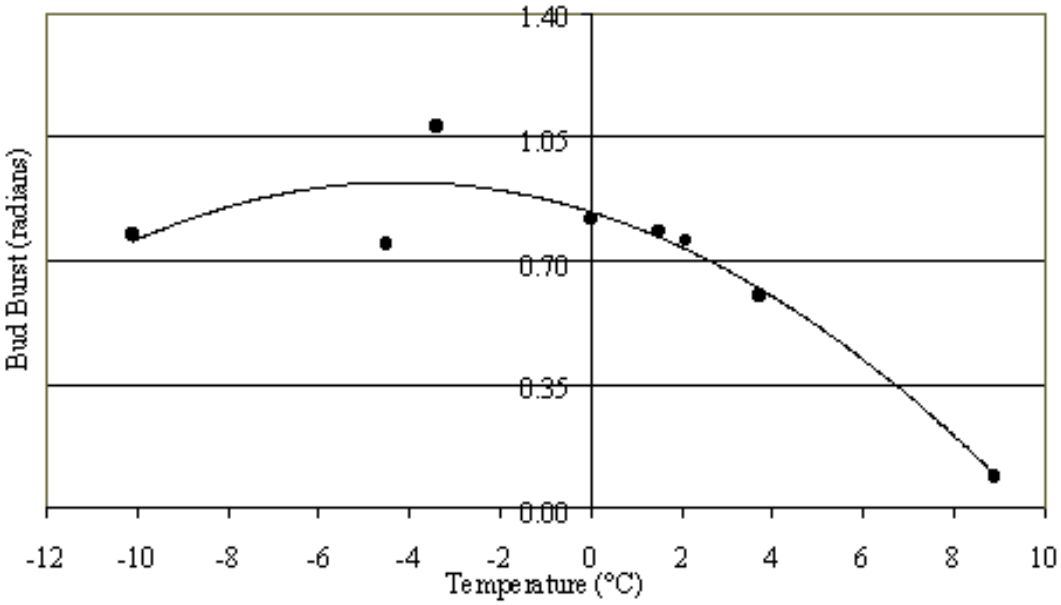
temperature. It took several hours and a complicated series of Excel equations to validate the Lantin model (as described previously). The difficulty of use, therefore, would be particularly off-putting to growers. The Utah model, again unknown to at least one commercial grower, was constructed using *Prunus persica*, so the direct application to *R. nigrum* may have not been apparent, but again validating the model with field data required a significant proportion of time and a thorough knowledge of Excel spreadsheets.

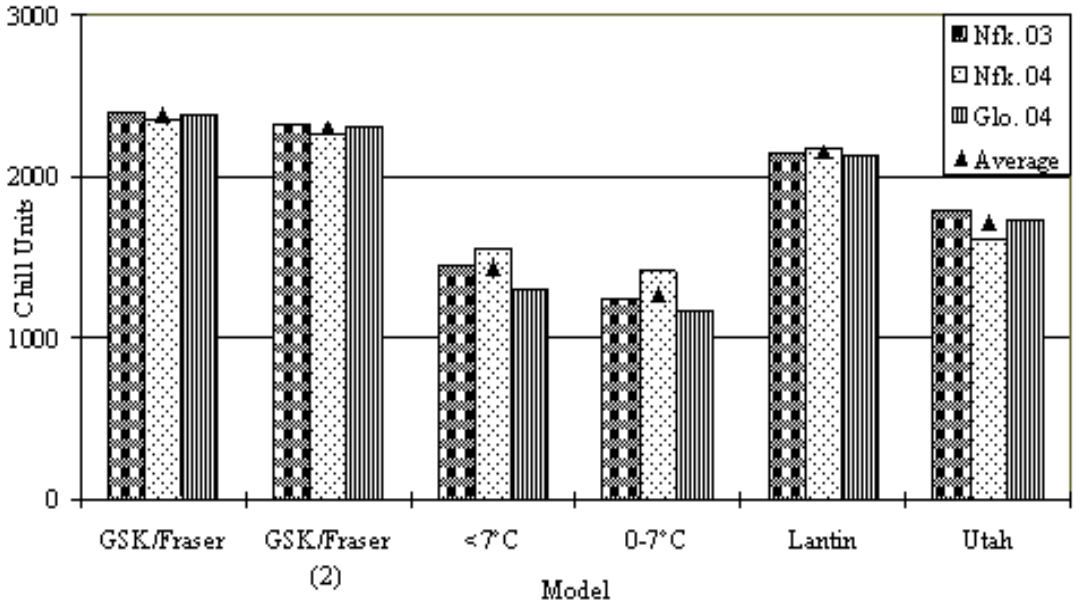
To date, few studies have compared the different chill units examined in this chapter, and no correlation between chilling satisfaction across geographical areas or time been established for *R. nigrum* cultivars. This was reported to be one of the main reasons why growers did not collect temperature and chilling satisfaction data (Keene, E. *Pers. comm.*). To be practical, therefore, a model must first be validated using field data and ideally should require minimum input from a grower. In an attempt to simplify the GSK/Fraser model, software was constructed (Harwood and Fraser, 2005) to allow the temperature data to be directly imported into an Excel spreadsheet from the TinyTalk data loggers and the chill units calculated automatically. This software will be available for all GSK growers.

Conclusions

Chill unit models were constructed, validated using field data and tested against existing models. Although data from Experiment 1 suggested temperatures below -3.4°C were as or more effective for satisfying the chilling requirement, this was not upheld in a subsequent experiment (Experiment 3). The results of Experiment 3, however, may have been compromised by an outbreak of *Puccinia*. The accuracy of the original GSK/Fraser chill unit models were reduced when the suspect data were included, which further supports the theory of the data being unreliable. Based on this evidence, the results of Experiment 3 should be disregarded in favour of Experiment 1. The optimum temperature for satisfying chilling and overcoming endodormancy of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' is -3.4ºC.

When validated against temperature and bud burst data collected for two consecutive years over two geographical regions, the original GSK/Fraser models were found to be as or most accurate than the <7ºC, 0-7ºC, Lantin and Utah models. The GSK/Fraser and Lantin models were most accurate for predicting the date of chilling satisfaction of 'Ben Gairn', the GSK/Fraser model was most accurate for 'Ben Hope' and the GSK/Fraser and <7ºC for 'Ben Tirran'. The availability of software, to further increase the grower friendliness of the GKS/Fraser models, should allow growers to monitor chilling of their cultivars. Further commercial feedback regarding the accuracy and ease of use of the models is required.





Chapter Five.
Climate Change Scenarios and Implications for the UK *Ribes nigrum* Industry

1.1. Introduction

Despite being a highly contentious area of research, there is wide-spread evidence that the planet's climate is changing. Between 1850 and 1950, the average global temperature increased by less than 0.5°C (Hulme *et al*., 2002). Thereafter the rate of increase doubled, and a further 0.5°C temperature rise was observed by 2000. Over the past 100 years oceanic temperature has risen by 1-2°C and in the UK the amphibian breeding period and bird migration season has advanced (Walther *et al*., 2002). Since 1960, the date of *Olea europaea* anthesis has advanced (Osborne *et al*., 2000), crop growing seasons have extended (Norby *et al*, 2003) and *Ribes nigrum* growers have reported earlier bud burst (Atkinson *et al*., 2004). All these examples have been attributed to increasing global temperature and climate change.

5.1.1. Predicted Climate Change Scenarios

Four climate change scenarios, Low, Medium-Low, Medium-High and High have been predicted, based on levels of carbon dioxide emissions (Hulme *et al*., 2002). By 2080, the average UK temperature is predicted to increase by 2°C – 4.5°C, which is two to three times faster than the current rate of increase (Bisgrove and Hadley, 2002). Under the Medium-High scenario, the number of hours that the UK winter temperature falls between 0ºC and 7ºC was predicted to decrease from 2500 hours to 2300 hours (-8%) by 2020; a further decline to 1700 hours (-32%) was predicted by 2080 (Hough, 2002). Several researchers predicted a shift in current crop production patterns as a result of increasing temperature and movement in a northerly direction would be evident (Walther *et al*., 2002). Production/distribution of *Picea abies*, *Pinus sylvestris*, *Alnus incana* (Sykes and Prentice, 1995), *Abies* spp. (Aussenac, 2002), *Malus domestica* and *Citrus unshiu* (Sugiura and Yakozawa, 2004) have been forecast to move Northwards.

5.1.2. Modeling Effects of Climate Change

When modeled, the effect of climate change was suggested to be directly related to the chilling received by a plant (Cannell and Smith, 1986). Exposure to warmer springs after fulfillment of the chilling requirement was associated with an advancement of bud burst. When the majority of the chilling requirement had been satisfied, no effect on bud burst was

observed, whereas large chilling deficits resulted in delayed bud burst. After exposure to warmer temperatures, chilling deficits of *Pyrus Communis* and *Malus domestica* 'Golden Delicious', 'Cox', 'Granny Smith' and 'Strumer' were partially compensated for by warmer spring temperatures (Atkins and Morgan, 1990).

In Scotland, *Ribes nigrum* currently receive sufficient chilling and the predicted temperature increase will continue to satisfy the crop's requirement (Atkinson *et al*., 2004). The potential for bushes to grow in the spring will be increased as a result of raised spring temperatures, and beneficial effects on crop growth, particularly yield, have been predicted (Atkinson *et al*., 2004). In England, however, particularly in the West Midlands and Norfolk, the crop's chilling requirement would not be fulfilled and detrimental effects on growth are expected (Atkinson *et al*., 2004). After exposure to a simulated climate change environment, a daily temperature increase of 3°C, as predicted by 2080 under the Medium-Low, Medium-High and High scenarios, resulted in the chilling requirement of *Pyrus Communis* and *Malus domestica* 'Golden Delicious', 'Cox', 'Granny Smith' and 'Strumer' being unfulfilled (Atkins and Morgan, 1990).

The primary objective of the experiment described in this chapter was to quantify the effects of elevated spring temperature, as forecast under climate change scenarios, on *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran'. In addition, the extent to which elevated spring temperatures could substitute for insufficient chilling was investigated.

5.2. Materials and Methods

5.2.1. Experiment 4. Elevated Spring Temperature

Plant material consisted of one-year old softwood cuttings of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', as described in Chapter 2. The plants were delivered to the University of Reading's Experimental Field Site on 3 June 2004, placed in double rows with 10cm between plants within a row and 80cm between rows and the stems were tied onto supporting wires to keep the pots upright. Pots were irrigated automatically with Avoncrop's Soft Fruit Mix 2 (6:11:31 N:P:K) nutrient solution four times a day for a total of one hour until the irrigation system was disconnected at the end of September.

Elevated spring temperatures were simulated by placing eight cultivars each of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' in an unheated polythene tunnel with mean temperature 3.2°C higher than outside (Figure 5.1) and in a heated polythene tunnel, mean temperature 4.3°C higher, to represent the predicted spring temperature rise under the High scenario in 2080. Control plants remained outside, experiencing natural chilling temperatures.

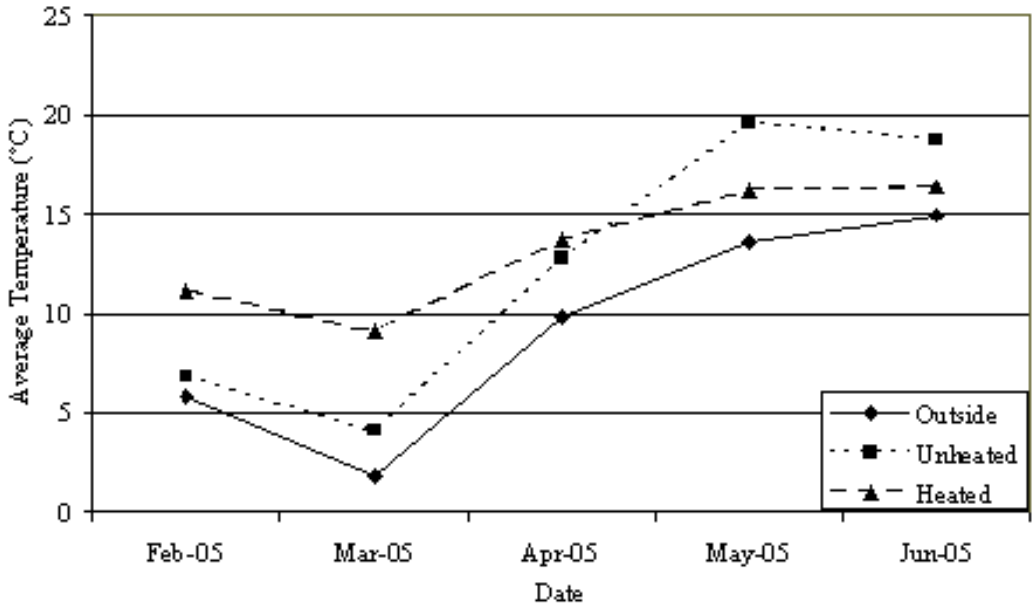


Figure 5.1. Average weekly temperatures of the simulated climate change scenarios.

When the temperature data were analysed, it was discovered that the mean temperature of the unheated polytunnel was higher than that of the heated tunnel from mid April. This was because the ventilation fan in the unheated tunnel was broken and was uneconomical to

repair. This is unlikely to have affected the bud burst behaviour of the plants as this occurred before mid April.

Plants were placed in the simulated environments on 1 February 2005, and accumulated chill units calculated using the GSK/Fraser models. By this date, 'Ben Gairn' had acquired 2616 of the required 2384 chill units (100%), 'Ben Hope' had received 2664 of 2604 units (100%) and 'Ben Tirran' had received 636 of the required 1211 units (53%). Plants were watered by hand when required. Bud burst, anthesis and fruit production were recorded at regular intervals and shoot extension was recorded at the termination of the experiment on 21 June 2005 (see Chapter 2 for a detailed description).

5.2.2. Experiment 5. Elevated Forcing Temperatures

Plant material consisted of one-year old softwood cuttings of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', as described in Chapter 2. The plants were delivered to the University of Reading's Experimental Field Unit on 2 July 2004, placed in double rows with 10cm between plants within a row and 80cm between rows and stems were tied onto supporting wires to keep the plants upright. Pots were automatically irrigated four times a day for a total of one hour using Avoncrop's Soft Fruit Mix 2 (6:11:31 N:P:K) until 30 September 2004.

Five plants of each cultivar were exposed to forcing temperatures of 13.7°C, 18.1°C, 19.9°C, 26.6°C and 29.70°C utilised (see table 5.1) under natural photoperiod. Field temperature was recorded via Tinytalk (-40ºC to +85ºC) data loggers from 1 October 2004 and data were regularly downloaded to monitor chill accumulation. The GSK/Fraser chill unit models were utilised to determine chill unit accumulation at regular intervals. 'Ben Gairn' were transferred into the forcing treatments on 29 November 2004 when 51% of the required chill units had accumulated (Table 5.2), and a second set of plants were transferred on 24 January 2005 when 100% of the required chill units had accumulated. Similarly, the first set of 'Ben Hope' plants

Table 5.1. Monthly temperature data for the elevated glasshouse compartments

Month/Temp	13.7ºC	18.1ºC	19.9ºC	26.6ºC	29.7ºC
January	13.1±0.2ºC	17.5±1.4ºC	18.2±1.2ºC	25.4±1.6ºC	26.3±3.0ºC
February	13.0±0.3ºC	17.7±1.2ºC	18.3±1.3ºC	25.7±0.8ºC	28.4±1.6ºC
March	13.3±0.4ºC	17.9±2.3ºC	19.3±2.5ºC	26.6±1.2ºC	29.8±1.8ºC
April	13.4±0.5ºC	18.0±1.7ºC	20.0±1.2ºC	26.7±0.9ºC	30.5±1.9ºC
May	14.9±1.2ºC	18.7±5.1ºC	22.4±4.1ºC	27.8±2.6ºC	31.8±1.4ºC
June	14.5±2.1ºC	18.7±1.3ºC	21.5±3.0ºC	27.6±1.8ºC	31.5±1.3ºC

were exposed to the elevated temperature regimes on 8 December 2004, when 55.4% of the required chill units had accumulated (Table 5.2), and a second set on 3 February 2005 when 100% of the required chill units had accumulated. Bud burst, flowering and fruiting were recorded at regular intervals and shoot extension was recorded at the termination of the experiment on 21 July 2005 (see Chapter 2 for a more detailed description).

Table 5.2. Chill accumulation on date of transfer into elevated temperatures

Cultivar	Date of Transfer	Accumulated Chill Units	Required Chill Units	Chill Requirement Satisfied
'Ben Gairn'	29 Nov. 2004	1218	2384	51%
'Ben Gairn'	24 Jan. 2005	2442	2384	100%
'Ben Hope'	8 Dec. 2004	1443	2604	55%
'Ben Hope'	3 Feb. 2005	2702	2604	100%

5.3. Results

5.3.1. Experiment 4. Simulated Climate Change Scenarios

The vegetative response to simulated climate change scenarios can be seen in Plates 5.1-5.3.

Plate 5.1. ***R. nigrum*** **'Ben Gairn' – effect of spring temperature**
L-R: natural chilling, +3.2ºC, +4.3ºC

Plate 5.2. ***R. nigrum*** **'Ben Hope' – effect of spring temperature**
L-R: natural chilling, +3.2ºC, +4.3ºC

Plate 5.3. *R. nigrum* 'Ben Tirran' – effect of spring temperature
L-R: natural chilling, +3.2ºC, +4.3ºC

Bud Burst

Time to first bud burst was highly dependant on cultivar, spring temperature and the interaction between these factors ($P<0.001$) so data were sub-divided and re-analysed. Compared to control plants exposed to natural chilling, bud burst was significantly advanced by 15 and 33 days ('Ben Gairn') and by 10 and 28 days ('Ben Hope') as the spring temperature increased by 3.2ºC and 4.3ºC respectively (Figure 5.2). Contrastingly, no effect of increasing spring temperature was observed for 'Ben Tirran'.

When final bud burst was analysed, there was no significant effect of cultivar ($P=0.1$) and no interaction between cultivar and spring temperature ($P=0.134$). A negative correlation ($P=0.001$) between final bud burst and elevated spring temperature was observed, although there was no difference between plants forced at +3.2ºC and +4.3ºC (Figure 5.3).

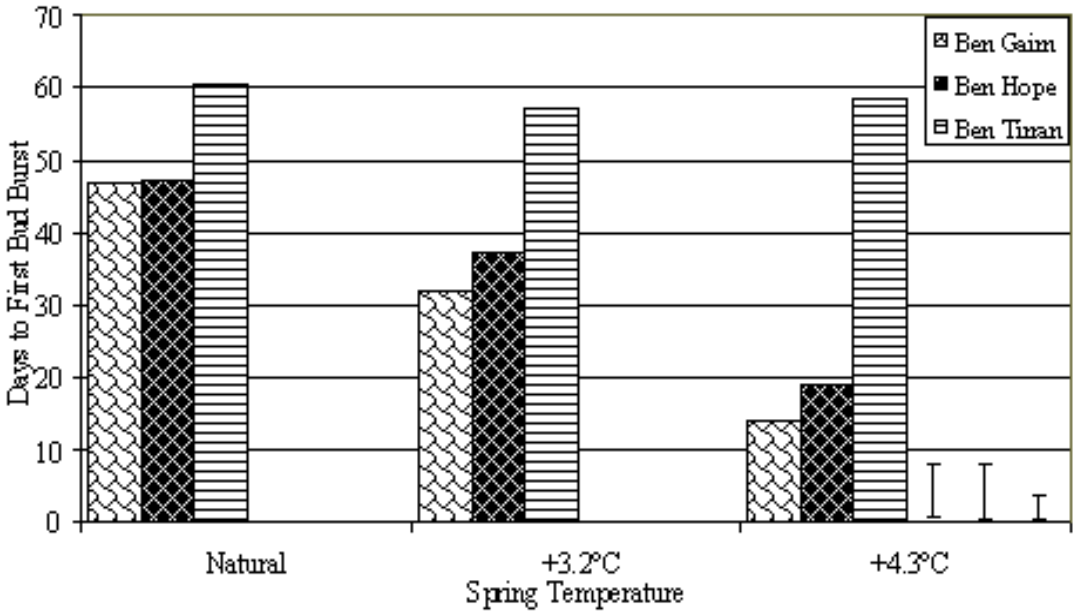


Figure 5.2. ***R. nigrum*** **– the effect of elevated spring temperature on time to first bud burst. First error bar represents 'Ben Gairn', second represents 'Ben Hope', third represents 'Ben Tirran', L.S.D (P<0.05), d.f. = 14.**

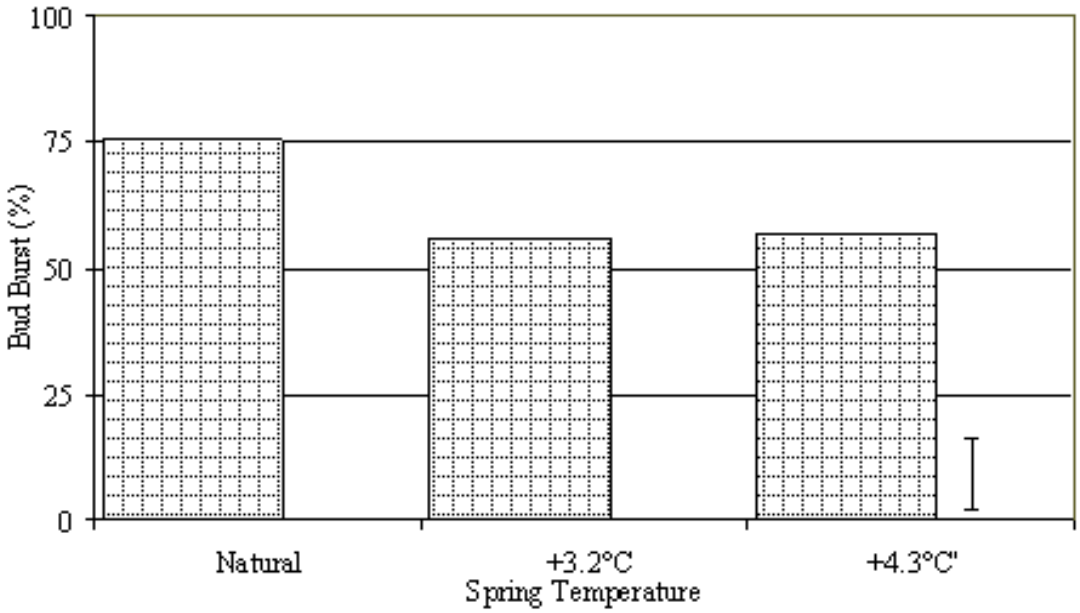


Figure 5.3. ***R. nigrum*** **– effect of elevated spring temperature on final percentage bud burst. Data pooled over all cultivars. Error bar represents L.S.D. (P<0.05), d.f. = 48.**

Final bud burst at different stem positions was highly dependant (P<0.001) on the interaction between cultivar and spring temperature, hence data were sub-divided and re-analysed. 'Ben Gairn' was unaffected by bud position (P=0.853), spring temperature (P=0.132) and the interaction (P=0.254). In general, bud burst of 'Ben Hope's' Terminal and Top buds was negatively correlated with increasing spring temperature, although this was not significant

(Figure 5.4.). Bud burst was lower in the middle and bottom stem sections after exposure to +3.2ºC, and although this effect was insignificant, further increasing the temperature to +4.3ºC significantly reduced bud burst in both sections.

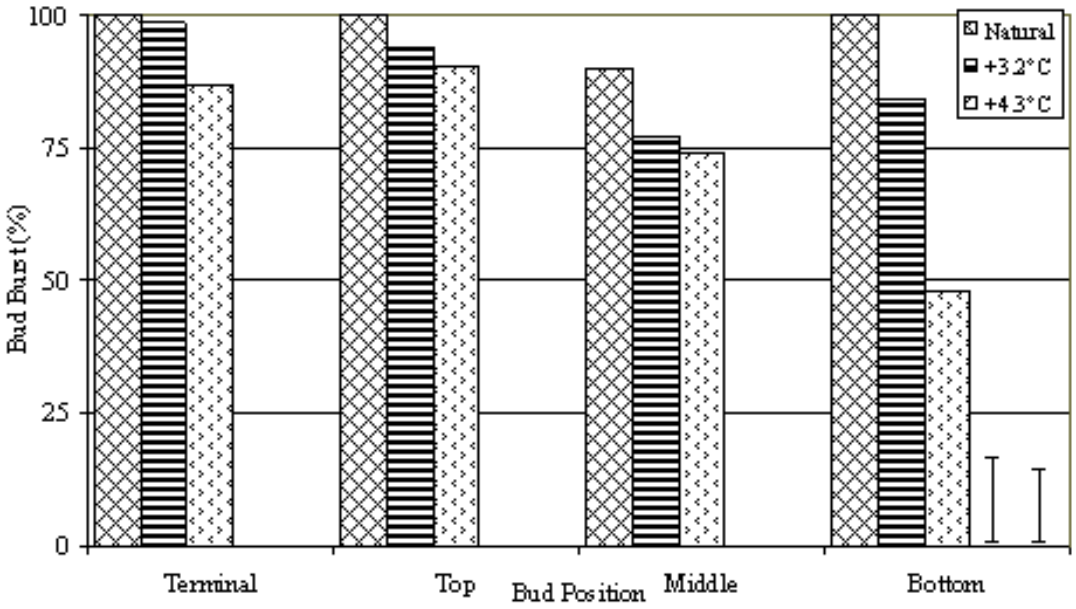


Figure 5.4. ***R. nigrum*** **'Ben Hope' – relationship between spring temperature and bud position. First error bar represents bud position, second represents spring temperature, L.S.D. (P<0.05), d.f. = 12.**

The effects of bud position, spring temperature and the interactions were highly significant (P<0.001) for 'Ben Tirran', hence data were sub-divided and re-analysed. There was no effect of spring temperature on Terminal or Middle bud burst (P=0.357), but as spring temperature increased, Top and Bottom bud burst decreased (Figure 5.5.).

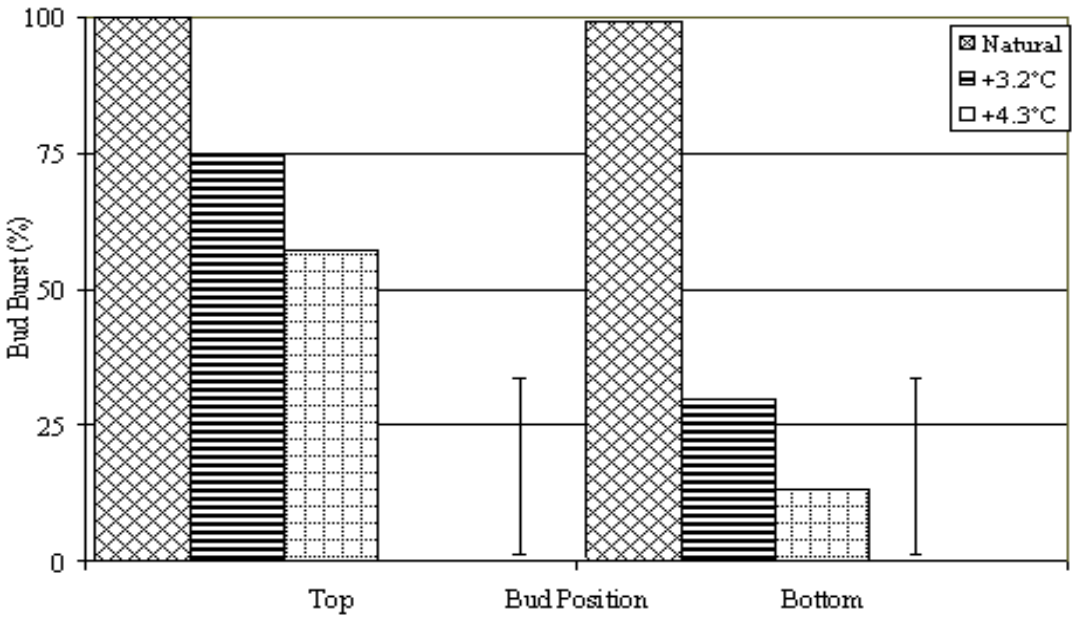


Figure 5.5. ***R. nigrum*** **'Ben Tirran' – relationship between spring temperature and bud position. Error bar represents L.S.D. (P<0.05), d.f. = 12**

In general, shoot extension was more vigorous for 'Ben Tirran' (23.7cm) compared to 'Ben Gairn' (14.9cm) and 'Ben Hope' (19.1cm). For all cultivars, shoot extension was negatively correlated with spring temperature (Figure 5.6).

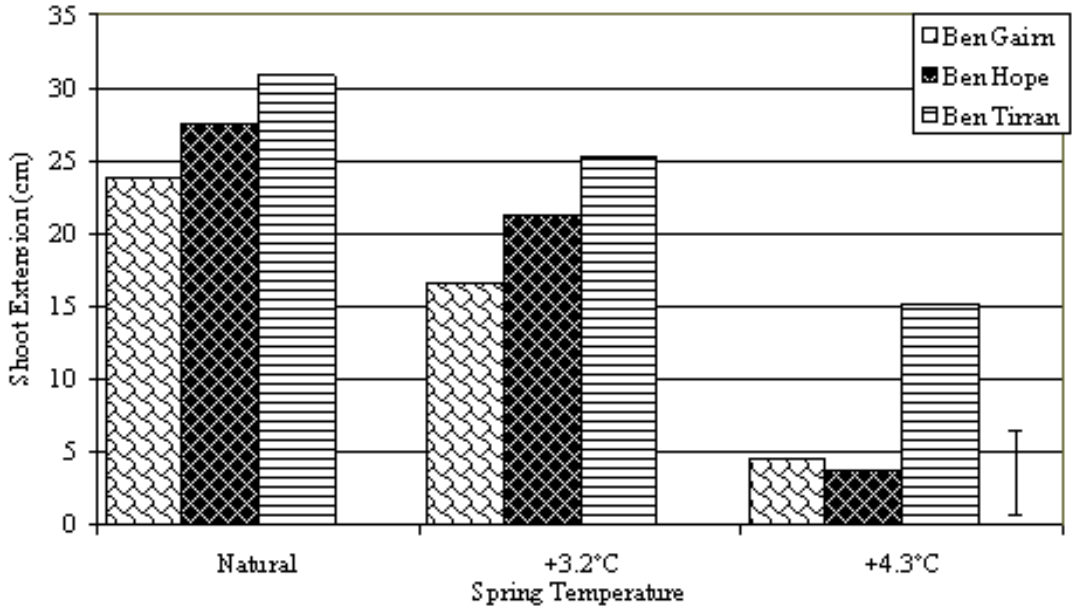


Figure 5.6. ***R. nigrum*** **– effect of elevated spring temperature on stem extension. Error bar represents L.S.D. (P<0.05), d.f. = 49.**

Anthesis

Time to anthesis was dependant on cultivar and spring temperature (P<0.001), but there was no interaction (P=0.563) between these factors. In general, 'Ben Gairn' and 'Ben Hope' flowered significantly (P<0.001) earlier than 'Ben Tirran'. For all cultivars, anthesis was advanced as the spring temperature increased to +3.2ºC but increasing the temperature to +4.3ºC had no further effect (Figure 5.7).

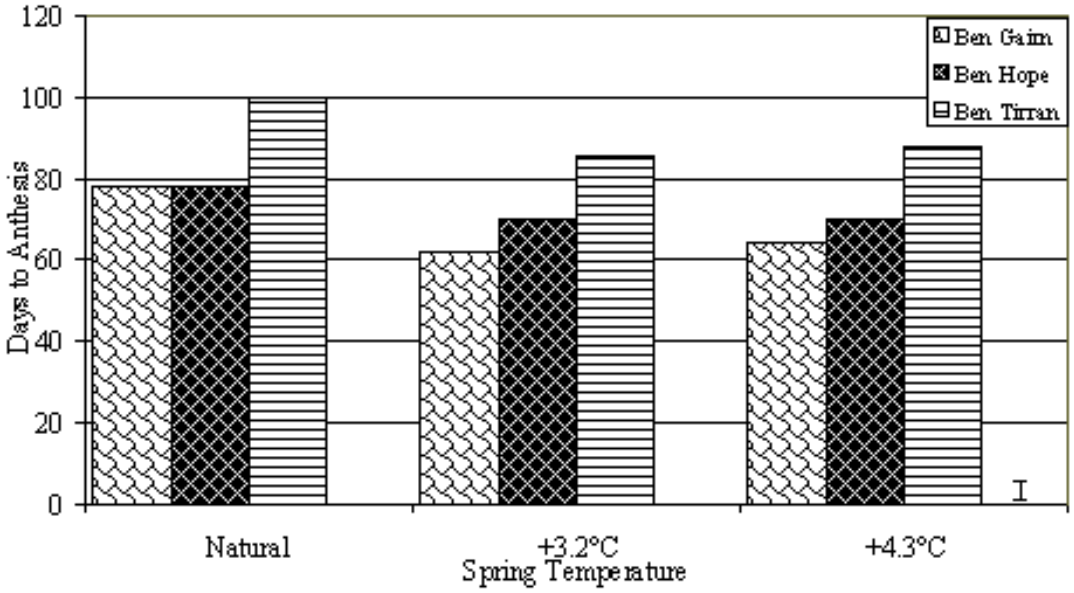


Figure 5.7. ***R. nigrum*** **- effect of elevated spring temperature on time to anthesis. Error bar represents L.S.D (P<0.05), d.f. = 40.**

The number of flowers that reached anthesis was dependant on cultivar, spring temperature and the interaction (P<0.001, P<0.001, P=0.012), hence data were sub-divided and re-analysed (Figure 5.8). Although exposure to +3.2ºC increased the number of 'Ben Gairn's' flowers that opened, this was not significant and further increasing the temperature to +4.3ºC significantly (P=0.01) reduced the number of flowers that opened. A negative correlation (P<0.001) between elevated spring temperature and the number of 'Ben Hope' flowers that reached anthesis was observed, but there was no effect of climate change scenario on 'Ben Tirran'.

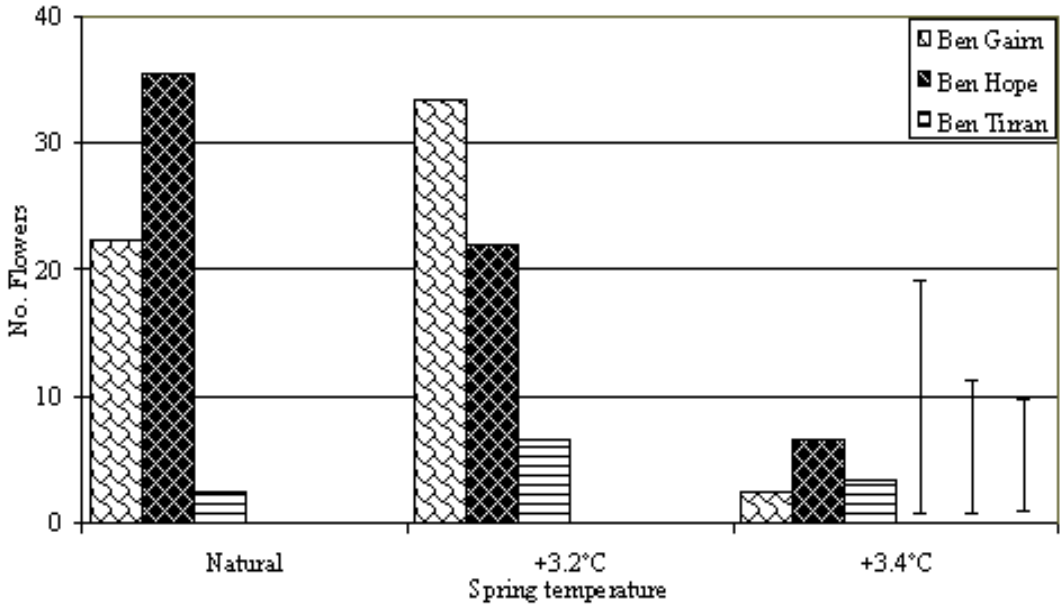


Figure 5.8. *R. nigrum* – effect of elevated spring temperature on the number of flowers that reached anthesis. First error bar represents L.S.D. (P<0.05) 'Ben Gairn', second represents 'Ben Hope', third represents 'Ben Tirran' d.f. = 12, 14 and 11 respectively.

Although time to first fruit was not dependant on spring temperature (P=0.1), the effect of cultivar and the interaction between cultivar and spring temperature was significant (P<0.001; P=0.037 respectively) hence data were sub-divided and re-analysed. A positive correlation between spring temperature and time to first fruit was evident for 'Ben Gairn' and 'Ben Hope', but no effect (P=0.061) was observed for 'Ben Tirran' (Figure 5.9).

Spring temperature was the only significant (P<0.001) factor affecting the number of fruit that formed. Although there was no difference between plants exposed to natural spring temperatures and those in +3.2°C, an increase of +4.3°C significantly reduced the number of fruit produced (Figure 5.10).

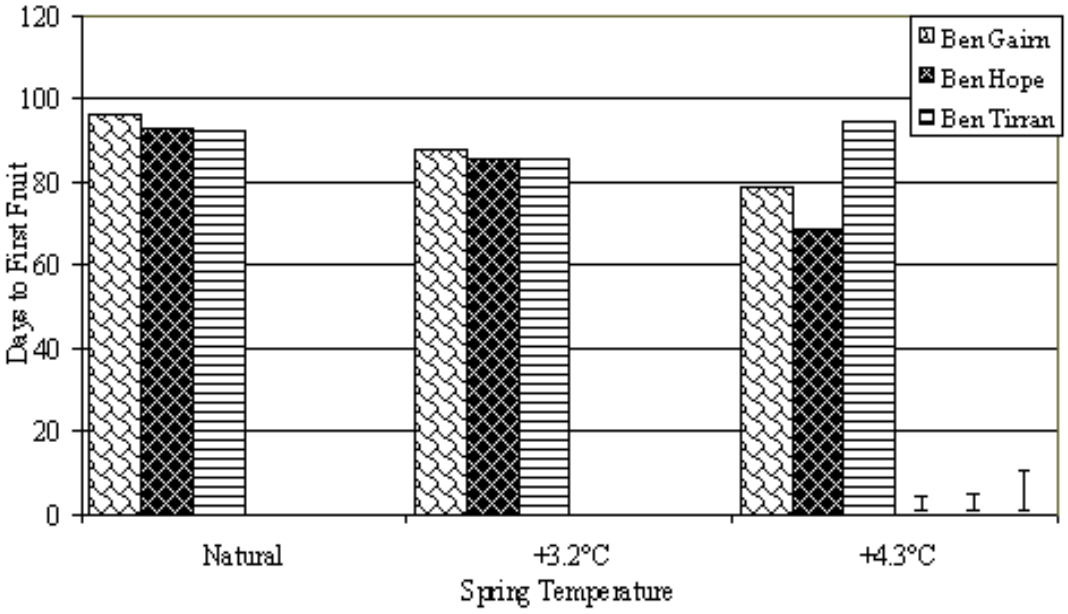


Figure 5.9. ***R. nigrum*** **- effect of elevated spring temperature on time to first fruit. First error bar represents 'Ben Gairn', second 'Ben Hope', third 'Ben Tirran', L.S.D (P<0.05), d.f. = 29, 32, 30 respectively.**

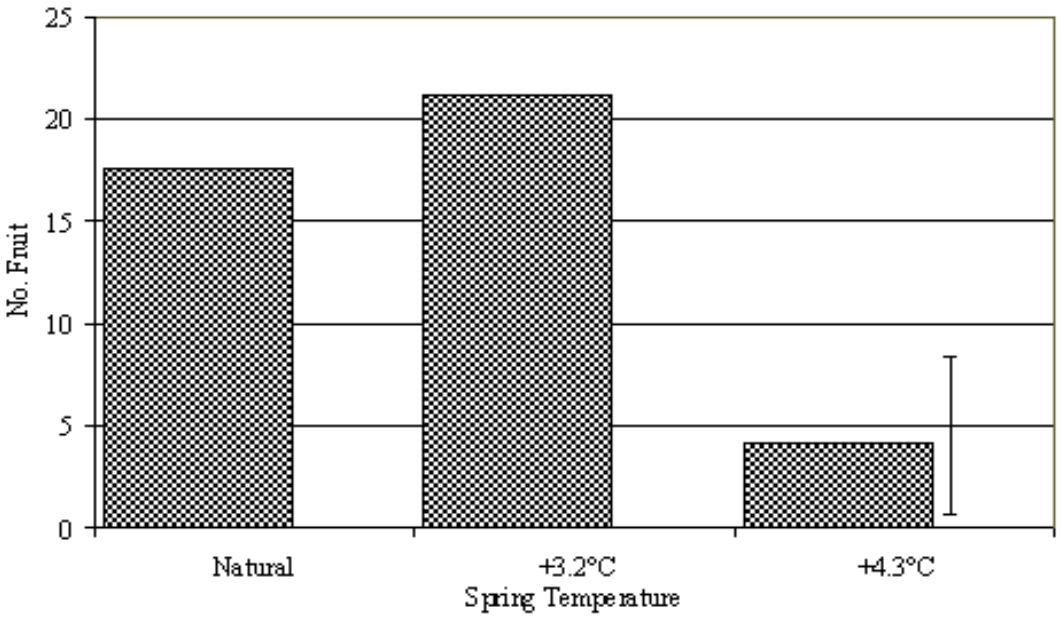


Figure 5.10. ***R. nigrum*** **– effect of elevated spring temperature on fruit production. Data pooled across cultivars. Error bar represents L.S.D. (P<0.05), d.f. = 33.**

5.3.2. Experiment 5. Elevated Forcing Temperatures

Bud Burst

In general, the greater the degree of chilling satisfaction (100% compared to 50%), the earlier (P<0.001) the bud burst – 9.9 days compared to 29.9 days. For both chill satisfaction treatments, the effect of forcing temperature was found to be highly significant (P<0.001) but cultivar (50% P=0.344, 100% P=0.822) and the interactions between cultivar and forcing temperature (50% P=0.31; 100% P=0.857) were not. For both cultivars, a positive correlation between forcing temperature and time to first bud burst was evident (Figure 5.11).

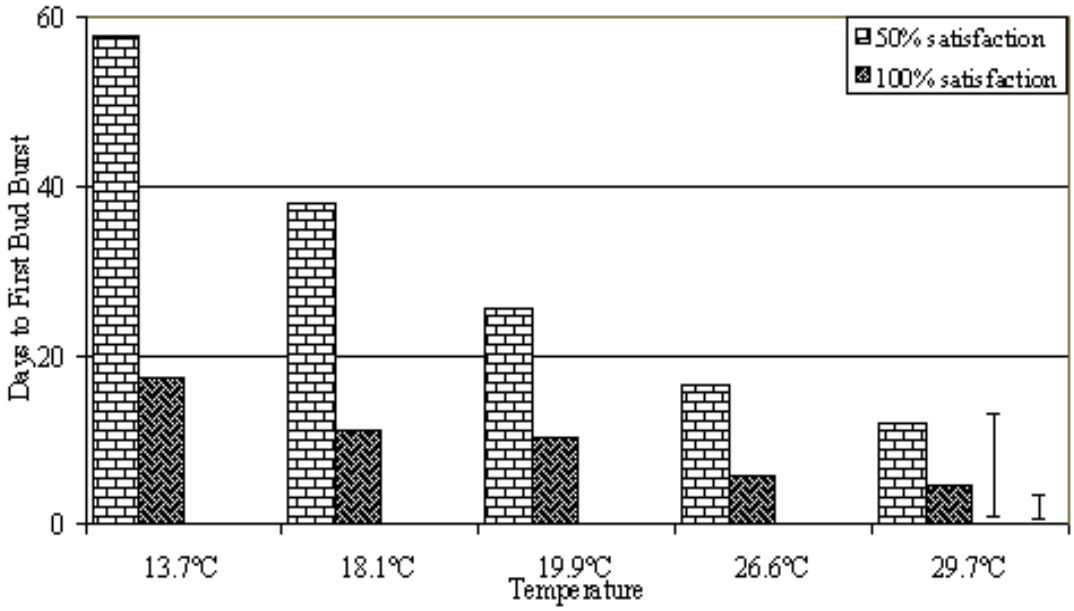


Figure 5.11. ***R. nigrum*** **– effect of forcing temperatures on time to first bud burst. Data pooled across cultivars. First error bar represents L.S.D. (P<0.05), 50% chilling requirement, second represents 100% chilling requirement, d.f. = 36.**

Significant interactions between the main treatments were recorded for final bud burst, hence data were divided by cultivar and re-analysed. Except for the 30ºC treatment, final bud burst of 'Ben Gairn' significantly increased as the chilling satisfaction increased from 50% to 100% (Figure 5.12). Although there was no effect on final bud burst as the temperature increased from 13º.7C to 26.6ºC, plants exposed to 29.7°C burst significantly more buds than those exposed to lower temperatures (Figure 5.12).

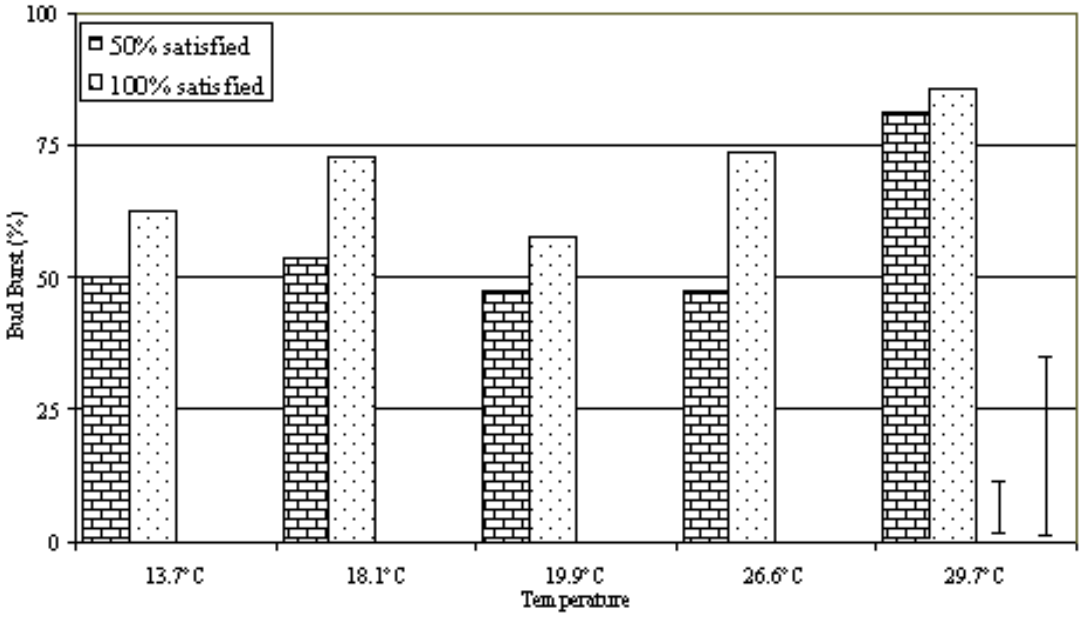


Figure 5.12. ***R. nigrum*** **'Ben Gairn' – effect of forcing temperature on final bud burst. First error bar represents chilling satisfaction, second represents temperature, L.S.D. (P<0.05), d.f. = 35.**

The effect of chilling satisfaction, forcing temperature and the interaction were highly significant (P<0.001, P=0.005, P<0.001) for 'Ben Hope' so data were further divided and re-analysed. After receiving 50% of the chilling requirement, bud burst was promoted when exposed to 19.9°C (Figure 5.13). As the chilling requirement was satisfied, however, 26.6°C was more efficient.

Significant interactions between bud position, cultivar, forcing temperature and chilling satisfaction were evident, hence data was divided by cultivar and re-analysed.

Significant effects of bud position and forcing temperature (P<0.001) were observed for 'Ben Gairn', but chilling satisfaction (P=0.113) and the interactions were not significant. Temperature had no effect on terminal bud burst and regardless of lateral bud position, exposure to 29.7ºC resulted in significantly higher bud burst (Figure 5.14). There was no clear relationship between bud position and forcing temperature for the remaining treatments. There was no effect of forcing temperature on 'Ben Hope' terminal bud burst and no clear relationship between bud position and temperature - in the top section of stems, forcing at 19.9ºC was the optimum treatment and in the middle and bottom sections, forcing at 26.6º or 13.7ºC produced the highest bud burst (Figure 5.15).

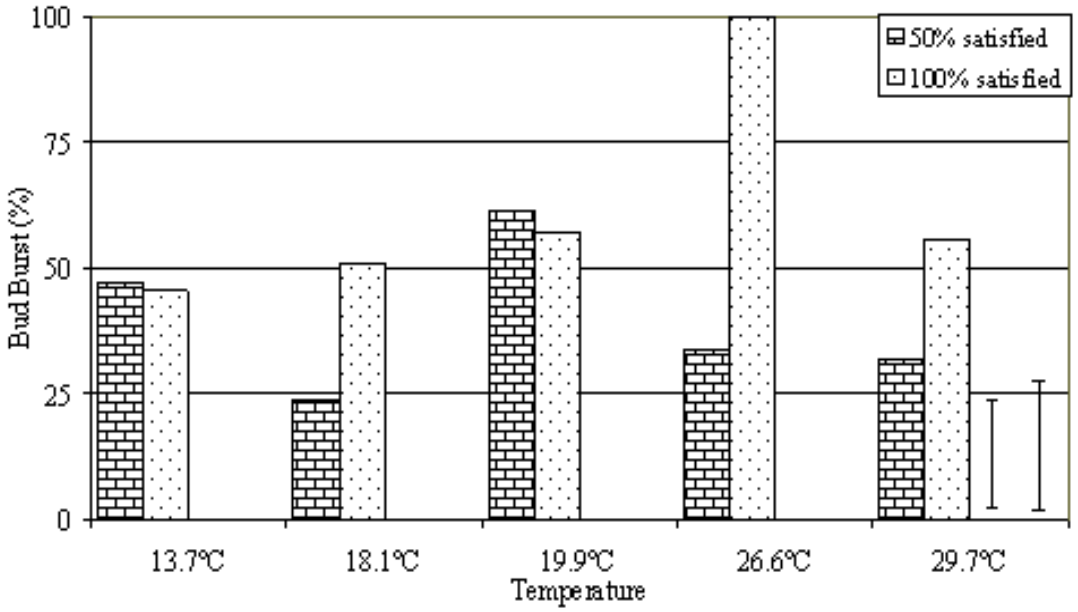


Figure 5.13. ***R. nigrum*** **'Ben Hope' – effect of effect of forcing temperature on final bud burst. First error bar represents 50% chilling satisfied, second represents 100% chilling satisfied, L.S.D. (P<0.05), d.f. = 16.**

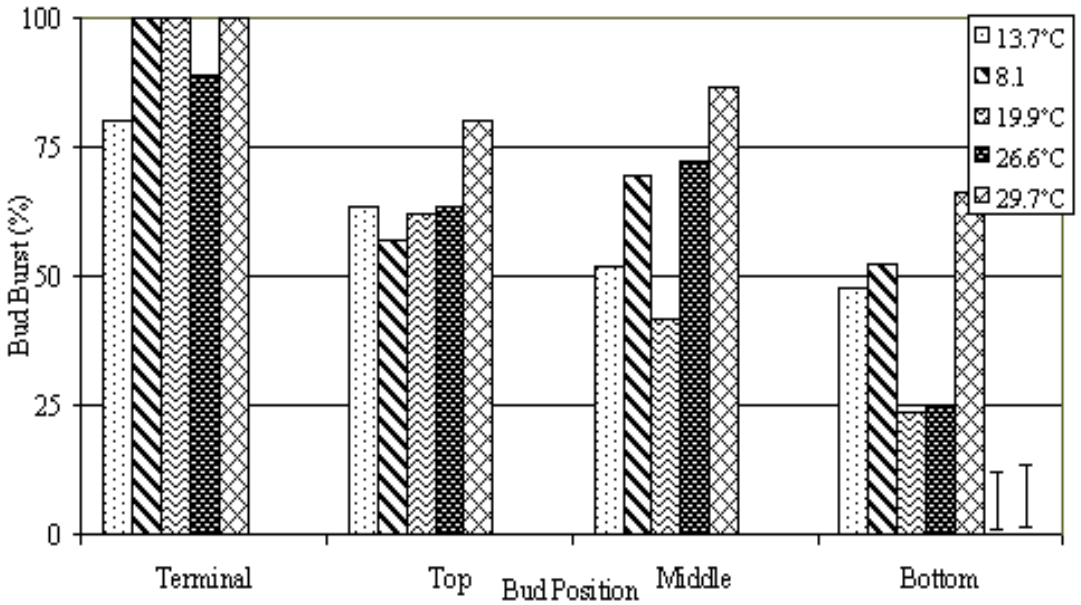


Figure 5.14. ***R. nigrum*** **'Ben Gairn' – relationship between forcing temperature and bud position. Data pooled across chilling treatments. First error bar represents L.S.D. bud position, second represents temperature, (P<0.05), d.f. = 152.**

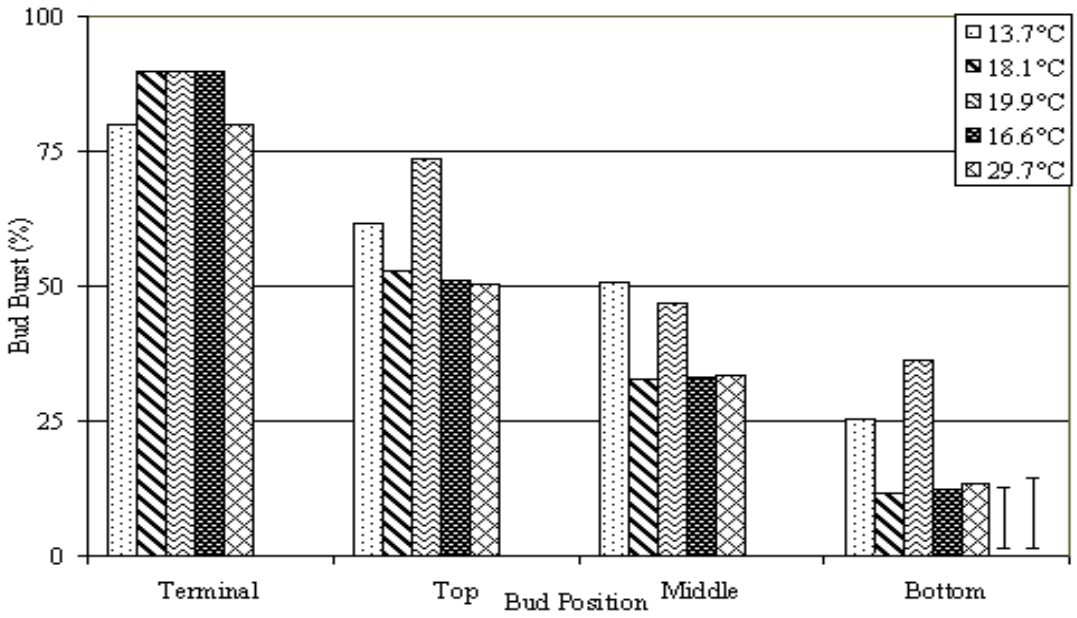


Figure 5.15. ***R. nigrum*** **'Ben Hope' – relationship between forcing temperature and bud position. Data pooled across chilling treatments. First error bar represents L.S.D. bud position, second represents temperature, (P<0.05), d.f. = 152.**

Shoot extension was affected by a number of significant interactions between factors and data were sub-divided and re-analysed. Chilling satisfaction had no effect (P=0.553) on 'Ben Gairn' but shoot extension was positively associated with increasing forcing temperature (Figure 5.16).

Although for 'Ben Hope' the effects of chilling requirement and forcing temperature were insignificant (P=0.811, P=0.285), the interactions were highly significant (P=0.01) hence data were further divided and re-analysed. After 50% of 'Ben Hope's chilling requirement had been satisfied, forcing temperature had no effect on shoot extension (P=0.408). As the chilling requirement was satisfied, however, extension was promoted by exposure to 19.9°C and temperatures above or below this were less effective (Figure 5.17).

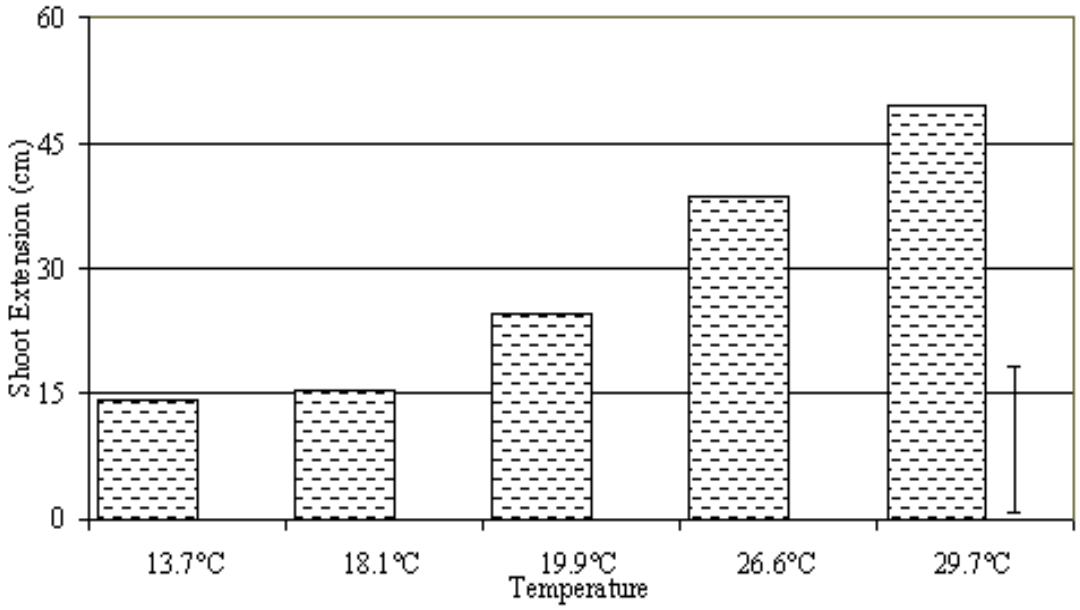


Figure 5.16. ***R. nigrum*** **'Ben Gairn' – effect of forcing temperature on shoot extension. Error bar represents L.S.D. (P<0.05), df. = 34.**

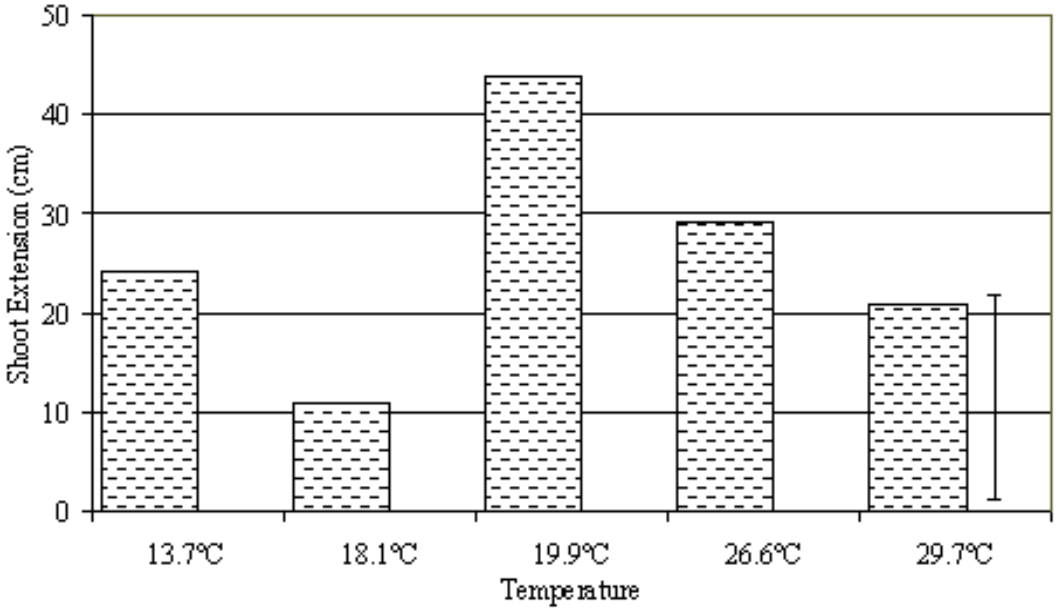


Figure 5.17. ***R. nigrum*** **'Ben Hope' – effect of forcing temperature on shoot extension. Error bar represents L.S.D. (P<0.05), d.f. = 14.**

Anthesis

After receiving 50% of the chilling requirement and forcing at 29.7°C, 26.6°C or 18.1°C, 'Ben Gairn' had not flowered by 21 July 2005. Of the five plants forced at 19.9°C, one produced 7 flowers after forcing for 42 days, of which one flower (14.3%) set fruit. Of the five plants exposed to 13.7°C, 4 flowered (Table 5.3) after an average of 124.5 days.

Table 5.3. *R. nigrum* 'Ben Gairn' – effect of forcing temperature on flower and fruit production after 50% chill satisfaction

Temperature	*Rep*	*Days to Flower*	*No. Flowers*	*No. Fruit*	*% Fruit Set*
13.7°C	2	119	8	4	50.0
13.7°C	3	130	8	2	25.0
13.7°C	4	119	3	3	100.0
13.7°C	5	130	7	3	42.9

After 100% of the chilling requirement had been fulfilled, no flowers had been produced by 21 July 2005 after forcing at 29.7°C, 26.6°C or 19.9°C and only two of the five plants exposed to 18.1°C and 13.7°C flowered (Table 5.4).

Table 5.4. *R. nigrum* 'Ben Gairn' – effect of forcing temperature on flower and fruit production after 100% chill satisfaction

Temperature	*Rep*	*Days to Flower*	*No. Flowers*	*No. Fruit*	*% Fruit Set*
18.1°C	2	32	5	3	60.0
18.1°C	3	32	6	2	33.3
13.7°C	1	39	13	7	53.9
13.7°C	3	63	4	1	25.0

No flowers emerged after exposure of 'Ben Hope' to 29.7°C therefore this data were omitted from further analyses. The effects of chilling satisfaction, forcing temperature and the interaction were found to be highly significant (P=0.003, P=0.002, P=0.006) so data were sub-divided and re-analysed. After 50% of the chilling requirement had been satisfied, delayed anthesis was associated with plants exposed to 13.7°C (Figure 5.18). No effect of forcing temperature was recorded when plants had received 100% of the chilling requirement and anthesis was recorded 33.5 days after forcing.

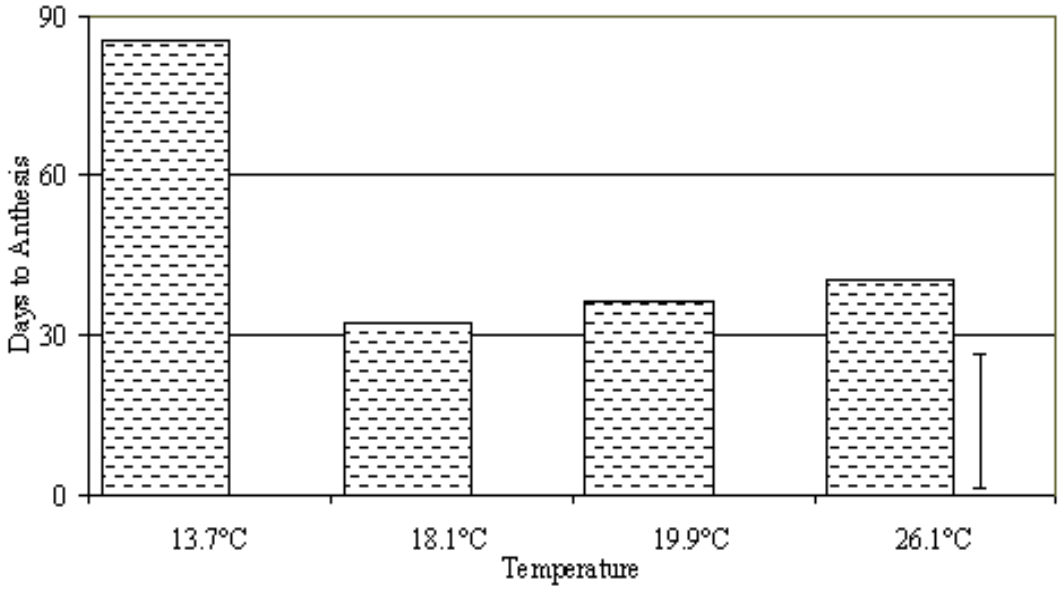


Figure 5.18. ***R. nigrum*** **'Ben Hope' – effect of forcing temperature on time to first flower. Error bar represents L.S.D. (P<0.05), d.f = 7.**

Flower and fruit production of 'Ben Hope' were unaffected by chilling satisfaction (P=0.440, P=0.142 respectively), but were highly dependant on forcing temperature (P=0.001, P=0.039), whereby 13.7°C promoted flower and fruit production compared to higher temperatures (Figure 5.19). After exposure to 26.6°C, all flowers aborted and no fruit were produced.

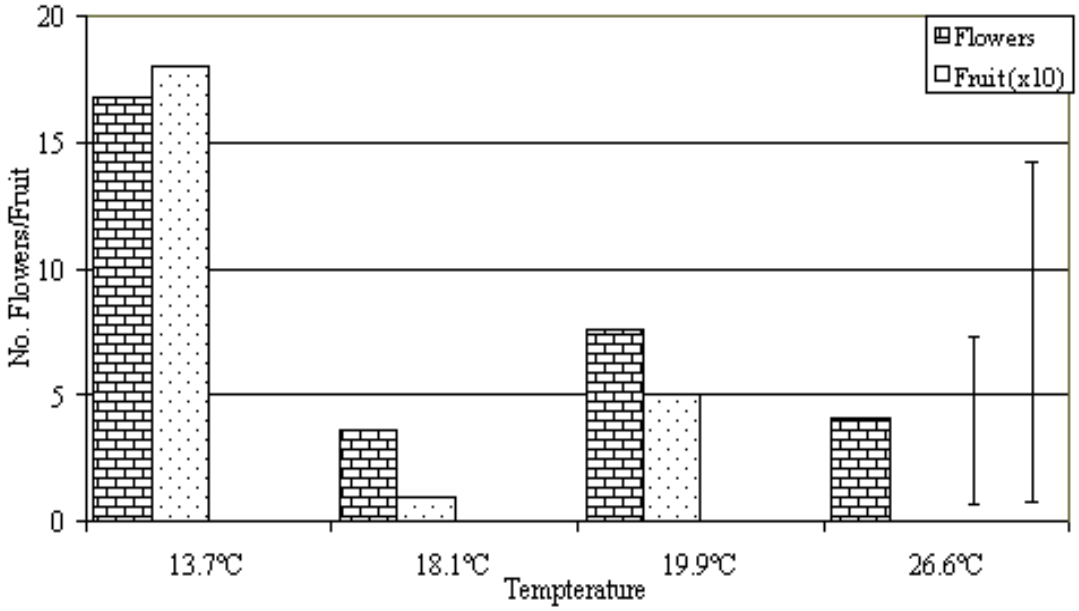


Figure 5.19. ***R. nigrum*** **'Ben Hope' – effect of forcing temperature on flower and fruit production. First error bar represents L.S.D. (P<0.05) flower production, second represents fruit production, d.f. =23 and 28 respectively.**

5.4. Discussion

Experiments were designed to quantify the effect of elevated spring temperatures, as predicted under the climate change scenarios, on bud burst characteristics, shoot extension, anthesis and frit production of *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran'.

5.4.1. The Effects of Elevated Spring Temperatures

Bud Burst

As the spring temperature increased by 4.3°C, predicted by 2080 under the High climate change scenario, bud burst was advanced for 'Ben Gairn' and 'Ben Hope' by 27-32 days. Bud burst of *Malus domestica* (Cannell and Smith, 1986), *Acer rubrum*, *Acer saccharum* (Norby *et al*., 2003) *Picea sitchensis* (Cannell and Smith, 1986), *Prunus armeniaca* 'Royal' (Brown, 1960), *Prunus avium* (Mahmood *et al*., 2000b) and 15 British tree species (Murray *et al*., 1989) was advanced after exposure to simulated climate change scenarios. Plants were placed in the elevated temperature regimes on 1 February, by which time 'Ben Gairn' and 'Ben Hope' had received 100% of their chilling requirements. The elevated temperature would fulfill the post-chilling heat requirement faster and promote earlier bud burst (Cannell and Smith, 1986). No such effect was observed for 'Ben Tirran', but the large chilling deficit (44.6%) may have prevented an accurate portrayal of the behaviour of the cultivar.

Despite the positive effects on time to bud burst, elevated spring temperature was negatively correlated to final bud burst and shoot extension. Similar findings were reported for *Actinidia chinensis* (Snelger *et al*., 1997), *Fragaria ananassa* (Kronenberg *et al*., 1976), *Rubus fructicosus* (Lopez-Medina and Moore, 1999) and *Prunus* spp. (Anderson *et al*., 1986; Erez and Couvillon, 1987; Mahmood *et al*., 2000a). Although initially a positive effect of elevated temperatures was observed for *Pseudotsuga menziesii* growth, a detrimental effect on final shoot height was observed, primarily due to premature cessation of growth (Olszyk *et al*., 1998a,b). The detrimental effects of elevated temperature on shoot extension and plant growth are contrary to the theories of Cannell and Smith (1986) and Artf *et al*. (1999) that after receiving sufficient chilling to overcome endodormancy in vegetative buds, elevated temperatures would increase general growth and development.

Response to climate change has been found to be dependant on species (Saxe *et al*., 2001) and provenance, with low arctic plants responding more than high arctic or alpine plants (Arft *et al*., 1999). Increasing the temperature by 2ºC had beneficial effects on the growth of

herbaceous species but was ineffective for woody species and it was concluded that herbaceous species were more adaptive to the external environment than woody species, and were better adapted to scavenge nutrients (Arft *et al*., 1999).

In effect, elevated spring temperatures, as forecasted under the simulated climate change scenarios, induced premature bud burst, but were detrimental to final bud burst and shoot extension. Plants are controlled by biological processes that are highly dependant on temperature and as the ambient temperature increases to a maximum, biological processes are enhanced and growth is promoted (Badeck *et al*., 2004). After fulfillment of the chilling requirement, plant growth was hypothesised to be dependant on temperature, and as this increases above a base level, growth and development increases (Richardson *et al.*, 1975). In theory, therefore, shoot extension under the elevated spring temperatures should have exceeded that of control plants exposed to lower temperatures. An environmental factor, other than temperature, may have limited the growth of the plants. After exposure to warm temperatures, initial growth rate of *Pseudotsuga menziesii* was accelerated but overall plant growth was negatively affected (Olszyk *et al*., 1998a). It was suggested that although the increase in temperature promoted vegetative production, the nutrient status of the plant could not sustain the accelerated growth. In support of this, the negative effect of increasing temperature on plant growth was concluded to be a result of resource limitation (Arft *et al*., 1999), a view which is further supported by Aerts *et al*. (2004). Although the nutrient status of the plants in this experiment was not determined, plants exposed to elevated spring temperatures appeared slightly chlorotic (Plates 5.1-5.3).

Alternatively, the lack of bud burst may have been a result of enhanced apical dominance. There was no effect of temperature on positional bud burst of ‘Ben Gairn’, however, increasing spring temperature reduced lateral bud burst of ‘Ben Hope’ and ‘Ben Tirran’. The chilling requirement of the apical bud is reported to be lower than that of lateral buds, yet differences between the bud types has not been quantified. The chilling requirements of the terminal buds may have been fulfilled earlier than that of lateral buds, thus the elevated temperatures would have satisfied the post-chilling heat requirement of terminal buds more rapidly than lateral buds. At the time of terminal bud burst, lateral buds may have been in the process of accumulating chilling/heating, and hence were not ready to burst. The subsequent inhibitory effect of the apical bud on the dormant buds may have prevented lateral bud from bursting and let to an overall decrease in final bud burst.

Anthesis

Compared to control plants, anthesis of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' was advanced from 84.5 days to 70.5 days as the spring temperature increased by 4.3°C. This supports the prediction of Atkinson *et al.* (2004) that by 2080 the date of *Ribes nigrum* anthesis would increase by 17% under the High climate change scenario. Time to first ripe fruit was similarly advanced from 94.6 days to 74.4 days (21.4%). A 4.3°C increase in spring temperature, however, proved to be detrimental to overall flower and fruit production. Previous research has proved conflicting, with a beneficial effect of increasing temperature reported for *Andromeda polifolia* and *Rubus chamaemorus* (Aerts *et al*., 2004), a detrimental effect for *Prunus avium* and *Prunus armeniaca* (Mahmood *et al*., 2000b; Brown, 1960) and no effect for *Fragaria ananassa* (Tehranifar *et al*., 1998). The maximum temperature *Fragaria ananassa* were exposed to (3ºC) however, was considerably lower than that reported to contribute to growth, and the ability of such low temperatures to promote growth and development is questionable.

As discussed above, the reduction in shoot extension of 'Ben Gairn' and 'Ben Hope' after exposure to elevated temperatures may have been a response to the nutrient status of the plant. Reductions in flower number and yield were recorded after nutrient deficiency of *Citrus reticulata* 'Okitsu Wase' (Inoue and Kataoka, 1992), *Capsicum annum* 'Jawala' (Roychoudhury *et al*., 1990) and *Vaccinium corymbosum* 'Berkley' (Gough and Abbott, 1984). Advancement of bud burst (27-32 days) was more distinct than advancement of anthesis (14 days). If anything, the increased temperature would be expected to have a similar if not more pronounced effect on flowering date. Delayed anthesis is an indication of insufficient chilling (Atkinson *et al*., 2004) and differences in the chilling requirements for bud burst and anthesis have been documented (Erez, 1995). The optimum chilling temperature for vegetative growth of *F. ananassa* 'Elsanta' was 2°C, yet the highest yield was produced when plants were chilled at -0.3°C (Tehranifar, 1997). The optimum temperature for fruit production of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' was found to be -0.1° to -3.4°C (see Chapter 3), which is significantly lower than that required for bud burst (see Chapter 4). Although the plants had received sufficient chilling to break endodormancy in vegetative buds, the chilling requirement of *R. nigrum* floral buds is unknown. If the chilling requirement for floral bud burst was higher than that of vegetative bud burst, 'Ben Gairn' and 'Ben Hope' would carry a floral chilling deficit which would not only delay the time to floral bud burst, but would have negative effects on flower production and fruit set (Atkinson *et al*.,

2004). The chilling requirement of *Ribes nigrum* flower/fruit production, however, is unknown and conclusions must be drawn with caution.

Advancing bud burst and hence fruiting is beneficial to many soft fruit crops, e.g. *Fragaria ananassa* and *Rubus idaeus* and growers receive a premium for the earlier harvest. However, the potential for frost damage is increased as the date of bud burst is advanced (Cannell and Smith, 1986). In the UK, *Ribes nigrum* is grown primarily under contract for the processing industry, and as such there is no financial incentive for advancing flower and fruit production.

5.4.2. Effect of Chilling Satisfaction

Bud Burst

Bud burst and anthesis were advanced in plants that had received 100% of the chilling requirement compared to those that had received only 50%. There was no difference, however, in the time to bud burst between 100% chilled plants forced at 13.7°C and chilling-deficient plants forced at 26.6°C or 29.7°C. There was also no difference in the timing of 'Ben Hope's floral bud burst when comparing 100% chilled plants forced at 13.7°C to chilling deficient plants forced at 18.1°C. This supports the theory of Cannell (1989) who hypothesised that the less chilling (e.g. the greater the chilling deficit), the more post-chilling heat requirement was required for growth and development. In effect, the chilling deficit can be overcome, at least in part, by increasing the exposure to warmer temperatures. A similar result was observed for when elevated temperatures compensated for insufficient chilling of *R. nigrum* 'Baldwin' (Plancher, 1983a) and *Vaccinium myrtillus* (Taulavuori *et al*., 2002). The difference between the temperature range for bud burst and anthesis is interesting, and suggests that the post-chilling heat requirement for bud burst is higher than that for anthesis. Bud burst occurs before flowering, therefore late frosts are more likely to damage vegetative buds. Breeding a higher post-chilling heat requirement for bud burst would delay bud burst and hence reduce the likelihood of being subjected to frost damage.

Although ABA was initially thought to be responsible for releasing bud dormancy (Mielke and Dennis, 1978; Pilate *et al*., 1989; Vince-Prue, 1975; Bondock *et al*., 1995) it has been suggested that the reduction in ABA concentration was as a result of, rather than the cause of, breaking dormancy (Ramina *et al*., 1995). After receiving insufficient chilling, *Prunus cerasus* 'Montmorency' bud burst was delayed and uneven yet the bud ABA concentration decreased to a minimum 4 weeks prior to bud burst, suggesting the lack of response was not

associated with ABA concentration (Miekle and Dennis, 1978). If dormancy was broken as result of cold temperature-induced ABA degradation, 'Ben Gairn' and 'Ben Hope' that had received only 50% of the chilling requirement should have remained in a dormant state. However, exposing the plants to elevated temperatures resulted in bud burst comparable to fully-chilled plants, indicating that elevated temperatures can overcome the effect of insufficient chilling. In response to elevated temperatures, ABA concentration has been found to increase (Weijun and Leul, 1999; Itai and Benzioni, 1974; Daie *et al*., 1981), which should increase intensity of dormancy of the unbroken buds and further prevent their bursting. The role of ABA in releasing dormancy, therefore seems increasingly less likely.

For any given forcing temperature, final bud burst of 'Ben Gairn' was lower when plants had received only 50% of the required chilling compared to those that had received 100%. This is supported by Plancher (1983a) and Scalabrelli and Couvillon (1986) who reported an increase in *Ribes nigrum* and *Prunus persica* 'Redhaven' bud burst with increasing chilling satisfaction. After receiving 50% of the required chilling, 'Ben Gairn' forced at 30°C burst more buds than those forced at 13°C after receiving all of the required chilling. There was no clear effect of chilling satisfaction and forcing temperature for 'Ben Hope'. Increasing the forcing temperature substituted for chilling deficits of *Betula pubescens*, *Betula pendula* and *Populus tremula* (Heide, 1993b).

Shoot extension was promoted as the forcing temperature of 'Ben Gairn' increased to 29.7°C. Although there was no significant effect on chilling deficit 'Ben Hope', shoot extension increased as the temperature rose to 19.9°C, yet further advancements were detrimental. Shoot extension of *Fragaria ananassa* (Bailey and Rossi, 1965; Tehranifar and Battey, 1997) and *Pseudotsuga menziesii* (Campbell and Sugano, 1975) was also promoted after exposure to elevated temperatures. Although growth of *Medicago sativa* was halted by exposure to elevated temperature, no adverse physical damage was apparent and growth resumed on transfer to lower temperature conditions, which is again indicative of thermodormancy (Levitt, 1980). *R. nigrum* would not typically be exposed to such high temperatures during the growing season, and detrimental effects on growth may have been expected.

Regardless of the degree of chilling satisfaction, bud burst lower down the stems were increased after forcing 'Ben Gairn' and 'Ben Hope' at 19.7ºC and 19.9ºC respectively. This suggests that the elevated temperatures compensated for the inhibitory effect of apical

dominance, which is responsible for preventing lateral buds bursting and is more severe lower down the stem. Previous research has not investigated this effect, but the high temperatures may have resulted in more even bud burst, e.g. terminal buds bursting at the same time as buds in the lower section of stems, which would nullify the effects of apical dominance.

Anthesis

Although the lack of data for flower and fruit production of 'Ben Gairn' does now allow for confident conclusions, but there was no effect of chilling satisfaction on flower number and fruit production of 'Ben Hope'. This result is further supported by research conducted on *Fragaria ananassa* (Bailey and Rossi, 1965) and *Vaccinium virgatum*, *Vaccinium darrowi*, and *V. virgatum* x *Vaccinium constablaei* (Lyrene, 1994). A reduction or cessation of *Fragaria ananassa* flower bud initiation and/or development was observed after exposure to elevated temperatures (Bailey and Rossi, 1965).

R. nigrum flowers are initiated the autumn before anthesis, hence floral initiation would have begun and some development would have occurred before the plants were exposed to the elevated forcing temperatures. Indeed, control plants in Experiment 4 had been exposed to identical chilling regimes as Experiment 5's plants, and successfully produced flowers and fruit, so it can be assumed that the plants had the potential to produce flowers. Although temperature-induced drought stress has been reported as a result of increased rates of evapo-transpiration (Levitt, 1980; Seeley, 1996), the plants were hand-watered when required and the peat was always damp, therefore drought stress is unlikely to have affected the results. The lack of flower production, therefore, is likely to be an adverse reaction to high temperatures.

'Ben Gairn' and 'Ben Hope' responded to elevated forcing temperature in one of two ways:

1. Flower production failed
2. Fruit set was low or non-existent

Low fruit set may be as a result of temperature-induced pollen damage. Low fruit set of *Phaseolus vulgaris* (Gross and Kigel, 1994), *Vigna unguiculata* (Warrag, 1983), *Aribidopsis thaliana* (Kim *et al*., 2001), *Prunus persica* (Walser *et al*., 1981) and *Annona cherimola* (Higuchi *et al*., 1998), after exposure to temperatures ranging from 32°C to 42°C, was attributed to male sterility. Alterations in pollen structure, possibly as a result of the mother

pollen cell failing to separate (Kim *et al*., 2001), and anther dehiscence have been observed by the above-mentioned authors. High temperatures have also been reported to affect the stigma, pollen tube growth, ovule fertilisation and seed set (Gross and Kigel, 1994; Higuchi *et al*., 1998). No further effect on flower morphology, including sepal, stamen, carpel or petal formation was evident after *A. thaliana's* exposure to 42°C (Kim *et al*., 2001). Pollen germination of *P. avium* increased as the forcing temperature increased from 10°C to 20°C, but a dramatic decrease was observed at 30°C (Mahmood, 1999). *Prunus avium* pollen tube growth was accelerated after exposure to 20°C, yet at 30°C the pollen tube failed to penetrate the stigma (Mahmood, 1999).

Alternatively, Wagstaffe and Battey (in press) observed increased incidences of *Fragaria anananssa* 'Everest' flower abortion in response to elevated temperature and attributed this to thermodormancy, whereby floral development failed in response to unfavourable temperature. This is further supported by Erez and Couvillon (1987). Flower production of *Prunus persica* (Erez and Couvillon, 1987), *Prunus cerasus* (Anderson *et al*., 1986), *Actinidia chinensis* (McPherson *et al*., 1995), *Brachycome hybrida*, *Sutera cordata* and *Argyranthemum frutescens* failed after exposure to elevated forcing temperatures. After 8 weeks of exposure to 33°C, *Brachycome hybrida*, *S. cordata* and *Argyranthemum frutescens* were returned to a lower temperature and flower production resumed. This suggests that although high temperatures inhibited floral development, this process was reversible. This is again indicative of thermodormancy, whereby flowering is impeded by elevated temperatures but can proceed when favourable conditions return.

The different reactions observed between 'Ben Gairn' and 'Ben Hope' may be due to cultivar specificity, as discovered throughout the previous experiments. The response of *Lycopersicon esculentum* to elevated temperatures (32°C compared to 26°C) was found to be cultivar dependant, with increased flower abortion as a result of high temperature for 'FLA7156', 'NC8288' and 'NC46E', but no effect on 'NC279HS', 'NC403HS' and 'Piedmont' (Sato *et al*., 2004). Alternatively, the susceptibility of *Arabidopsis thaliana* to elevated temperature damage was dependant on the stage of flower development (Kim *et al*., 2001). 'Ben Gairn' may have been at a more susceptible stage than 'Ben Hope' when the treatments were applied.

5.4.3. Effect of Bud Position

In both experiments, bud position significantly affected bud burst, with the greatest bud burst recorded for terminal buds and the least bud burst for basal buds. This is a classic symptom of paradormancy, whereby lateral bud growth is inhibited by apical dominance, and even after the chilling requirement has been satisfied, buds may fail to burst (Lang *et al*., 1987). Although endodormancy was thought to be the limiting factor in *R. nigrum* bud burst (Saunders, R., *Pers. Comm*.), the results of this experiment suggest that paradormancy may be as detrimental to bud burst, resulting in a 75% decrease in bud burst of 'Ben Tirran' under the simulated climate change scenarios. Paradormancy was alleviated in *Rubus ideaus* 'Glen Clova' by horizontal training of branches or tip pruning (White *et al*., 1999). These techniques, however, are time and labour intensive and have not been previous utilised with *R. nigrum*, hence the physiological effect of altering the growth of the plants is unknown. Possible adverse side-effects may be reduced crop yield or growth habits that are unsuitable for traditional machine harvesting. A reduction in apical dominance of *Annona* spp. 'African Pride' in response to drought was observed, although the detrimental effects of drought on flower and fruit production should be considered before such treatments is applied (George and Nissen, 2002). Alternatively, application of dormancy breaking chemicals was concluded to induce lateral bud burst as a result of inflicting damage on apical buds (Bautista *et al*., 1987; George *et al*., 2002), and *R. nigrum* dormancy breaking experiments are being trialled on-farm. Although not recorded in these experiments, reduced bud burst lower down the stems would presumably result in reduced flower and fruit production. Alleviating the effects of apical dominance, therefore, has the potential to increase crop yield and hence is an important subject that warrants further research.

5.5. Conclusions

After fulfillment of the chilling requirement, elevated spring temperatures, as predicted under the climate change scenarios, hastened bud burst and anthesis but were detrimental to shoot extension, flower and fruit production. The elevated temperatures were found to have less of an effect on 'Ben Tirran', most likely due to the large chilling deficit of this cultivar. On fulfillment of the chilling requirement, bud burst was dependant on satisfaction of a post-chilling heat requirement, therefore cooling buds, via overhead sprinkler systems, may delay heat accumulation and thus bud burst. For cultivars that have higher chilling requirements, e.g. 'Ben Tirran', cooling the buds would have the added advantage of allowing more chilling to accumulate and hence increasing the productivity of the crop.

Elevated temperatures compensated for 50% of 'Ben Gairn' and 'Ben Hope's chilling requirement, resulting in advanced vegetative and floral bud burst and increased final bud burst. Reproductive processes, however, were negatively affected. In particular, dramatic effects on the numbers of flower and fruit that were produced were observed, likely to be as a result of temperature-induced pollen damage. The timing of floral initiation and development in modern-day *R. nigrum* cultivars is unknown. The detrimental effects of elevated temperatures, therefore, cannot be estimated until the development process is understood.

The inhibitory effect of apical dominance on lateral bud burst was evident in both experiments, suggesting that paradormancy may inhibit bud burst after endodormancy has been broken. Crop management techniques have proved successful at alleviating the effects of paradormancy in fruit crops, but often rely on methods not commonly employed in *Ribes nigrum* production. Further research on the effectiveness of such treatments, as well as potential beneficial/detrimental effects, is required.

The detrimental effects of elevated spring temperatures were clearly evident in these experiments, and decreases in flower production and yield should be expected. In the long-term, low-chill cultivars will be bred, but growers are already reporting classic signals of lack of chilling and in the short term, this problem must be tackled to keep the UK blackcurrant industry profitable.

Chapter Six.
Inducing Premature Bud Burst

6.1. Introduction

The effects of insufficient winter chilling of *Ribes nigrum* were examined in previous chapters. While plant breeders are in the process of breeding low chill requirement into the crop, traditional methods are time-intensive and new cultivars with sufficiently low chilling requirements may take in excess of ten years to become commercially available. In the meantime, previous work (Delap, 1966; Delap, 1967; George and Nissen, 1992) suggested that altering crop management techniques and/or chemical application may be used to aid the forcing of bud burst in crops, and has been successfully utilised for *Malus domestica* and *Prunus persica*.

6.1.1. Post-Harvest Nitrogen Application

There have been conflicting reports regarding the effects of post-harvest nitrogen application on the depth of dormancy. Time to bud burst decreased following nitrogen application to *Malus domestica* 'Lord Lambourne' (Delap, 1966) and Cox's Orange Pippin (Delap, 1967), but no effect was recorded for 'Golden Delicious' (Terblanche *et al*., 1979) and bud burst of *Prunus persica* 'Flordaprince' was delayed (George and Nissen, 1992). Flowers are reported to be more fertile following autumnal nitrogen application (Delap, 1966), which increased fruit set and yield (Terblanche *et al*., 1979; George and Nissen, 1992). Excess nitrogen may increase the photosynthetic capacity of the leaves, allowing rapid bud burst in the spring (George and Nissen, 1992)

6.1.2. Premature defoliation

Premature defoliation has been applied to several crops, including *Malus domestica* (Swartz *et al*., 1984) and *Ribes nigrum* (Tinklin and Schwabe, 1970) in an attempt to manipulate the duration and depth of dormancy. Treatment has resulted in successful cultivation of temperate-zone crops out-with their geographical boundaries, where warm winter temperatures do not satisfy the crops' chilling requirements. Bud burst of *Prunus persica* 'Flordaprince' and 'Flordagold' was observed before any chilling, based on the Utah and <7.2°C chilling models, had accumulated (Lloyd and Firth, 1990). However, premature defoliation of was ineffective when applied to *Actinidia chinensis* 'Hayward' (Snelgar *et al*., 1997), *Olea europaea* (Rallo and Martin, 1991) and *R. nigrum* 'White Bud' (Westmore,

2004). Detrimental effects of treatment have been reported on flower development of *A. chinensis* (Snelgar *et al*., 1997) and *R. nigrum* 'Baldwin' (Corke and Wilson, 1963) and final bud burst of *R. nigrum* 'Vija' (Plancher, 1983b).

During the lead-up to dormancy, an inhibitory substance, perhaps ABA, is synthesized in leaves and transported to buds (Mielke and Dennis, 1975; Masia *et al*., 1993). After premature defoliation of *Prunus cerasus*, the concentration of ABA did not increase in treated buds as it did in control plants (Mielke and Dennis, 1978). This suggests that ABA is produced at least in part by the leaves and transported to the buds, as proposed by Eagles and Wareing (1964). Premature leaf removal therefore, would prevent ABA accumulating and hence would positively affect the depth of dormancy.

6.1.3. Photoperiodic Lighting

It has been reported that extended photoperiods can substitute for insufficient chilling (Roberts *et al*., 1974; Campbell and Sugano, 1975; Thomas and Vince-Pru, 1997). After receiving insufficient chilling, time to first bud burst was advanced and final bud burst increased after exposure of *Ribes nigrum* 'Boskoop Giant' (Hoyle, 1960), *Alnus glutinosa, Alnus incana, Betula pubescens, Betula pendula, Prunus padus, Populus tremula* (Heide, 1993b), *Fagus sylvatica*, *Betula pubescens*, *Larix decidua* (Wareing, 1954b), *Picea abies* (Heide, 1973) and *Acer saccharum* (Olmsted, 1951) to extended photoperiods (from 17 to 24 hour photoperiods). However, extending the photoperiod had no effect on these parameters when *Cornus alba* (Walley and Cockshull, 1976), *Robinia pseudoacacia* and *Acer pseudoplatanus* (Wareing, 1954b) were insufficiently chilled. It may be that response to extended photoperiod is species-specific, or that the chilling deficit of *C. alba*, *R. pseudoacacia* and *A. pseudoplatanus* was too large and could not be overcome by extending the photoperiod. The effect of photoperiod was found to decrease as the chilling requirement was increasingly satisfied (Heide, 1993b).

6.1.4. Dormancy Breaking Chemicals

Dormancy breaking chemicals have been utilised to induce bud burst in many crop species, primarily when temperate-zone crops have been grown in warmer climates and hence do not receive sufficient chilling to satisfy the requirement for bud burst e.g. *Malus domestica* spp. grown in Yemen (Finetto, 1993) and *Prunus armeniaca* grown in Turkey (Kuden and Son, 1997). There are several commercially-available products that rely either on oil, nutrient or

chemical activity to force bud burst. The major drawback in using oils and chemicals, however, is the rigorous and expensive registration process that must be undertaken in the UK before application to a crop is permitted. Treatment has also been reported to have detrimental effects on the crop - hydrogen cyanamide can damage flowers and dinitrocresol (DNOC) oil is phytotoxic (Erez and Yablowitz, 1997).

Success has been achieved in the use of chemical adjuvants for the breaking for dormancy. 'Armobreak' (International Agro Additive Specialities, Naarde, Netherlands) is marketed as an adjuvant to be used in conjunction with other dormancy breaking chemicals (Anon, 2004) and GlaxoSmithKline growers have utilised the adjuvant Abacus (Loveland Industries, Cambridge, UK) for in-house dormancy breaking experiments, with encouraging results (Saunders, R., *Pers. Comm.*). The mode of action of adjuvants is unclear. The Pesticide Directorate has deemed that such chemicals appear to induce bud burst by sealing the surface of the bud, thus preventing transpiration and causing a stress reaction (Mitchell, S., *Pers. comm.*), but there is no evidence to support this theory. The degradation pathway of alkylphenyl hydroxypolyoxyethylene, the active ingredient of several adjuvants, is relatively unknown. One identified step involves the loss of ethoxylate (C_2H_40) side groups (Jonkers *et al*., 2001). This compound may degrade to produce ethylene (C_2H_4) and hence result in bud burst. The advantage of using adjuvants to aid the dormancy breaking process is that the mode of action is deemed to be physical and hence they are exempt from the Control of Pesticides Regulations 1986 (Mitchell, S., *Pers. Comm.*).

The objective of the experiments described in this Chapter was to determine the extent to which altering crop management techniques could advance and promote *R. nigrum* growth and development.

6.2. Materials and Methods

6.2.1. Experiment 4. Post-Harvest Nitrogen Application

Plant Material

Softwood cuttings were taken from *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' stock plants in April 2003, delivered to the University of Reading's Experimental Field Site on 2 July 2003, tied into the supporting wires and connected to the irrigation system, as described in Chapter 2. Pots were irrigated using Avoncrop's Soft Fruit Feed Mix 2, four times a day for a total of one hour until 29 September.

Experimental Protocol

On 5 September 2003, 60 plants of 'Ben Gairn' and 'Ben Hope' were chosen at random. Twenty plants of each cultivar were arranged in double rows with spacings of 10cm between plants within a row and 80cm between rows and an irrigation line running down the middle of each row. One irrigation dripper was inserted into each pot and the irrigation lines were connected to separate Dosatrons. One line of plants was supplied with a nutrient solution containing 50ppm nitrogen (sub-optimal), a second line of plants was supplied with 100ppm nitrogen (optimal) and the third line with 150ppm nitrogen (supra-optimal) (Table 6.1). The pH of the solutions was maintained between 6.3 and 6.5 and the E.C. between 1.7mS and 1.8mS. The irrigation timer was re-set to irrigate for 10 minutes four times a day until 11 November when the system was shut down.

Cold Storage and Randomisation

On 22 December 2003, 15 plants of each cultivar from each nitrogen treatment were placed in a cold store set at 4.11°C (see Table 6.2) and wrapped in black plastic to prevent the buds desiccating in the cool, dry air of the cold store.

As advised by the University of Reading's Statistical Advice Centre, the forcing glasshouse, set at 17.1°C (Table 6.2), was split into five positional blocks, and each block was further split into 100 positions (see Chapter 2). One plant of each cultivar from each nitrogen treatment was, using random number tables, placed in each of the five glasshouse blocks under natural conditions on 22 December 2003 to act as a control. Plants were monitored daily until bud burst occurred on all plants, thereafter bud burst was recorded three times a week until 15 June 2004.

Table 6.1. Composition of the nutrient solutions

Nutrient	*Chemical Formula*	*50ppm*	*100ppm*	*150ppm*
Calcium nitrate	$Ca(NO_3)_2$	0.457kg	0.457kg	0.457kg
Nitric acid	HNO_3	1.035L	1.035L	1.035L
Potassium nitrate	KNO_3	0.110kg	1.368kg	3.220kg
Potassium Sulphate	K_2SO_4	3.040kg	1.881kg	0.180kg
Mono potassium phosphate	KH_2PO_4	0.646kg	0.646kg	0.646kg
Magnesium Sulphate	$MgSO_4$	1.410kg	1.410kg	1.410kg
Iron ethylenediaminetetraacetic acid	$C_{10}H_{12}FeNaO_8$	2.060g	2.060g	2.060g
Manganese Sulphate	$MnSO_4$	0.550g	0.550g	0.550g
Disodium octaborate tetrahydrate	$Na_2B_8O_{13}.4H_2O$	0.205g	0.205g	0.205g
Zinc sulphate	$ZnSO_4$	0.165g	0.165g	0.165g
Copper sulphate	$CuSO_4$	0.095g	0.095g	0.095g
Ammonium molybdate	$(NH_4)2(Mo_4)_{13}$	0.040g	0.040g	0.040g
Water	H_2O	50L	50L	50L

Table 6.2. Average two-weekly temperature recordings from the cold store and glasshouse

Temp/ Week	4.11°C	17.17 ± 6°C
0		16.89 ± 4°C
2	4.19 ± 0.6°C	17.02 ± 4°C
4	3.78 ± 0.6°C	17.32 ± 6°C
6	4.27 ± 0.3°C	17.24 ± 6°C
8	4.13 ± 0.6°C	17.16 ± 5°C
10	4.19 ± 0.6°C	17.39 ± 6°C

.

6.2.2. Experiment 5. Premature Defoliation

Two-year old plants of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' (as described in Experiment 4) were utilised in this experiment. Sixty plants of each cultivar were chosen at random and colour-coded tags, to differentiate between the defoliation dates, were placed around each plant. Twenty plants of each cultivar were defoliated by hand on either 1 September or 25 September 2003. Care had to be taken during this process to ensure that buds were not removed accidentally. A further 20 plants of each cultivar were left to allow leaf senescence to occur naturally and by 18 November 2003 leaf abscission was complete.

Cold Storage

On 22 December 2003, fifteen plants of each cultivar from all of the defoliation treatments were placed in a cold store set at 4.11°C and wrapped in black plastic to prevent bud desiccation. Control plants were randomly placed in the glasshouse (as described in Experiment 4) set at 17.1°C. Five plants of each cultivar from each defoliation treatment were removed after receiving 2, 6 or 10 weeks of chilling and were randomly placed in the glasshouse. Using random number tables, one cultivar from each treatment was randomly placed in each of the five glasshouse blocks when removed from the cold stores.

6.2.3. Experiment 6. Photoperiodic Lighting

Mature budsticks of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' (supplied by William Price, Bradfields Farm, Taunton, Somerset) were cut from certified stock plants on 18 December 2003, collected and placed immediately into the 2°C cold store in black polythene bags with a water saturated sponge to prevent the budsticks dessicating. Ninety budsticks of each cultivar were removed after 2 weeks of cold storage (5 January 2004) and 8 weeks of storage (4 March 2004) and separated into three bundles of 30 budsticks. Each bundle was then placed into a beaker of water and one beaker was placed on a trolley in the 8-hour photoperiodic compartment, one in a 17-hour photoperiodic compartment and the third was placed under natural photoperiod as a control. The temperature was maintained at a constant 22°C for all treatments. Glasshouse staff wheeled the trolleys out of the photoperiodic bays at 8am and the plants were exposed to natural light until 3.30pm when the trolleys were wheeled into their sealed bays. Broad spectrum, low intensity tungsten bulbs supplemented the daylength until the required duration had been achieved.

Budsticks were checked twice a week and time to first bud burst recorded. Thereafter, the number of buds that had burst was counted once a week. The beakers were originally randomly placed on the trolleys/bench in the photoperiodic glasshouse. Every week, when monitoring bud burst, the beakers were re-randomised. To avoid the accumulation of bacteria and other contaminants, the water in which the budsticks were maintained was replaced three times weekly.

6.2.4. Experiment 7. Dormancy Breaking Chemicals

Mature budsticks (as in Experiment 6) of 'Ben Tirran' were removed from the 2°C cold store on 20 January. Three adjuvents were utililsed – Abacus (alkylphenyl hydroxypolyoxyethylene, esterified rape seed oil and natural fatty acids), which has been identified by GSK as having bud-bursting properties, Activator 90 (alkylphenyl hydroxypolyoxyethylene and natural fatty acids) and Celect (natural fatty acids) at concentrations of 2%, 1% or 0.5% v/v. Each solution was sprayed on ten cuttings until run off. The cuttings were left to dry in the glasshouse then placed into beakers of water. The beakers were placed on a bench in the heated glasshouse at 20°C and bud burst was recorded three times a week. As in the previous experiment, the water in the beakers was replaced three times a week.

6.3. Results

Initially, data covering all cultivars were analysed. Where significant interactions between cultivar and treatment were discovered, the data were divided by cultivar and re-analysed.

6.3.1. Experiment 4. Post-Harvest Nitrogen Application

Time to First Bud Burst

There were no significant interactions between the main treatments, however, a significant ($P<0.001$) effect of cultivar and an interaction ($P<0.001$) between cultivar and chilling duration was observed, hence the data were divided by cultivar and re-analysed.

After 0 weeks of chilling, 'Ben Gairn' burst bud significantly ($P<0.001$) faster than 'Ben Hope' (Figure 6.1). As the chilling duration increased, however, and the chilling requirement of each cultivar was increasingly satisfied, this effect became non-significant. As noted in the previous chapter, when the chilling duration increased for 'Ben Gairn' (0-6 weeks) and 'Ben Hope' (0-10 weeks), the time to bud burst significantly ($P<0.001$) decreased.

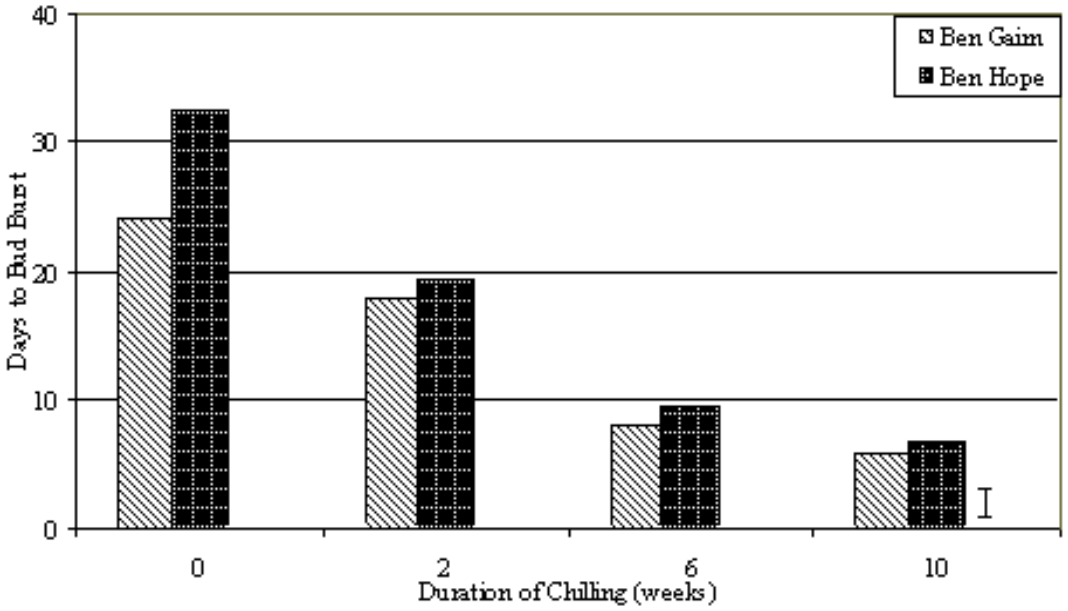


Figure 6.1. *R. nigrum* – effect of chilling duration on time to first bud burst. Data pooled across defoliation treatments. Error bar represents LSD (P=0.05), d.f. = 86

Final Bud Burst

For both cultivars final bud burst decreased as the nitrogen concentration increased to 150ppm, but this was deemed to be statistically non-significant (P=0.113). As with the previous data, the effect of cultivar was highly significant (P=0.006), as was the interaction between chilling duration and cultivar so data were sub-divided and re-analysed.

Cultivar had no effect on final bud burst until plants were chilled for 10 weeks, after which time 'Ben Gairn' plants demonstrated a significantly (P=0.005) higher final percentage bud burst than 'Ben Hope' plants (Figure 6.2). As the chilling duration increased for 'Ben Gairn' (from 0-6 weeks) and 'Ben Hope' (0-10 weeks), bud burst significantly (P<0.001) increased.

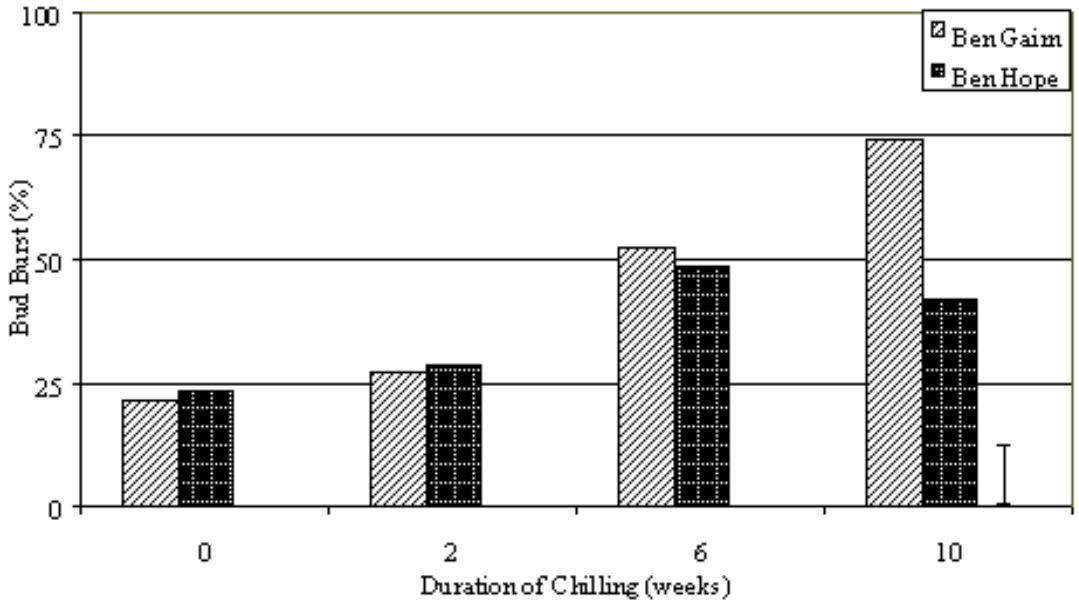


Figure 6.2. *R. nigrum* – effect of chilling duration on final bud burst. Data pooled across defoliation treatments. Error bar represents LSD (P=0.05), d.f. = 87.

6.3.2. Experiment 5. Premature Defoliation

The effect of cultivar was deemed to be highly significant (P<0.001), as were the interactions between cultivar and defoliation date (P<0.001) and cultivar and chilling duration (P=0.004) so data were sub-divided and re-analysed.

Time to Bud Burst

'Ben Gairn'

The effects of chilling duration and defoliation date and the interaction were highly significant (P<0.001; Figure 6.3) so data were sub-divided and re-analysed.

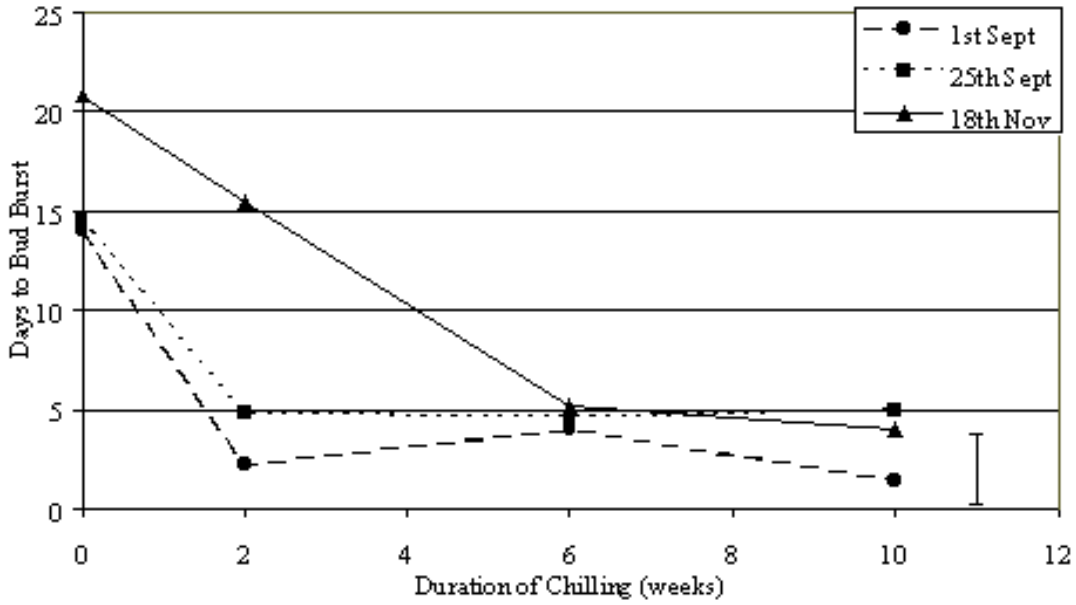


Figure 6.3. ***R. nigrum*** **'Ben Gairn' - effect of chilling duration and defoliation date on the time to first bud burst. Error bar represents L.S.D. (P=0.05), df = 39.**

Plants defoliated on 1 September or 25 September then chilled for a maximum of 2 weeks burst bud significantly faster than those defoliated on 18 November. As the chilling requirement was increasingly satisfied, the effect of defoliation date was reduced and after 6 weeks of chilling defoliation had no effect. Bud burst was significantly advanced as the chilling duration increased from 0 to 2 weeks (1 September and 25 September) and from 0 to 6 weeks (18 November).

'Ben Hope'

Chilling duration, defoliation date and the interactions (Figure 6.4) were highly significant ($P<0.001$) so data were further divided and re-analysed.

Bud burst was significantly ($P<0.001$) advanced, when chilled for a maximum of 2 weeks, after defoliation on 1 September. As the chilling requirement of 'Ben Hope' was increasingly satisfied, however, the effect of defoliation became less apparent and after 6 weeks of chilling there was no treatment effect. Bud burst was increased as the chilling duration increased from 0 to 2 weeks (1 September) or from 0 to 6 weeks (25 September and 18 November).

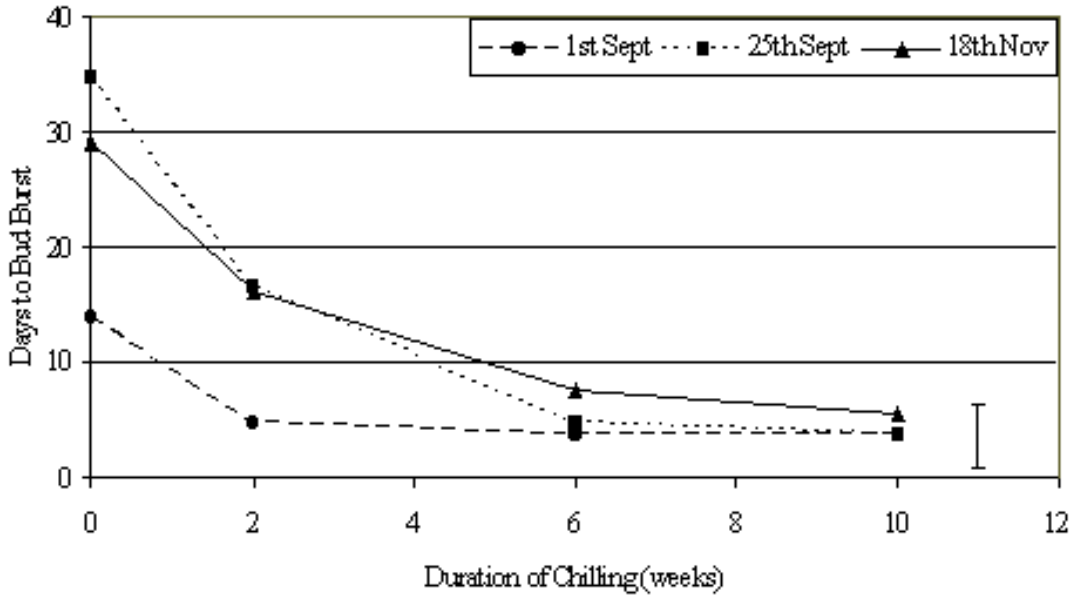


Figure 6.4. ***R. nigrum*** **'Ben Hope' - effect of chilling duration and defoliation date on the time to first bud burst. Error bar represents L.S.D. (P=0.05), df = 38.**

'Ben Tirran'

Chilling duration and defoliation date had significant (P<0.001) effects on time to first bud burst, but there was no significant (P=0.135) interaction. Bud burst was advanced after defoliation on 1 September (Figure 6.5.), but as the date of defoliation was delayed, the effect on time to bud burst was reduced. Increasing the chilling duration from 0 to 10 weeks significantly advanced bud burst.

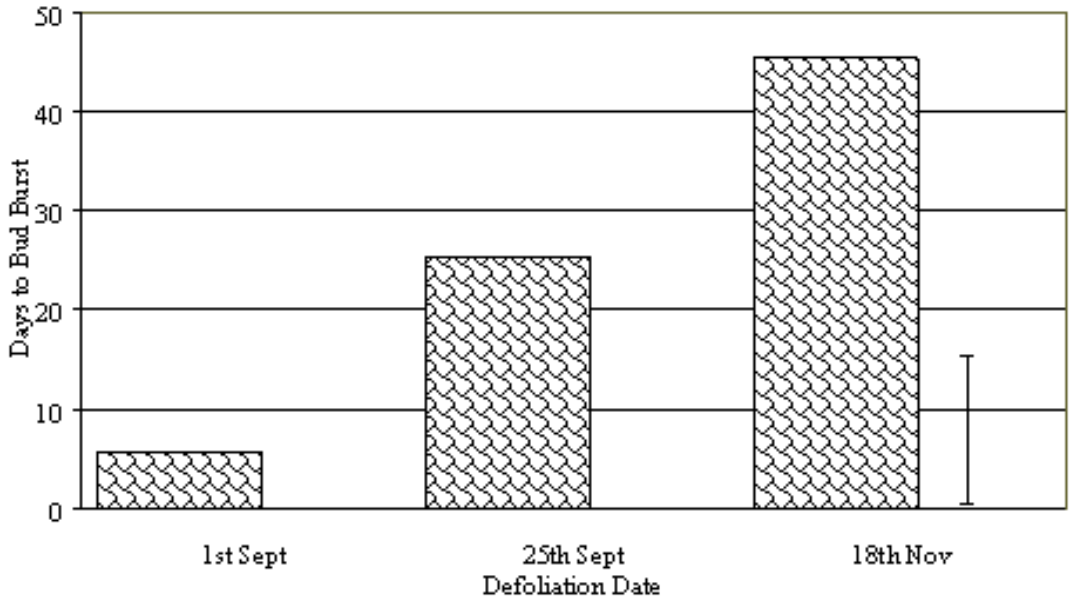


Figure 6.5. ***R. nigrum*** **'Ben Tirran' - effect of defoliation date on time to first bud burst. Data pooled across chilling durations. Error bar represents L.S.D. (P=0.05), df = 4.**

Final Bud Burst

Although the effects of chilling duration and defoliation date were highly significant (P<0.001), there were no significant interactions between the main treatment factors: cultivar and defoliation date (P=0.178), cultivar and chilling duration (P=0.295) or cultivar, defoliation date and chilling duration (P=0.220). Regardless of treatment, there was no significant difference between 'Ben Gairn' and 'Ben Hope', whereas final bud burst of 'Ben Tirran' was significantly (P<0.001) lower.

Defoliating on 1 September significantly (P<0.001) improved bud burst but as the defoliation date was delayed, final bud burst significantly (P<0.001) decreased (Figure 6.6). As the chilling duration increased from 2 to 10 weeks, final bud burst significantly (P<0.001) increased.

Every plant defoliated on 1 September suffered tip die-back on every stem. Initially, the terminal buds only seemed affected, but as the growing season progressed, the necrosis continued down the stem. Had the plants been carried on another season, it is likely that whole plant die-back would have been recorded.

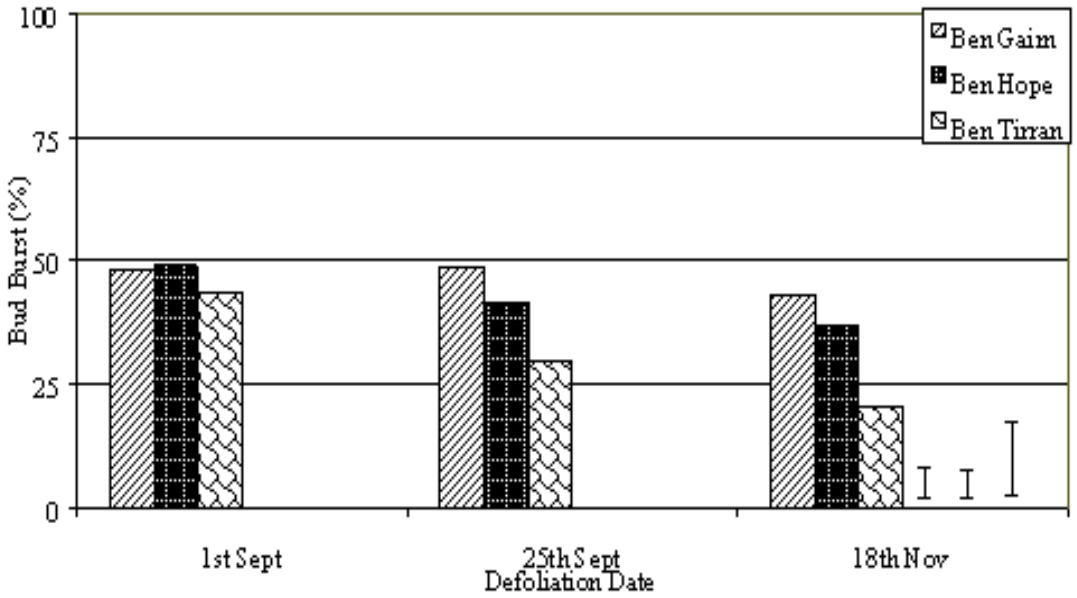


Figure 6.6. ***R. nigrum*** **- effect of defoliation date on final bud burst. Data pooled across chilling durations. First error bar represents 'Ben Gairn', second represents 'Ben Hope', third represents 'Ben Tirran', L.S.D (P<0.05) df.=130.**

6.3.3. Experiment 6. Photoperiodic Lighting

Time to First Bud Burst

Cultivar, photoperiodic regime, chilling duration and the interactions between these factors were found to be highly significant (P<0.001) so data were re-analysed on an individual cultivar basis.

'Ben Gairn'

Photoperiod and the interaction (Figure 6.7) between photoperiod and chilling temperature were significant (P=0.027; P=0.011 respectively), hence data were further sub-divided and re-analysed.

Faster bud burst was associated with budsticks chilled for 2 weeks then exposure to natural or 17-hour photoperiods. After 8 weeks of chilling, budsticks exposed to either 8-hour or 17-hour photoperiods burst bud significantly faster than those exposed to natural daylength.

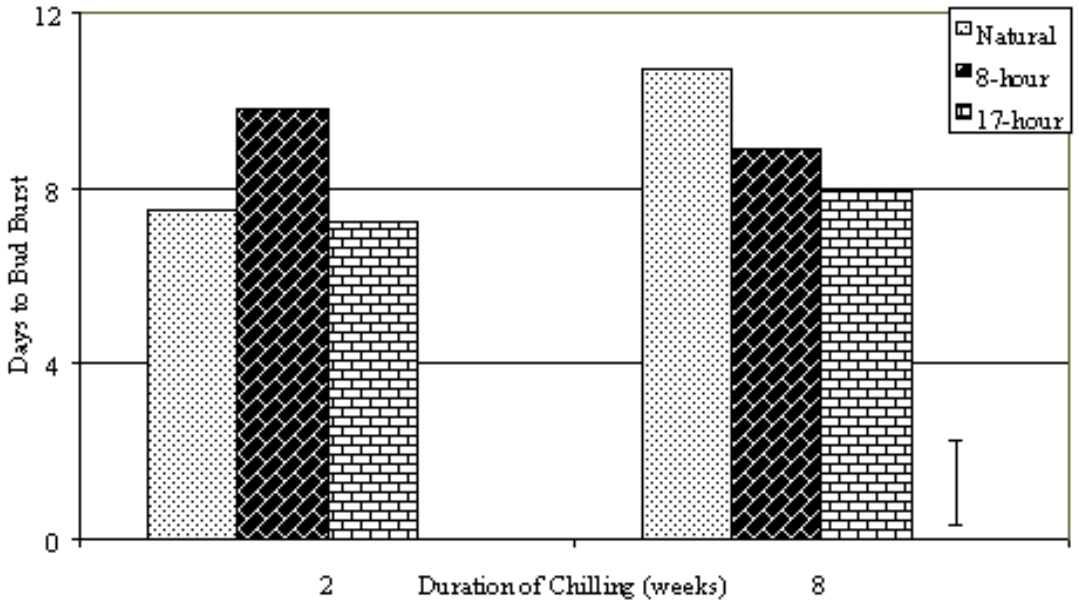


Figure 6.7. ***R. nigrum*** **'Ben Gairn' - effect of chilling duration and photoperiodic regime on time to first bud burst. Error bar represents L.S.D. (P=0.05), df = 138.**

'Ben Hope'

There was a significant effect of chilling duration (P<0.001), photoperiod (P=0.015) and a strong interaction (P<0.001) between the two factors (Figure 6.8).

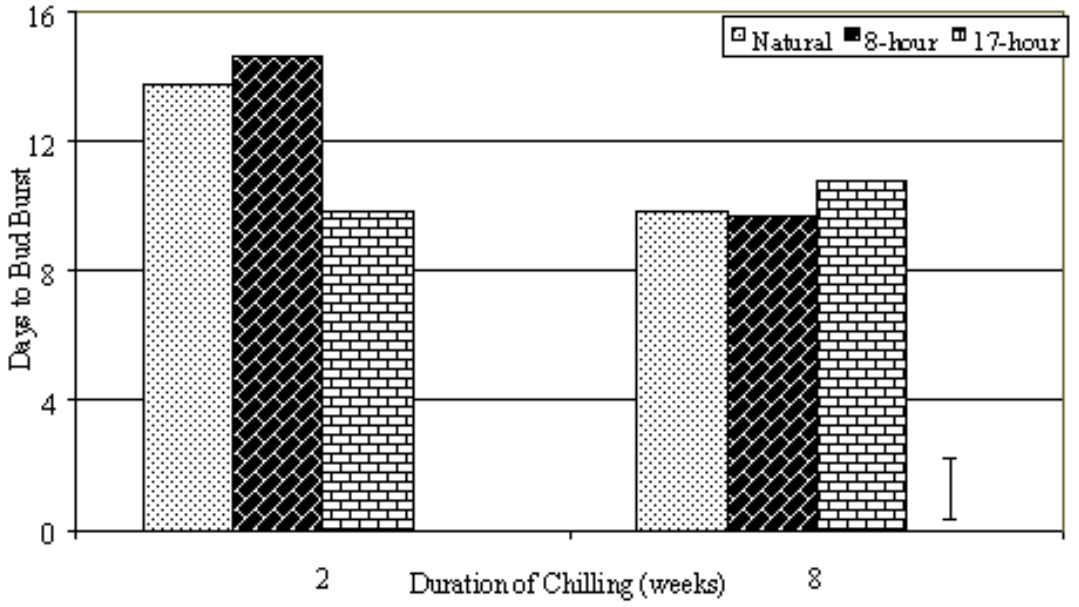


Figure 6.8. ***R. nigrum*** **'Ben Hope' - effect of chilling duration and photoperiodic regime on time to first bud burst. Error bar represents L.S.D. (P=0.05), df = 171.**

After two weeks of chilling, budsticks exposed to the 17-hour regime bud burst faster than those exposed to shorter photoperiods. As the chilling requirement was increasingly satisfied, the effect of photoperiod became insignificant.

'Ben Tirran'

The effects of chilling duration and photoperiod were highly significant (P<0.001) but there was no significant (P=0.307) interaction between the two factors (Figure 6.9).

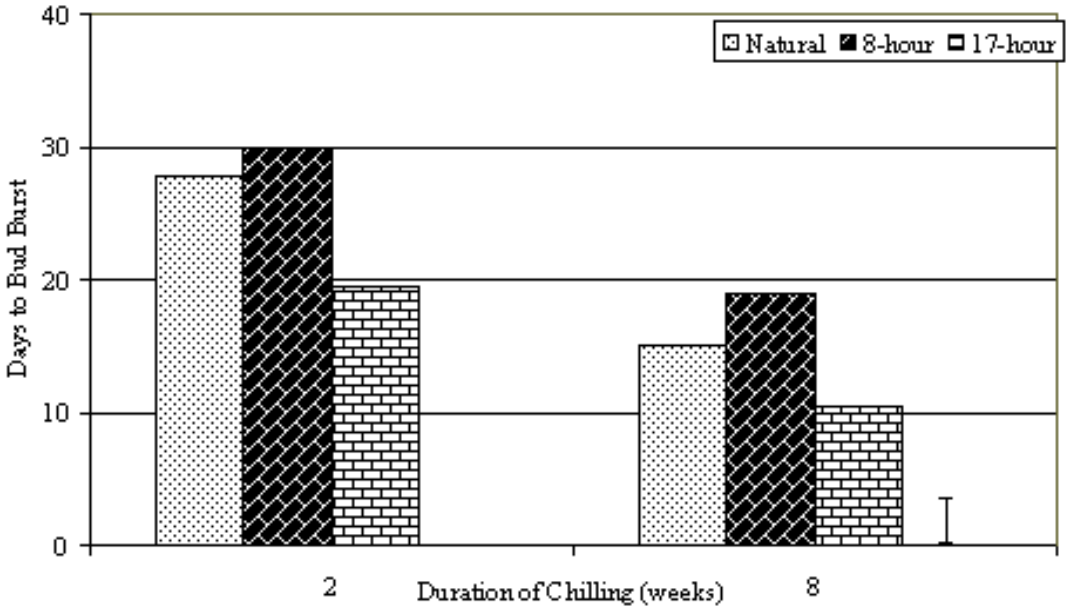


Figure 6.9. ***R. nigrum*** **'Ben Tirran' - effect of chilling duration and photoperiodic regime on time to first bud burst. Error bar represents L.S.D. (P=0.05), df = 129**

Regardless of chilling duration, bud burst occurred significantly (P<0.001) faster after exposure to 17-hour photoperiods. For all photoperiodic treatments, increasing the chilling duration from 2 to 8 weeks resulted in faster bud burst.

Final Bud Burst

The three-way interaction between cultivar, photoperiod and chilling duration was highly significant (P<0.001). As such, data were sub-divided and re-analysed.

'Ben Gairn'

A strong interaction between chilling duration and photoperiod (P<0.001, Figure 6.10) was evident and data were sub-divided and re-analysed. After chilling for 2 weeks, bud burst was promoted by extending the photoperiod to 17-hours. In contrast, after 8 weeks of chilling although generally more uniform, higher levels of bud burst were associated with budsticks exposed to the 8-hour photoperiod.

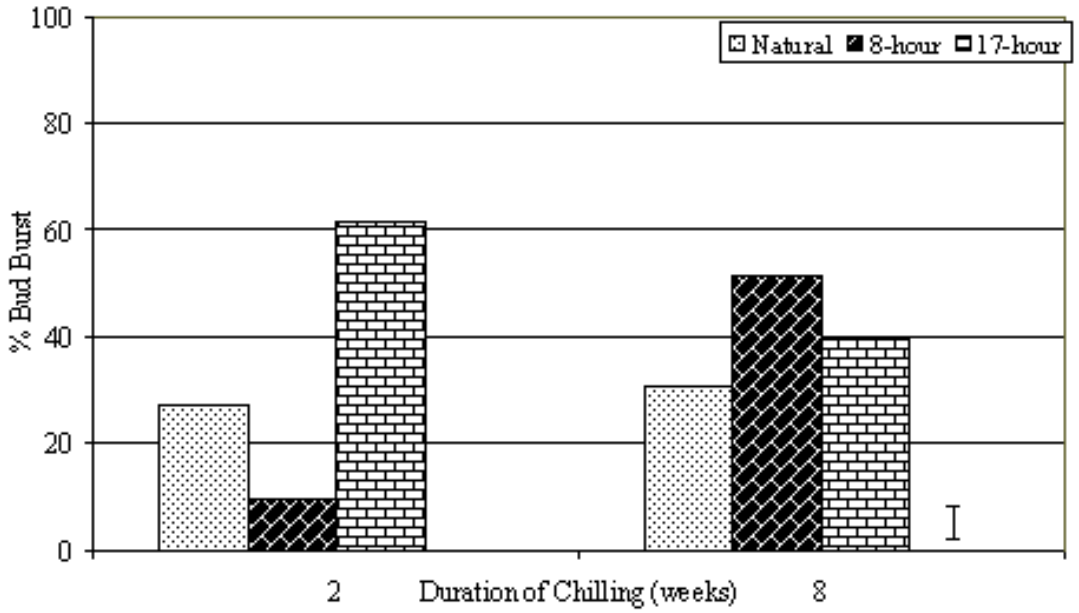


Figure 6.10. ***R. nigrum*** **'Ben Gairn' - effect of chilling duration and photoperiodic regime on final bud burst. Error bar represents L.S.D (P=0.05), df = 174.**

'Ben Hope'

Highly significant effects of chilling duration and photoperiod (P=0.001) were recorded. As the interaction between the two factors was also significant (P=0.031), data were re-analysed (Figure 6.11).

When budsticks were placed under natural or 8-hour photoperiods, increasing chilling duration promoted significantly (P<0.001) higher levels of bud burst. After two weeks of chilling, exposure to extended photoperiod (17-hours) promoted bud burst shorter-day

treatments. As the chilling duration increased and the chilling requirement was increasingly satisfied, the effect of photoperiod became insignificant.

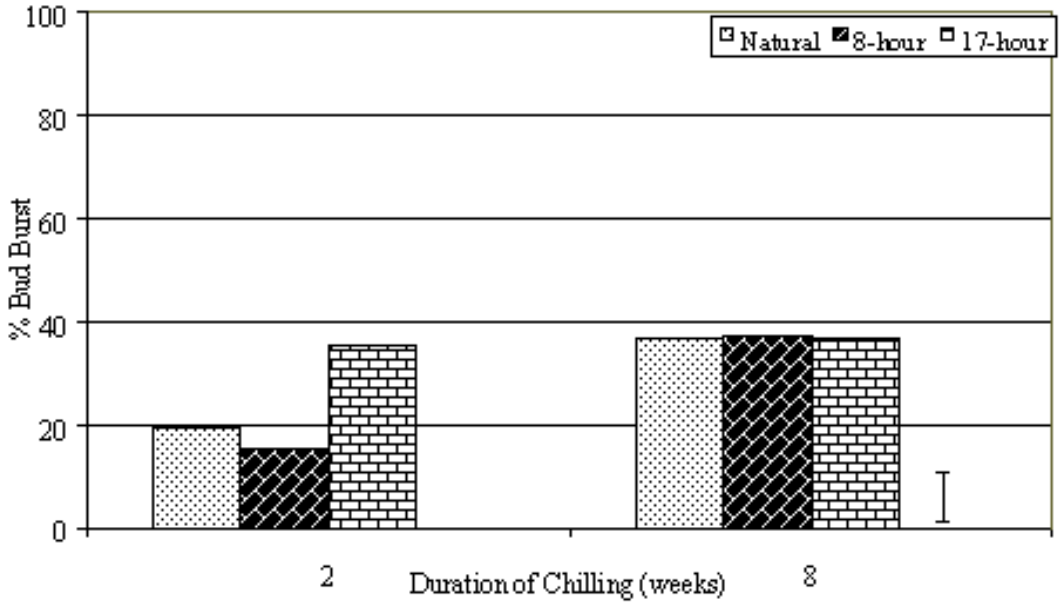


Figure 6.11. ***R. nigrum*** **'Ben Hope' - effect of chilling duration and photoperiodic regime on final bud burst. Error bar represents L.S.D. (P=0.05), df = 174.**

'Ben Tirran'

There was a significant effect of chilling duration, photoperiodic regime (P<0.001) and a strong interaction (P<0.001) between the two factors (Figure 6.12).

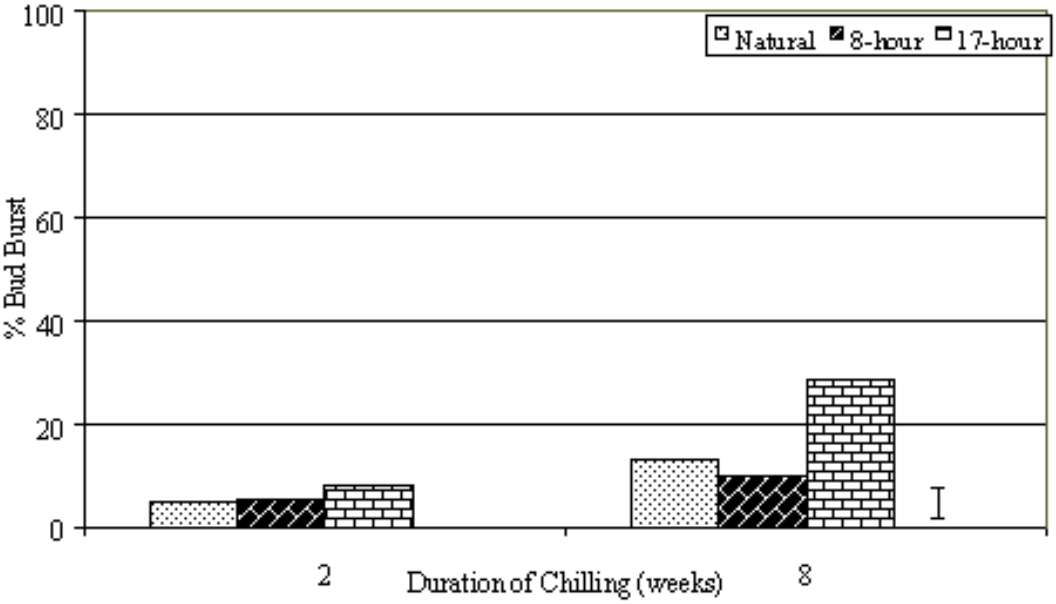


Figure 6.12. ***R. nigrum*** **'Ben Tirran' : effect of chilling duration and photoperiodic regime on final bud burst. Error bar represents L.S.D. (P=0.05), df = 179.**

After two weeks of chilling, final bud burst was extremely low and there was no significant effect of photoperiod. As the chilling requirement was increasingly satisfied, exposure to a 17-hour photoperiod significantly increased bud burst. A positive effect of increasing chilling duration was recorded for all treatments.

Experiment 7. Dormancy Breaking Chemicals

Control budsticks, sprayed with water, did not burst bud and hence were excluded from statistical analyses. Adjuvant and concentration were statistically significant ($P<0.001$; $P=0.009$ respectively) as was the interaction ($P=0.033$, Figure 6.13) so data were sub-divided and re-analysed.

Application of Activator 90 resulted in a higher final percentage bud burst compared to the other two chemicals and increasing the concentration of both Activator 90 and Abacus from 0.5% to 2% increased bud burst significantly. Bud burst was generally poor, regardless of chemical or concentration.

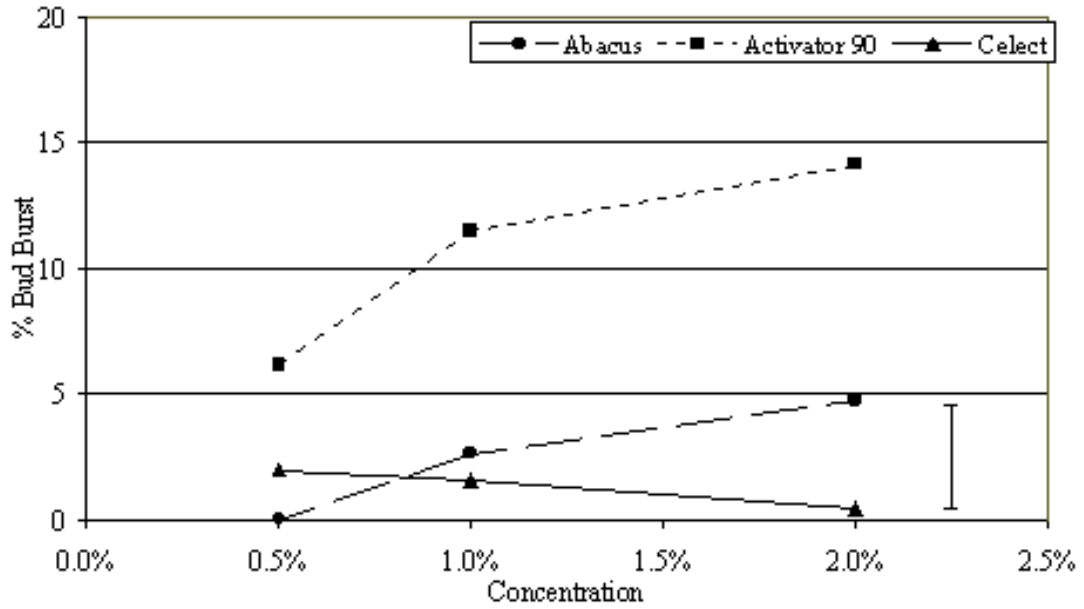


Figure 6.13. ***R. nigrum*** **'Ben Tirran' - effect of dormancy breaking chemical and concentration on final percentage bud burst. Error bar represents L.S.D. (P=0.05), df = 81.**

6.4. Discussion

6.4.1. Bud Burst Advancement

Variable results were achieved with these experiments. While post-harvest nitrogen application did not affect bud burst, premature defoliation and extended photoperiods were extremely effective.

Post-harvest nitrogen application had no effect on the date of bud burst of 'Ben Gairn' and 'Ben Hope', regardless of concentration. The results of previous research on this topic has proved contradictory. Nitrogen application to *Malus domestica* Cox's Orange Pippin (Delap, 1967) and *M. domestica* 'Golden Delicious' (Terblanche *et al*., 1979) advanced bud burst by a maximum of 30 days. However, application to *Prunus persica* 'Flordaprince' (George and Nissen, 1992) and *M. domestica* 'Lord Lambourne' (Hill-Cottingham and Williams, 1967) delayed bud burst by 17 and 28 days respectively.

Date of nitrogen application appears to be significant and it is possible that treatment was applied too late in the growing season for the *Ribes nigrum* plants to benefit. April applications of nitrogen advanced bud burst of *M. domestica* 'Lord Lambourne' by 28 days (Hill-Cottingham and Williams, 1967). As the application date was delayed, beneficial effects became less apparent - August application advanced bud burst by 14 days and November application had no effect (Hill-Cottingham and Williams, 1967). Similarly, winter (September-December) applications of nitrogen to *M. domestica* 'Cox's Orange Pippin' decreased the time to first bud burst whereas spring (January-March) and summer (May-July) applications had no significant effect (Delap, 1967).

When chilled for a maximum of 2 weeks, defoliation on 1 September advanced bud burst compared to plants that senesced naturally. Response to defoliation on 25 September, however, was found to be dependant on cultivar – bud burst was significantly advanced for 'Ben Gairn' and 'Ben Tirran', but no effect was evident for 'Ben Hope'. Premature defoliation of *Prunus persica* 'Flordaprince' and 'Flordagold' significantly advanced bud burst, but earlier (April) defoliation was more effective than later (May) defoliation (Lloyd and Firth, 1990). Previous *Ribes nigrum* research has reported no effect of defoliation on time to bud burst (Plancher, 1983b; Westmore, 2004). Both authors had concluded, however, that defoliation was conducted too late in the season to be of benefit to plants and Plancher (1983b) defoliated *R. nigrum* 'Klone 8' and 'Vija' just 4 weeks before natural senescence.

As the chilling duration of 'Ben Gairn' and 'Ben Hope' increased to 6 weeks, and the chilling requirement increasingly satisfied, defoliation had no effect on time to bud burst. A similar result was recorded for *Ribes nigrum* 'White Bud' (Westmore, 2004). After 10 weeks of chilling, defoliation date was still significant for 'Ben Tirran', which is perhaps a reflection of this cultivar's higher chilling requirement. This treatment, therefore, may be particularly advantageous for cultivars with higher chilling requirements.

When applied in tropical countries, premature defoliation of *Malus domestica* induced bud burst by 4 weeks whereas control plants failed to burst, suggesting that the plants were carrying a large chilling deficit (Edwards, 1987). Premature defoliation was suggested to compensate for 100% of the chilling requirement of *Prunus persica* 'Flordaprince' which, according to the Utah model, had received none of its 150 chill units (Lloyd and Firth, 1990). Had this been the case in this experiment, after defoliation on 1 or 25 September, the date of bud burst should have been comparable within a cultivar, regardless of the chilling duration supplied. Control plants that received no artificial chilling, however, bud burst significantly later than plants that had been chilled, suggesting that although defoliation could partly substitute for insufficient chilling, it could not be used as a substitute for 100% of *Ribes nigrum's* chilling requirement.

Bud ABA concentration increased to a maximum at the onset of dormancy then steadily decreased to a minimum in spring, at which time bud burst occurred (Mielke and Dennis, 1978). Bud burst advancement, as a result of premature defoliation, supports the theory that ABA is transported from the leaves to the buds. Premature defoliation would reduce the concentration of ABA transported from leaves to the buds, resulting in an initial low level and a decrease in the depth of dormancy (Mielke and Dennis, 1978). Depth of *Prunus persica* 'Gleason Elberta' dormancy was determined by the GA_3 concentration required to promote bud bust and defoliated trees required 75% less GA_3 than trees that were not defoliated (Walser *et al*., 1981), indicating that the depth of dormancy was significantly lower than that of cold-treated plants.

Bud burst response to photoperiodic lighting was cultivar-dependant. For both chilling durations (2 weeks and 8 weeks) and for all three cultivars, exposure to long (17-hours) photoperiods was more effective than exposure to short photoperiods (8-hours). The

exception to this was 'Ben Hope', where photoperiod had no effect on bud burst after 8 weeks of chilling. This result is supported by Hoyle (1960), who reported that long day conditions induced bud burst of *Ribes nigrum* compared to short day conditions. Similarly, bud burst of *Fagus sylvatica* (Wareing, 1954a) *Betula pubescens*, *Betula pendula*, *Alnus incana*, *Alnus glutinosa*, *Prunus padus*, *Populus tremula* (Heide, 1993a) and *Acer saccharum* (Olsted, 1951) was advanced after exposure to long days compared to short days. However, *Cornus alba* seedlings transferred from short day to long day conditions did not bud burst faster than those exposed only to short days (Whalley and Cockshull, 1976). It is possible that the behaviour of plants when exposed to long day conditions is species-specific, as demonstrated by Wareing (1954b). *Fagus sylvatica, Larix decidua* and *B. pubescens* exposed to long day conditions burst bud whereas *Robina pseudoacacia* and *Acer pseudoplatanus* remained dormant. The effect of provenance may explain the species/cultivar specificity reported. Previous research with *Pseudotsuga menziesii* concluded that the relationship between provenance and photoperiod was complex and significant (Campbell and Sugano, 1975). However, few researchers have investigated this topic to provide definitive answers.

6.4.2. Bud Burst Promotion

Nitrogen application had no effect on final bud burst of *Ribes nigrum* 'Ben Gairn' and 'Ben Hope'. Given the lack of response described in the previous section, this is not surprising. However, beneficial effects of nitrogen fertilisation on plant growth have been reported. Compared to untreated controls, nitrogen application to *Prunus persica* 'Flordaprince' increased the number of leaves produced, leaf dry matter, tree height, tree spread and over-all dry weight (George and Nissen, 1992). When applied to *Malus domestica* 'Lord Lambourne' in April, an increase in the number of nodes produced, scion dry weight and leaf production was recorded, but August and October applications had no effect (Hill-Cottingham and Williams, 1967).

Further evidence to support the theory that the nitrogen was applied too late to be of benefit in this experiment is that the date of senescence of nitrogen-treated plants was the same as that of control plants. Had the nitrogen been taken up, senescence should have been delayed, as reported for *P. persica* 'Flordaprince' (George and Nissen, 1992) and *M. domestica* 'Lord Lambourne' (Hill-Cottingham and Williams, 1967). No effect of nitrogen application on *M. domestica* 'Lord Lambourne' was evident after application in November, however and the authors hypothesised that treatment was applied too late and not utilised by the trees, hence

the lack of effect. Prior to delayed abscission, elevated leaf nitrogen concentrations were reported in treated *P. persica* 'Flordaprince' (George and Nissen, 1992). Similarly, *M. domestica* 'Lord Lambourne' given nitrogen in April and August had a higher leaf nitrogen content than those supplied with nitrogen in October (Hill-Cottingham and Williams, 1967). Although there was no visible difference in *R. nigrum* leaf colour, enhanced leaf nitrogen content may not have been discernable to the eye.

Unlike *Fragaria ananassa*, *Oleo europaea* and *Ribes nigrum*, there was no cultivar-specific effect of premature defoliation on the final bud burst of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' (Guttridge and Mason, 1966; Rallo and Martin, 1991; Plancher, 1983b). Maximum bud burst of all three cultivars occurred after defoliation on 1 September and as the defoliation date was delayed, the beneficial effect became less significant. This result should not have been expected as it is widely supported by previous research on *F. ananassa* (Guttridge and Mason, 1966; Guttridge *et al*., 1961), *R. nigrum* (Tinklin and Schwabe, 1970; Plancher, 1983b) and *Prunus persica* 'Flordaprince' (Lloyd and Firth, 1990).

Final bud burst of *Ribes nigrum* 'White Bud' (Westmore, 2004), *R. nigrum* 'Klone 8' (Plancher, 1983b) and *Oleo europaea* (Rallo and Martin, 1991) were unaffected by premature defoliation. However, in all of these experiments, defoliation was conducted late in the season ('Klone 8' was defoliated mid-October, only a month before natural senescence) and Westmore (2004) suggested that advancing the date of defoliation may prove to be more effective at promoting bud burst. Although, in terms of final bud burst, the optimum treatment was defoliation on 1 September, all plants in this treatment suffered from tip die-back, the severity of which increased as the growing season progressed. Initially the terminal bud seemed only to be affected, and the buds below this burst as normal. As the season progressed however, the die-back continued down the stems, with the previously-burst buds dying. This effect was also reported by Corke and Wilson (1963) who discovered that defoliation of *R. nigrum* 'Baldwin' prior to 22 August resulted in tip die-back. Premature defoliation of *P. persica* 'Gleason Elberta' resulted in limb die-back, and high incidences of flower abscission were reported (Walser *et al*., 1981). Similar damage had been reported after premature defoliation by mites or severe weather (Westwood, 1978). Damage was reported to prematurely-defoliated *F. ananassa*, but further investigation revealed it was as a result of mechanical or pathogen attack. (Guttridge and Mason, 1966). Die-back may occur as a result of buds not gaining cold-hardiness before defoliation. Westwood (1978) reported that

premature defoliation prevented or delayed tissue maturity and hence negatively affected the ability of the plant to acclimatise to cold winter conditions. After defoliation of *P. persica* 'Gleason Elberta', temperatures fell to -20°C, which may account for the vegetative and floral die-back reported (Walser *et al*., 1981).

Exposure to 17-hour photoperiods after chilling 'Ben Gairn' and 'Ben Hope' for 2 weeks and 'Ben Tirran' for 8 weeks resulted in an increase in final bud burst. As the chilling requirement of 'Ben Gairn' was satisfied, plants exposed to the 8-hour photoperiod burst more buds. There was no effect of photoperiod when 'Ben Hope' and 'Ben Tirran' were chilled for 8 and 2 weeks respectively. The results obtained are partly supported by Hoyle (1960), who reported that *Ribes nigrum* exposed to 17-hour photoperiods burst significantly more buds than those exposed to 8-hour photoperiods. Heide (1993b) discovered that when *Alnus glutinosa*, *Alnus incana*, *Betula pendula*, *Betula pubescens*, *Fagus sylvatica*, *Populus tremula* and *Prunus padus* were insufficiently chilled, plants exposed to 20-hour photoperiods had a higher final percentage bud burst than those that were subjected to 8-hour photoperiods. As the chilling requirements of the trees were fulfilled, however, the effect of photoperiod decreased, as reported for 'Ben Hope' in this experiment

After exposure to 17-hour photoperiods, *Ribes nigrum* burst bud whereas plants under an 8-hour photoperiod did not, hence it was concluded that photoperiod was a substitute for chilling deprivation (Hoyle, 1960). The results produced by 'Ben Tirran' in this experiment, however, do not support this theory. The generally low final bud burst values after chilling for 2 weeks suggests that the chilling requirement of 'Ben Tirran' had not been fulfilled. Indeed, the lack of effect of photoperiod is an indication that the chilling deficit was too large to overcome. After a further 6 weeks of chilling, however, an effect of photoperiod was observed. The effect of photoperiod is reported to be species-specific (Wareing, 1954a; Heide, 1974a). The results of this experiment suggest that photoperiodic response may also be cultivar-specific.

Application of 2% Activator resulted in the highest final bud burst, and there was no difference between budsticks treated with either Abacus or Celect, regardless of concentration. Concentrations tested in the field have ranged from 10% Abacus to 5% with very little difference in the bud burst potential being recorded (Saunders, R., *Pers. □inim.*). The low percentage bud burst reported in this experiment, therefore, may be a result of the

low concentrations applied. Adjuvant application was successful in that bud burst was promoted compared to the controls, but final bud burst values were significantly lower (16%) than the commercially acceptable 75%. This may partly be attributed to the fact that 'Ben Tirran' had only received a very small proportion (circa. 30%) of the chilling requirement. As in the photoperiodic experiment, the chilling deficit may have been too great for chemical application to overcome. Additionally, this experiment was conducted using mature budsticks and as reported in Chapter 3, the bud burst potential of budsticks may be significantly lower than that of whole plants. Therefore, the low bud burst reported is likely to be a combination of a high chilling deficit, low adjuvant concentration and choice of plant material.

6.4.3. Comparison of Treatments

The most successful treatments for advancing bud burst were premature defoliation and extended photoperiod. Premature defoliation advanced bud burst of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' by 13, 14 and 40 days, whereas photoperiodic extension was less effective, but still advanced bud bust by 2, 5 and 20 days respectively. In contrast, photoperiod extension increased final bud burst of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' by 50%, 20% and 20%, whereas premature defoliation was less effective and increased bud burst by 5%, 12% and 23%. For all cultivars, bud burst was advanced most and final bud burst greatest after exposure to premature defoliation compared to extended photoperiodic lighting. This treatment is also, practically, the most easiest to apply

6.4.4. Additional Effects

In all experiments aimed at inducing premature bud burst, careful consideration must be given to the implications of any treatment. In particular, care must be taken to ensure that bud burst is not advanced so much that the newly emerging leaves/flowers are at risk of being damaged by frost. This is of particular importance to 'Ben Tirran', where bud burst was advanced by 60 days after defoliation on 1 September. It was not possible to determine the effects of treatment on flower and fruit production, and since the crop is harvested for profit, any effects must be quantified before being recommended to growers. Although nitrogen application increased *Malus domestica* 'Golden Delicious' yield (Terblanche *et al*., 1979), detrimental effects of premature defoliation on *Ribes nigrum* yield were observed (Corke and Wilson, 1963). Post-harvest nitrogen application may result in excess growth and prevent the plantation attaining cold-hardiness. Detrimental effects on bud hardiness may result in plant

death. Premature defoliation may reduce available carbohydrate reserves for the following spring and hence have an adverse affect on flowering and fruit production. Further experiments are essential to determine such effects.

6.4.5. Practical Applications

From a practical perspective, if future research proves post-harvest nitrogen application to be of benefit, it is a relatively easy process to conduct and may be combined with autumn pest management practices.

Similarly, premature defoliation would be relatively easy to apply. Mechanical harvesting of *Ribes nigrum* is in itself a destructive process and results in partial defoliation of bushes. Post-harvest pest infestation can often be a problem in *R. nigrum* plantations and growers regularly implement post-harvest pest and disease practices. Insect damage e.g. *Nematus ribesii* (sawfly) may result in premature defoliation, as might disease e.g. *Pucciniastrum epilobi* (rust) and *Septoria ribus* (leaf spot). If such pests/diseases are present in a crop, a cost-effective solution may involve not spraying the crop and allowing the pest/disease to defoliate the bushes (Saunders, R., *Pers. Comm.*). Further research must be conducted, however, to ensure that such treatment, or lack of, does not have adverse reactions on the following year's bud burst and cropping potential. Alternatively, chemical application e.g. copper sulphate and zinc sulphate has been □inimize to promote premature defoliation of *Malus domestica* (Hermano *et al*., 1987, and such treatment may be prove effective for *R. nigrum*. Again, research must be conducted to ensure such application does not adversely affect the following year's bush performance.

In reality although extending the photoperiod had beneficial effects on bud burst the cultivar-specificity demonstrated in this experiment would require further research to be conducted, □inimize□ a range of *Ribes nigrum* cultivars. Extending the natural photoperiod by an hour either side of sunset/sunrise would be difficult but not impossible for growers to implement, but extending the photoperiod to 17-hours is not a practical solution. The financial implications, including set-up costs and running fees, are likely to out-weight any bud burst and yield benefits.

Application of dormancy-breaking chemicals perhaps holds the most potential. Growers have accepted the practice of applying such chemicals, and many have started small in-house

experiments using different active ingredients and concentrations. Future research should be directed towards quantifying how much of the chilling deficit such applications can substitute for. In addition, the time-lag between application and bud burst must be determined, again in order to minimize the risk of frost damage to newly expanding leaves and flowers.

6.5. Conclusions

Post-harvest nitrogen application had no beneficial effect on either the time to first bud burst or final bud burst of 'Ben Gairn' and 'Ben Hope'. The insignificant effect, however, on date of senescence between nitrogen-treated plants, suggests that application was applied too late in the season for the plants to have benefited.

Premature defoliation on 1 September significantly advanced bud burst of all cultivars but defoliation on 25 September was cultivar specific, with significant advances being recorded for 'Ben Gairn' and 'Ben Tirran'. Premature defoliation significantly increased final bud burst, with early defoliations more effective than later treatments. Every plant exposed to defoliation on 1 September, however suffered tip die-back that worsened as the season progressed. Delaying defoliation until 25 September increased bud burst, but to a lesser degree than the earlier treatment and did not result in tip die-back.
Photoperiod extension was found to be cultivar-specific, but generally exposure to 17-hour photoperiods advanced and increased bud burst compared to exposure to 8-hour photoperiods.

Overall, bud burst of the adjuvant-treated budsticks was extremely low, perhaps due to the use of budsticks compared to whole plants, the large chilling deficit or the low concentration. Application of 2% Abacus was the optimum treatment, which suggests that higher concentrations may improve the results.

While application of post-harvest nitrogen, premature defoliation or dormancy-breaking chemical application may be relatively easy to apply to *Ribes nigrum* plantations, extending the photoperiod in the field to 17-hours is less easy. This treatment, therefore has limited practical applications. In general, further research is advised using a wider range of *R. nigrum* cultivars, to determine differences due to cultivar. Additionally, care must be taken not to advance bud burst so much that frost damage becomes a risk and future research should concentrate on quantifying the effects on flower and fruit production. The process of *R.*

nigrum flower initiation and development, however, are lacking, and must be quantified to understand the effects of dormancy-breaking treatments.

Chapter Seven. *Ribes nigrum* Flower Initiation and Development

Introduction

Detrimental effects of flower production in response to elevated spring temperatures were observed (Chapter 5), but in order to fully understand the effects of predicted climate change scenarios on flower production, the timing and rate of floral development in modern-day cultivars under natural chilling conditions must first be established.

7.1.1. Stages of Flower Formation

Flower initiation is typically identified by stages, the number and description of which varies with author and species. Booij *et al*. (1992) and Mathers (1952) listed six stages for *Apium graveolens* and *Rubus ideas* respectively, whereas Robertson (1957) and Horridge and Cockshull (1974) listed 11 stages for *Rubus* spp. and *Rosa* spp. Regardless of species, the first two stages of flower initiation appear to be uniform.

In the first stage (Figure 7.1), referred to as 0 or 1, the apex is vegetative, characterized by a flat or slightly convex apex (Roefolfse and Hand, 1989) and surrounded by newly produced leaves (Taylor *et al*., 1997).

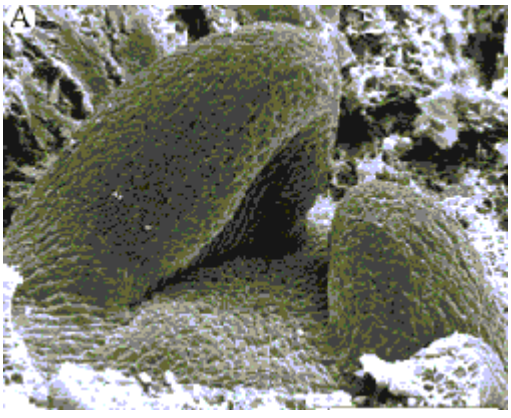


Figure 7.1. Scanning electron microscope image of *Prunus persica* 'Redhaven' flower initiation Stage 0/1. (after Engin and Iqbal, 2004)

The second stage, termed 1 or 2, is identified by a raised and elongated apex (Cathey and Borthwick 1957; Roefolfse and Hand, 1989; Taylor *et al*., 1997). Formation of the perianth ring has also been classed as the second stage of initiation (Mathers, 1952; Robertson, 1957). Floral development beyond this may be dependant on species, but also on the frequency of dissections and author - Mathers (1952) listed six stages of *Rubus ideaus* flower development but Robertson (1957) observed 11 stages for *Rubus* spp.

7.1.2. Flower Formation in *Ribes nigrum*

Nasr and Wareing (1961) investigated the timing of flower initiation in *Ribes nigrum* 'Victoria' and found that 19% of buds had initiated flowers by 15 August, and 86% by 29 August. In a similar study, Vestrheim (1972) investigated the time of floral initiation in *R. nigrum* 'Bang Up', 'Boskoop', 'Brodtorp', 'Silvergieter' and 'Wellington XXX' and discovered all cultivars except 'Bang Up' had initiated flowers by 15 August. Wilson and Adam (1966), however, reported that *R. nigrum* cultivars had initiated floral primordia nine days earlier than reported by Nasr and Wareing (1961) and Vestrheim (1972). These discrepancies may be explained by the theory that early flowering cultivars initiate flowers earlier than later flowering cultivars (Vestrheim, 1972). Further experimentation investigated the timing of floral initiation at three different heights from the base – bottom (nodes 0-6), middle (7-12) or top (13-18) (Nasr and Wareing, 1961). Flowers were not initiated in buds 0-6, and by 22 August circa. 50% of the buds in the middle section had initiated flowers compared to 30% of buds in the top section. All buds in the top and middle sections contained flower initials by 5 September (Nasr and Wareing, 1961). The authors suggested that lower buds failed to initiate flowers because they had entered into a state of dormancy prior to 22 August.

The aim of the experiment reported here was to investigate the timing and rate of floral initiation and development in early, mid, and late flowering *Ribes nigrum* cultivars, and to determine the effect of bud position.

Materials and Methods

Plant material consisted of two-year old softwood cuttings of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', as described in Chapter 2. The plants were delivered to the University of Reading's Experimental Field Site on 2 July 2003, tied onto supporting wires to keep the pots upright and irrigated automatically with Avoncrop's Soft Fruit Mix 2 (6:11:31 N:P:K) nutrient solution four times a day for a total of one hour until 28 September 2004.

Spider mites were observed in the centre of the buds on 11 April 2005 and plants were originally treated with an overhead spray of bifenthrin (Talstar®) at a rate of $0.4mlL^{-1}$. Mites were again observed the following week and plants were treated with thiachloroprid (Calypso®) at a rate of $1mlL^{-1}$ on 22 April 2005 and 29 April 2005. A follow-up spray of abamectin (Dynamec®) on 12 May 2005 at a rate of $0.3mlL^{-1}$ successfully eradicated the mites.

Using a pair of sharpened secateurs, cutting material containing the current year's growth was taken from 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' and cuttings were labeled and placed into beakers of water. Buds at nodes 2, 4, 6 and 10 from the base of the budstick were dissected by slicing away the bud scales and leaves, and then exposing the apex at the base of the bud. Samples were viewed using an M400 Polyvar dissecting microscope. Number of leaves, flower stage and number of flowers were recorded. The first dissection was carried out on 25 May 2004 and subsequent dissections conducted at regular intervals.

Data relating to the stage of flower development over time was analysed in two parts. Initially, data encompassing 25 May to 16 July 2005 were analysed, then data from 16 July to the termination of the experiment were analysed.

Statistical analyses were conducted using Genstat V's ANOVA to determine differences between cultivars, bud position and dissection date.

Results

Total Flower Production

The effects of dissection date and bud position were highly significant (P<0.001; P=0.002), but the interaction was not (P=0.961). Significantly fewer flowers were produced by buds at node two compared to nodes higher up the stem (Figure 7.2).

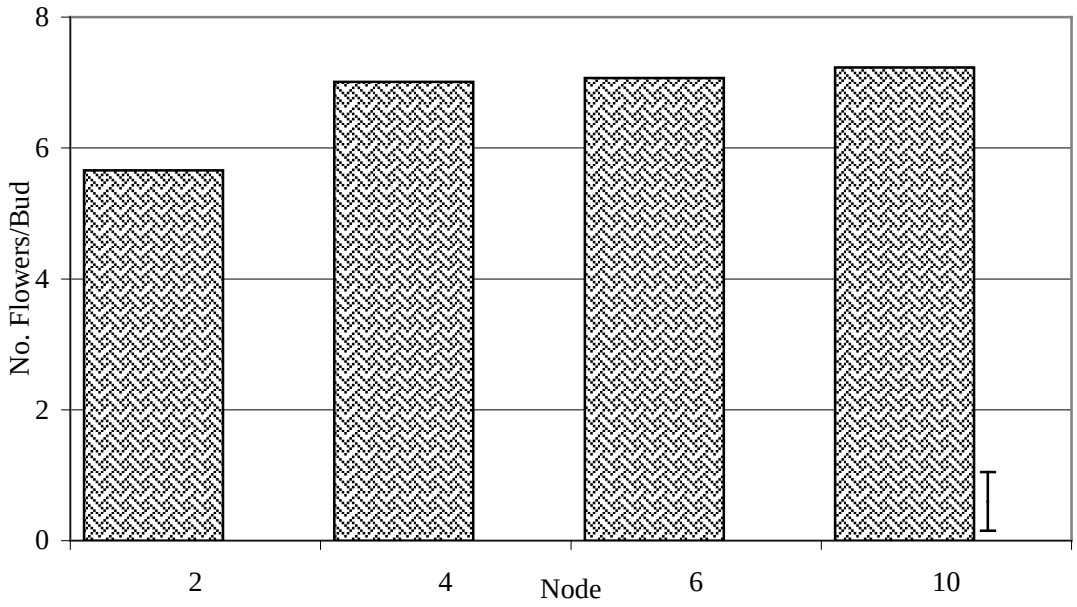


Figure 7.2. ***R. nigrum*** **'Ben Gairn' – effect of bud position on flower production. Data pooled across bud dissection dates. Error bar represents L.S.D. (P<0.05), d.f. = 461**

There was a large degree of variation within the results, but in general the number of flowers produced by each bud increased between 21 September 2004 and 16 February 2005, after which there was a significant decrease (Figure 7.3). Prior to flower emergence, there was a significant increase in the number of flowers produced from 16 February 2005 until 29 April 2005, after which there was again a significant decrease.

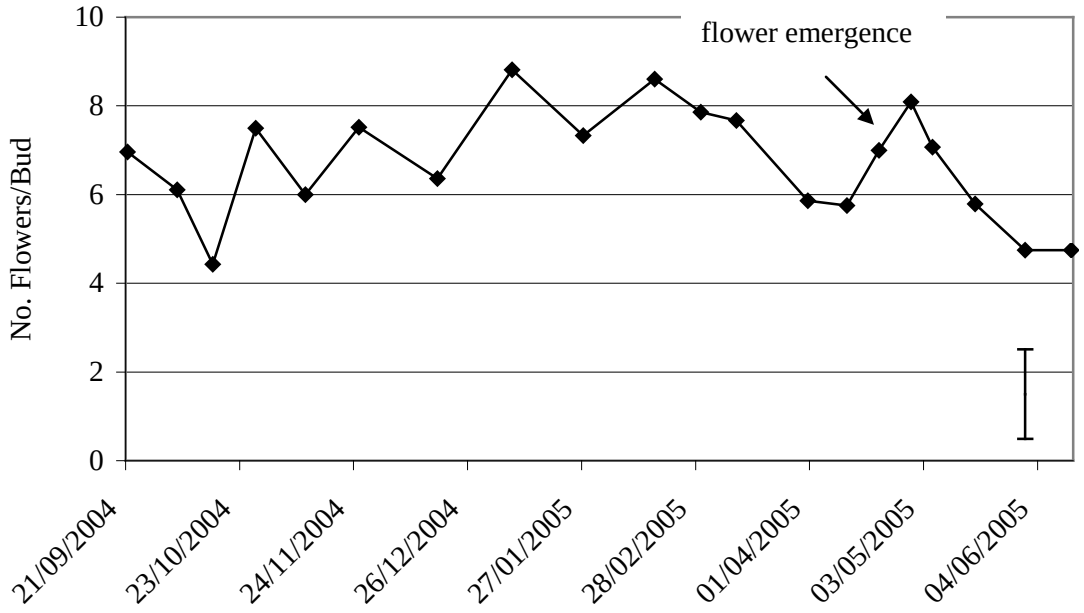


Figure 7.3. ***R. nigrum*** **'Ben Gairn' – effect of dissection date on flower production. Data pooled across bud positions. Error bar represents L.S.D. (P<0.05), d.f. = 461**

For 'Ben Hope', the effects of dissection date and bud position were highly significant (P<0.001; P=0.005), but the interaction was not (P=0.961). Buds at node 2 produced significantly fewer flowers (P<0.001) than those higher up (Figure 7.4).

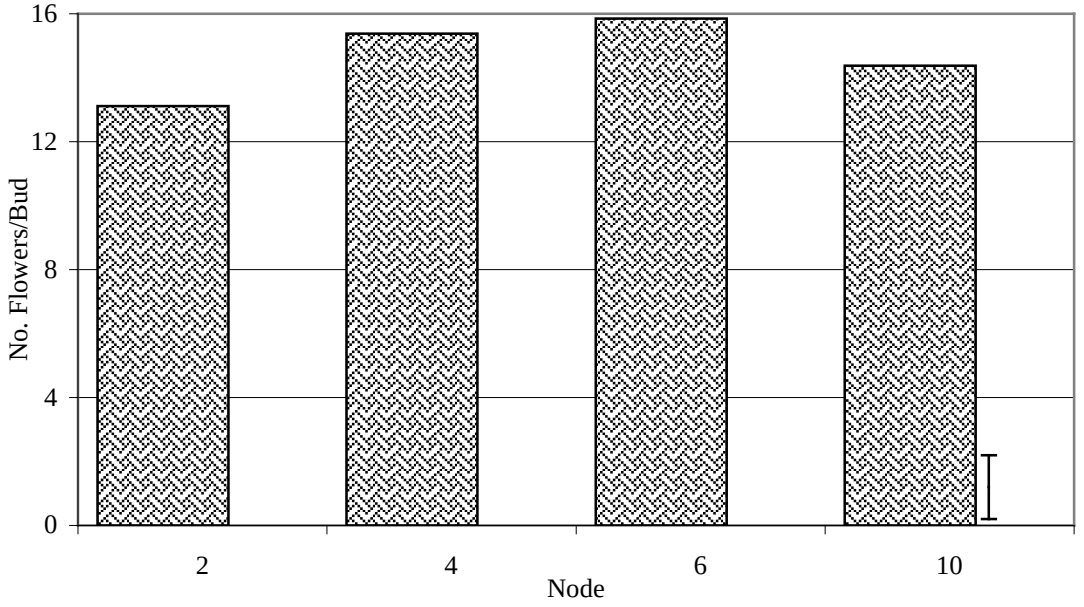


Figure 7.4. ***R. nigrum*** **'Ben Hope' – effect of dissection date on flower production. Data pooled across bud dissection dates. Error bar represents L.S.D. (P<0.05), d.f. = 455**

There was a large degree of variation within the results, but in general the number of flowers produced by each bud significantly increased between 21 September 2004 and 27 January 2005 (Figure 7.5).

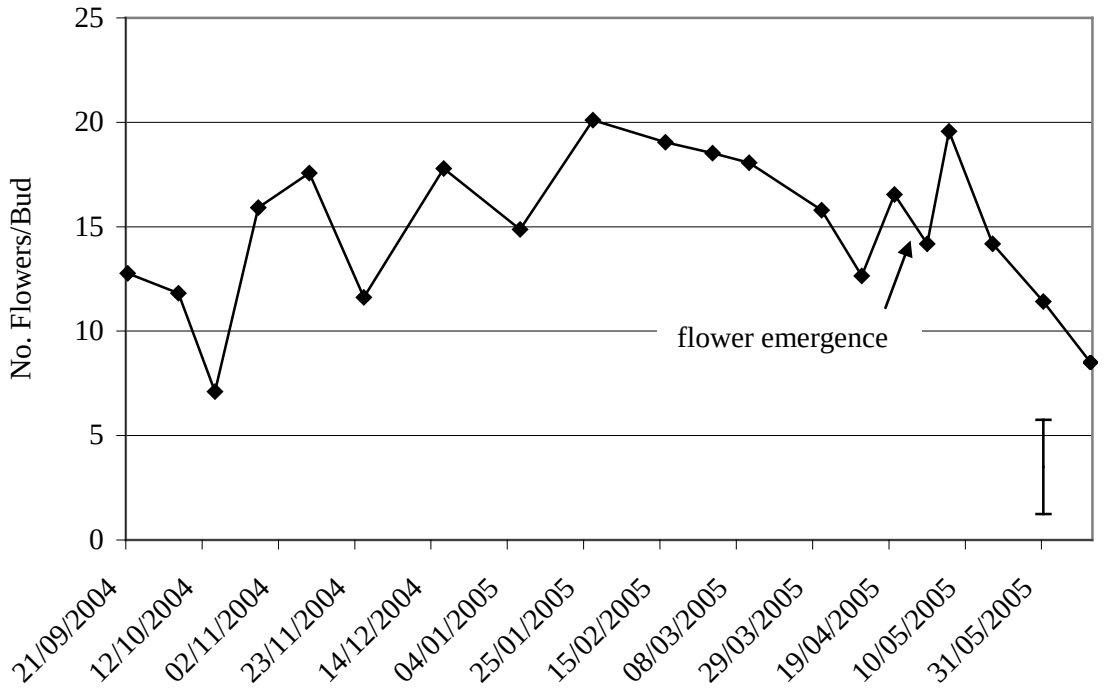


Figure 7.5. *R. nigrum* 'Ben Hope' – effect of dissection date on flower production. Data pooled across bud positions Error bar represents L.S.D. (P<0.05), d.f. = 455

Between 27 January 2005 and 11 April 2005, the number of flowers present in each bud significantly decreased. Prior to flower emergence, and until 5 May 2005, an increase in flower number was observed, but this was short lived and decreased after 5 May 2005.

As with the other cultivars, the effects of bud position and dissection date were highly significant (P<0.001) for 'Ben Tirran', but the interaction was not significant (P=0.972). Buds at node 2 produced significantly fewer flowers than those at higher bud positions. Again, there was a large degree of variation within the results, but in general the number of flowers present in each bud increased to a maximum on 31 March 2005, after which there was a significant decrease (Figure 7.7).

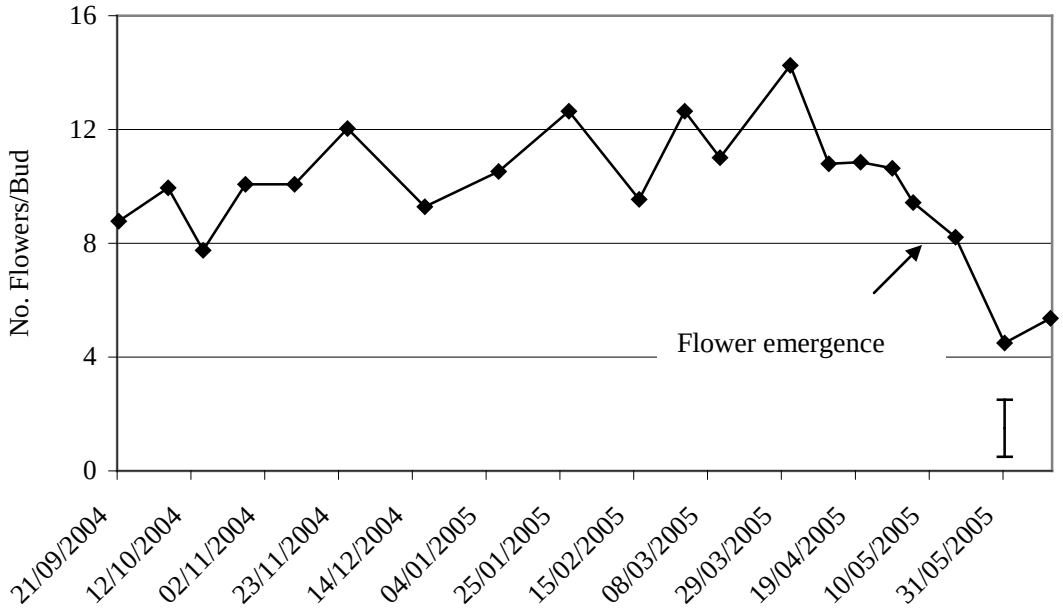


Figure 7.7. ***R.nigrum*** **'Ben Tirran' – effect of dissection date on flower number. Data pooled across bud positions. Error bar represents L.S.D. (P<0.05), d.f. = 457**

Flower Death

The extent of flower death, recorded from 20 April 2005, is depicted in Figure 7.8. Of the 8.74 flowers present in 'Ben Gairn' buds, only 3.81 (43.6%) were alive and only 41.5% (8.21 out of a possible 9.8) of 'Ben Hope's' flowers were alive. Similarly, of 'Ben Tirran's' 11.46 flowers, an average of 6.8 (59.5%) were alive in each bud.

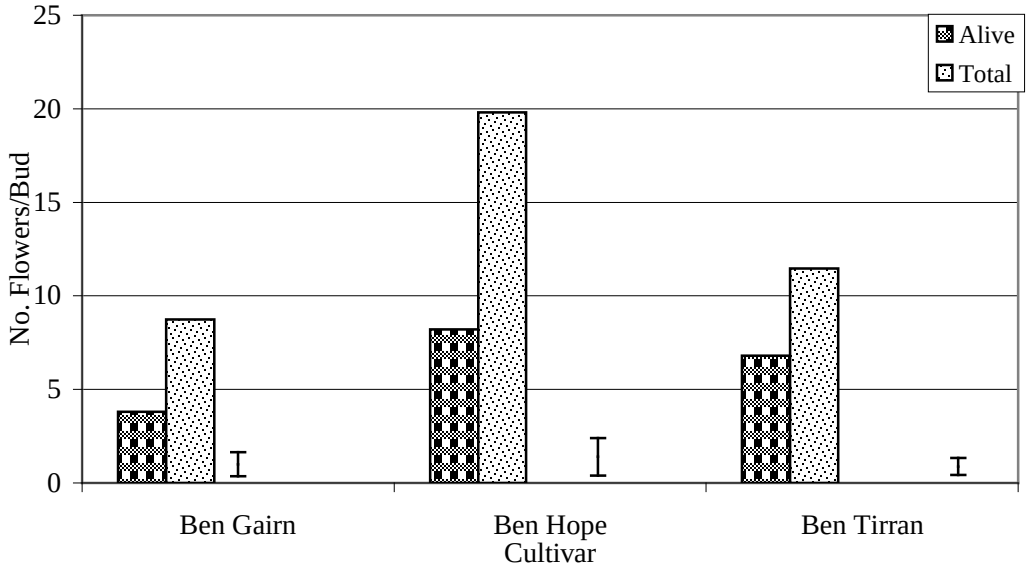
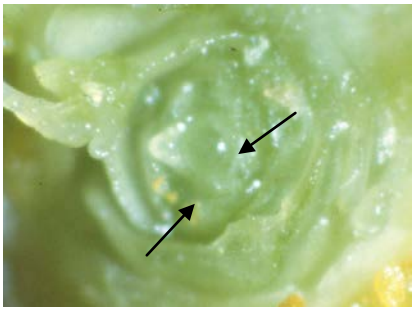


Figure 7.8. ***R. nigrum*** **– comparison of bud flower composition. Error bars represent L.S.D. (P<0.05), d.f. = 192, 191 and 191 respectively.**

Floral development

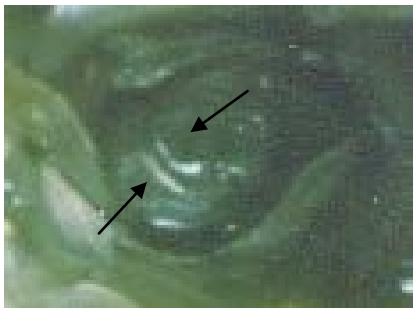
Stages of floral development were rated on a scale of 0 – 13 (Figures 7.9a-g, Table 7.1).

a



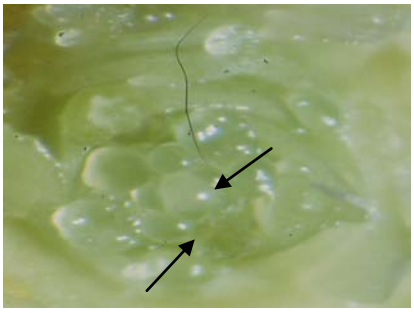
Stage 0. Flat, round vegetative apex

b



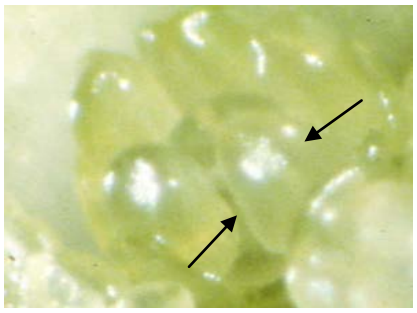
Stage 1. Apex raised

c



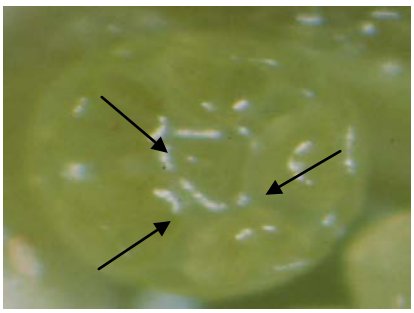
Stage 3. Individual umbletts formed

d



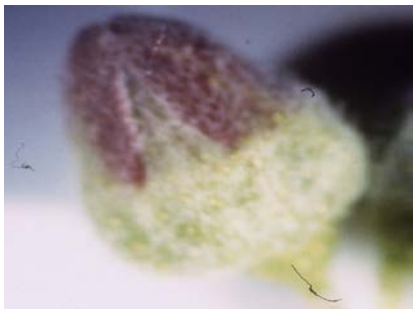
Stage 4. Formation of anthers

e



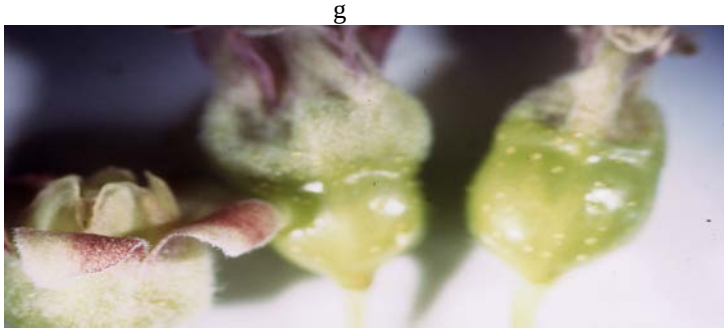
Stage 5. Style discernable

f



Stage 8. Flowers hairy and pink

g



Stage 10. Flowers open

Stage 11. Petals touching, ovary enlarged

Stage 12. Petals dying, ovary swollen

Figure 7.9. *R. nigrum* floral development stages

Table 7.1. Definition of flower stages

Stage	Definition
0	Vegetative, apex flat
1	Apex slightly raised
2	Apex dividing
3	Individual umblettes discernable
4	Formation of 5 anthers, all touching at the tip
5	Flowers swell and anthers separate, circular style, slightly raised in flower centre
6	Petals present at base of anthers
7	Anthers enclosed by petals, slightly raised style triangular in shape. Individual flowers just visible to the naked eye.
8	Flowers partly covered by hairs, tinged pink, anthers swollen and touching, style elongating.
9	Flowers enlarged, covered completely by hairs, style elongated. Flowers emerge
10	Flowers open, pollen visible on anthers
11	Petal tips touching and pointing upwards, ovary swollen, contains green, immature seeds, style dying
12	Petals dead and brown, ovary further enlarged, containing immature seeds, style dead
13	Seeds maturing

No flowers were present, regardless of cultivar or bud position, until 3 August 2004. The effects of cultivar and dissection date were highly significant ($P<0.001$; $P<0.001$; $P<0.001$), as were the interactions. Data were further divided by cultivar and re-analysed.

Ben Gairn

The effect of dissection date was highly significant ($P<0.001$) but bud position and the interaction were not ($P=0.059$; $P=0.185$). Flower development progressed steadily (Figure 7.7) and developed from Stage 2/3 on 3 August to Stage 5 by 10 September where they remained until 25 November.

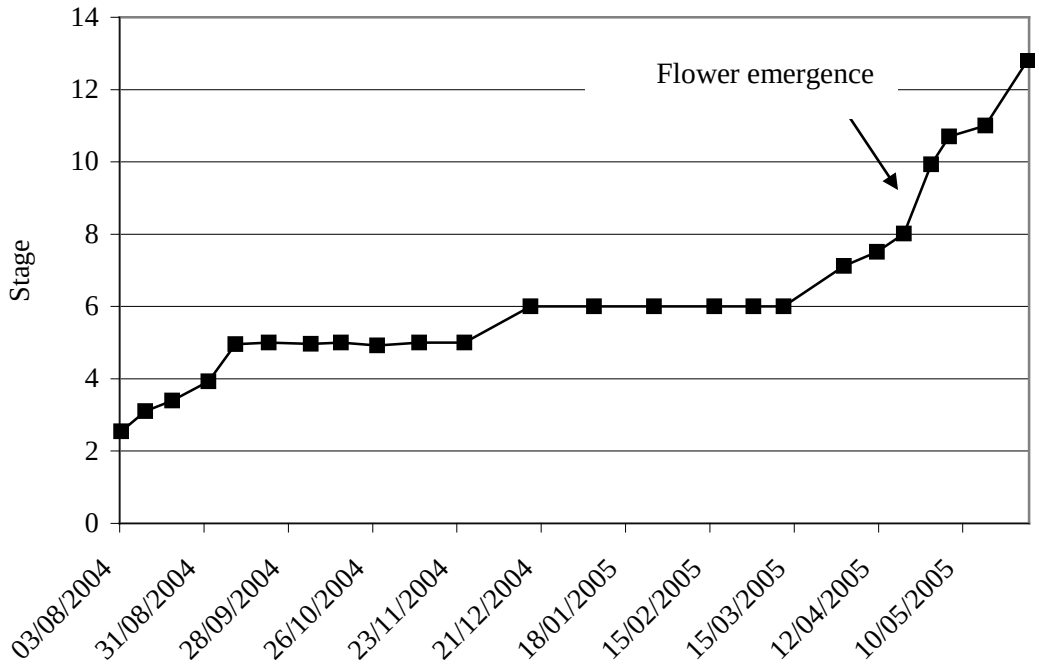


Figure 7.7. ***R. nigrum*** **'Ben Gairn' – floral development in relation to time. Data pooled across all bud positions.**

Buds then progressed to Stage 6 where they over-wintered. Floral development began again on 11 March and progressed rapidly.

Ben Hope

Flower stage was highly dependant on dissection date (P<0.001), but bud position and the interaction between these factors were insignificant (P=0.751; P=1). Flowers had developed from Stage 3 to Stage 5 by 21 September then further progressed to Stage 6 between 25 November and 17 December, where they over-wintered. Development began again on 11 March and progressed rapidly (Figure 7.8).

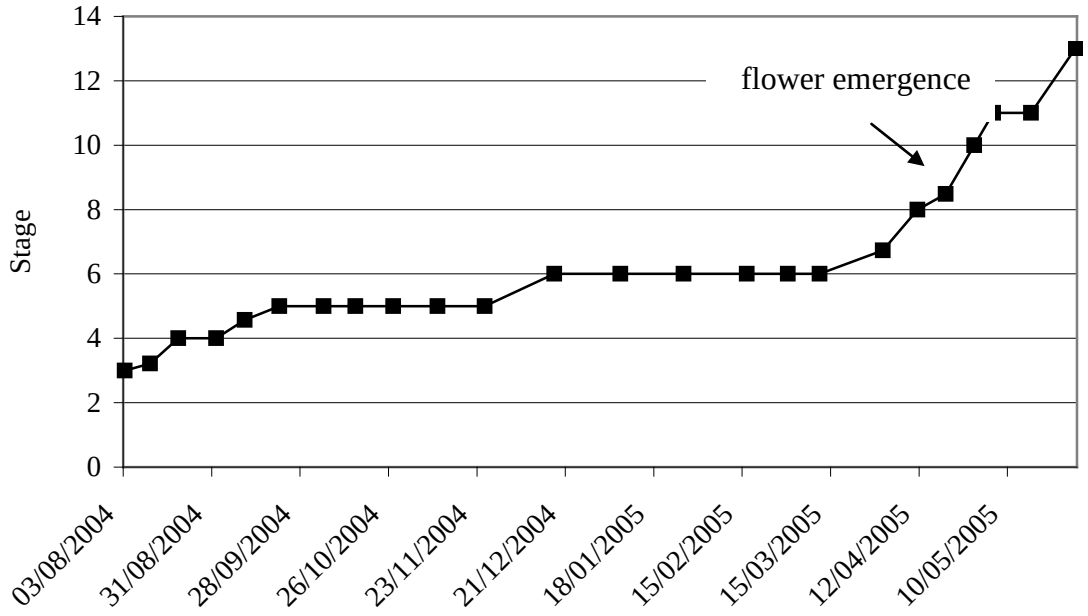


Figure 7.8. ***R. nigrum*** **'Ben Hope' – floral development in relation to time. Data pooled across all bud positions.**

Ben Tirran

As with the other cultivars, the effect of dissection date was highly significant (P<0.001) but bud position and the interaction were not (P=0.966; P=1). Flowers developed from Stage 3 to Stage 5 by 10 September and progressed to Stage 6 between 25 November and 17 December, where they over-wintered. Development began again on 20 April and progressed rapidly (Figure 7.9).

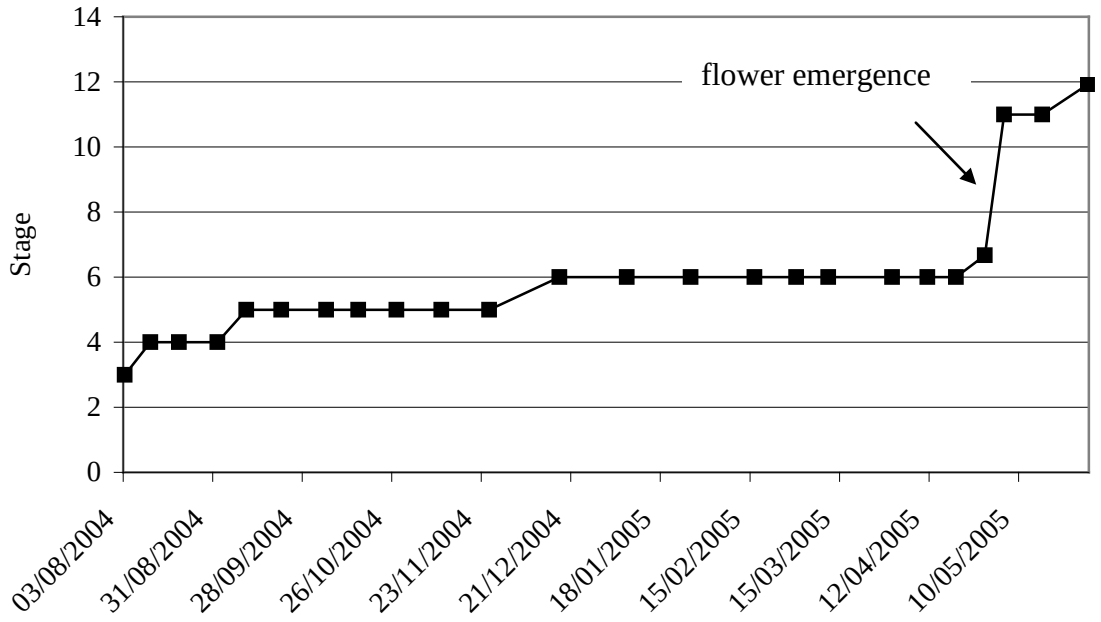


Figure 7.9. ***R. nigrum*** **'Ben Tirran' – floral development in relation to time. Data pooled across all bud positions.**

Discussion

The aim of this experiment was to determine the date of floral initiation in 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', and to monitor the development of the flowers. All buds remained in a vegetative state until July 16 and between this and August 3, I was otherwise indisposed. During this period, flowers were initiated and developed to Stage 3. The date of flower initiation, therefore, was not recorded to the desired accuracy.

7.4.1. Flower Production

Unexpectedly, high rates of flower abortion were observed towards the end of the experiment. In general, each *R. nigrum* bud has been reported to express between 6.6 and 9.6 flowers (Brennan, 1996) which is considerably higher than the final number of berries produced by the cultivars. To ensure adequate pollination and fruit set, it is not uncommon for crops to over-produce flowers, then abort a proportion of the available flowers (Ito and Kikuzawa, 2003). This is unlikely to explain the reduction in reported in this experiment however, as the number of flowers produced by 'Ben Gairn' was within the normal limits and the degree of abortion was too high (Brennan, 1996).

For all cultivars, there was a significant decrease in the total number of flowers (alive and dead) produced by each bud in the months immediately prior to flower emergence, this reduction, however, may be attributed to flower abortion due to the spider mite infestation. The flowers that aborted became desssicated, appeared brown in colour and detached from the floral apex at the slightest touch. Such flowers may have become detached from the apex prior to dissection, and individual flowers would not havbe been easily discernable in the bud. The reduction in flower number after emergence is likely to be due to the flowers becoming detached from the buds, and although great care was taken, it is inevitable that some flowers would habe been lost when the stems were being transported from the Experimental Field Site to the main University Campus.

Flower abortion was reported in *Rubus chamaemorus* (Jean and Lapointe, 2001) and *V. vinifera* (Lavee, 1985) in response to drought and nutrient stress (Jean and Lapointe, 2001). In this experiment, however, the plants were regularly irrigated and did not visually appear to be suffering from nutrient stress. Alternatively, flowers may have aborted due to unfavourable growing conditions, in that the plants were maintained in pots and hence the root systems were more constricted than if they had been grown under field conditions. It is more likely, however, that the high levels of dead flowers are due to the infestation of spider mite, which were mainly observed in the centre of the buds. Although the mites were chemically treated, control was not achieved instantly and the mites were not eradicated, until 20 May 2005. Mite damage to newly-developed flowers has resulted in flower death of *Fragaria ananassa* (Grasselly, 1995), *Vaccinium vinifera* (Roberto *et al*., 2001), *Quercus* spp. (Scutareanu and Roques, 1993) and *Baptisia australis* (Evans *et al*., 1989). Flower death as an indirect result of mite infestation has been reported, whereby presence of mites was found to alter the cold-hardiness temperature of *Ribes nigrum* cultivars by as much as 10ºC, resulting in flower death at relatively high temperatures (Carter and Hummer, 1999).

7.4.2. Floral Development

Although the exact date of flower initiation was not determined, buds had developed from Stage 0 on 16 July to Stage 3 by 3 August, which is considerably earlier than reported for other fruit species e.g. *Rubus ideaus* 'Lloyd George', 4 September (Robertson, 1957), *Rubus occidentalis*, 16 October (Robertson, 1957) and *R. ideaus*, mid-September (Mathers, 1952). The date of *Ribes nigrum* flower initiation had previously been reported to be 1 August (Nasr and Wareing, 1961) and 15 August (Vestrheim, 1972). Germplasm exchange and current breeding objectives e.g. low chilling requirement, may have altered the timing of floral initiation of modern-day *R. nigrum*, compared to the previous research that relied on older cultivars. Alternatively, the timing of flower initiation may be dependant on environmental parameters and during the past 50 years, global temperature has increased by an average of 1ºC (Landsberg, 1974; Lavee, 1985; Booij *et al*., 1992). Flower formation of *Fragaria ananassa* was advanced after exposure to elevated temperatures (Pietila *et al*., 2002; LeMiere *et al*., 1996) therefore the earlier bud burst reported in this experiment may be due to the temperature differential between the current climate and that when the previous research was conducted.

Floral development of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' progressed slowly from late September and stopped between mid-December and March/April. Suspension of flower development over winter has been reported for several species, but differences in the duration of stoppage are evident. *Rubus occidentalis* 'Cumberland' ceased developing early October, *Rubus ursinus loganobaccus* and *Rubus ideaus* 'Lloyd George' late October, and *Rubus fruticosus* 'Ashton Cross' and 'Himalayan Giant' mid-November (Robertson, 1957). Flower initiation of *R. ideaus* 'Malling Promise' and 'Ranere' stopped in December, as in this experiment, but re-commenced in January and February respectively (Williams, 1959; Waldo, 1933). The predominant factor controlling suspension of *R. ideaus* flower formation was found to be low temperature (Williams, 1959). Although not recorded in this experiment, *Ribes nigrum* flower development may have been prolonged in comparison to previous research due to the increase in global temperature, as described above. In spring, re-

commencement of *Vaccinium vinifera* (Lavee, 1985), *Actinidia chinensis* (Linsley-Noakes and Allan, 1987), *R. nigrum* (Vestrheim, 1975) and *Anethum graveolens* (Booij *et al*., 1992) floral development was advanced by exposure to warmer temperatures. In particular, March/April temperatures appeared to be most important for this process (Vestrheim, 1975). Colder winter temperatures may therefore be associated with suspension of flower development and warmer spring temperatures with advancement of development.

Initially it appeared as if *Ribes nigrum* buds would over-winter at Stage 5, but all cultivars had progressed to Stage 6 by 17 December 2004. It is relatively difficult to compare this with previous research, due to the very different scales employed by authors; however, translation of other scales into that employed in this Chapter was attempted. *Rubus ideaus* 'Malling Promise' flowers were well-defined and appeared to over-winter in a mature stage, corresponding to Stage 7 or 8 (Williams, 1959). In contrast, *R. ideaus* 'Ranere' were immature and had reached approximately Stage 2 when growth ceased over winter (Waldo, 1933). *Actinidia chinenesis* over-wintered in Stage 1 (Linsley-Noakes and Allan, 1987), *Rubus fructicosus* 'Himalayan Giant' and 'Ashton Cross' at Stage 2, *R. ideaus* 'Lloyd George' at Stage 4, *Rubus occidentalis* x *R. ideaus* at Stage 6 and *Rubus. ursinus loganobaccus* at Stage 7 (Robertson, 1957). The over-wintering stage of *Anethum graveolens* was found to be dependant on autumn temperature, with initiation and development being accelerated by exposure to 12ºC compared to 8ºC (Booij *et al*., 1992). Therefore, the warmer the autumn temperature, the more advanced the over-wintering floral stage, which supports the theory of Williams (1959) that flower cessation over winter was a reaction to low temperature.

Flower initiation and development of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' was found to be independent of bud position. In contradiction to this, bud position affected flower initiation in *Ribes nigrum* (Nasr and Wareing, 1961), *Rubus ideaus* (Waldo, 1933; Mathers, 1952; Williams, 1959), *Rubus ursinus loganobacus* and *Rubus fructinosus* (Robertson, 1957). These authors reported a time-lag in development, whereby flowers formed firstly in buds closest to the apical bud, followed by those in the middle section of the stem, and basal buds lastly. Positional differences have been suggested to be a result of apical

dominance and flower initiation was deemed to start when lateral buds were released from correlative inhibition (Waldo, 1933; Williams, 1959; Nasr and Wareing, 1961a).

Although in general 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' behaved similarly, at several stages minor differences were apparent. 'Ben Gairn' buds were marginally less developed by 3 August, 'Ben Hope' entered Stage 5 11 days later and 'Ben Tirran' remained in an over-wintering state for 31 days longer than the other cultivars. By the end of the experiment, 'Ben Tirran' had reached Stage 12 whereas 'Ben Gairn' and 'Ben Hope' were at Stage 13. More significant differences were observed in previous *Ribes nigrum* research, whereby flower initiation was delayed in 'Bang Up' compared to 'Boskoop', 'Silvergeiter', 'Wellington XXX' and 'Brodtorp' (Vestrheim, 1972). *Rubus ideaus* cultivar differences were far more apparent, with the date of flower initiation varying from 30 August for 'Ranere' and 'Lloyd George' to 8 February for 'Latham' and 'Chief' (Waldo, 1933).

It had been reported that early flowering *Ribes nigrum* and *Vaccinium vinifera* cultivars initiated flowers earlier than later flowering cultivars (Vestrheim, 1972; Lavee, 1985). In this experiment, however, flower initiation for all cultivars occurred between 16 July and 3 August. Differences between cultivars, however, were observed in the spring, when the early and mid flowering 'Ben Gairn' and 'Ben Hope' re-commenced flower development before the later flowering 'Ben Tirran'. Floral development progressed rapidly after spring bud burst, and this is when cultivar differences were most apparent. Little difference was observed between 'Ben Gairn' and 'Ben Hope', but this would be expected as these cultivars bud burst at similar times (Atwood, 2004). 'Ben Tirran' on the other hands, bursts bud circa. 4 weeks later than the other cultivars, and a similar delay in the re-commencement of flower development would be expected.

The lack of effect of bud position and cultivar-specificity, especially during the stage changes, may be due to the extended time-periods between observations. If dissections were carried out are more frequent intervals, subtle differences between bud position and cultivars may become apparent.

Ideally, this experiment should be repeated, firstly to clarify the date of flower initiation, but also to determine the extent of variation between years. When repeating this experiment, however, it is important to bear in mind that crop management techniques may affect the timing and rate of flower development e.g. summer pruning advanced *Prunus avium* flower initiation by 31 days (Guimond *et al*., 1998). Williams (1959) stated that discrepancies in the timing of *Rubus ideaus* flower initiation compared to earlier literature were due to climatic differences. Similarly, differences in the date of *Rubus fructicosus* flower initiation in Oregon and Maryland were explained in terms of temperature differences (Waldo, 1933). Year to year variations were observed for *R. ideaus*, whereby floral initiation began on 5 October in 1932 and 25 September the following year (Waldo, 1933).

Conclusions

Flowers were initiated in *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' between 16 July and 3 August 2004. Flower development progressed slowly until 17 December and the buds over-wintered in Stage 6 then developed rapidly from March/April. Flower initiation was unaffected by bud position, and only slight cultivar differences were apparent, but further experimentation at more frequent intervals may record differences in both parameters. Repetition would also allow a more detailed record of flower abortion. Care should be taken to ensure the plants are kept pest and disease free to ensure infestation does not contaminate the results. The controlling factor in flower initiation and development appeared to be temperature, and future research should investigate the effects of proposed climate change scenarios on the timing and rate of floral development.

Chapter Seven.
Ribes nigrum Flower Initiation and Development

7.1. Introduction

Detrimental effects of flower production in response to elevated spring temperatures were observed (Chapter 5), but in order to fully understand the effects of predicted climate change scenarios on flower production, the timing and rate of floral development in modern-day cultivars under natural chilling conditions must first be established.

7.1.1. Stages of Flower Formation

Flower initiation is typically identified by stages, the number and description of which varies with author and species. Booij *et al*. (1992) and Mathers (1952) listed six stages for *Apium graveolens* and *Rubus ideas* respectively, whereas Robertson (1957) and Horridge and Cockshull (1974) listed 11 stages for *Rubus* spp. and *Rosa* spp. Regardless of species, the first two stages of flower initiation appear to be uniform.

In the first stage (Plate 7.1), referred to as 0 or 1, the apex is vegetative, characterized by a flat or slightly convex apex (Roefolfse and Hand, 1989) and surrounded by newly produced leaves (Taylor *et al*., 1997).

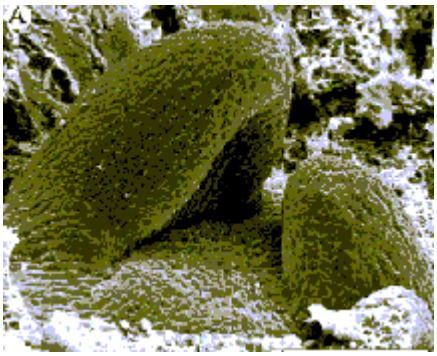


Plate 7.1. Scanning electron microscope image of *Prunus persica* 'Redhaven' flower initiation Stage 0/1. (after Engin and Iqbal, 2004)

The second stage, termed 1 or 2, is identified by a raised and elongated apex (Cathey and Borthwick 1957; Roefolfse and Hand, 1989; Taylor *et al*., 1997). Formation of the perianth ring has also been classed as the second stage of initiation (Mathers, 1952; Robertson, 1957).

Floral development beyond this may be dependant on species, but also on the frequency of dissections and author - Mathers (1952) listed six stages of *Rubus ideaus* flower development but Robertson (1957) observed 11 stages for *Rubus* spp.

7.1.2. Flower Formation in *Ribes nigrum*

Nasr and Wareing (1961) investigated the timing of flower initiation in *Ribes nigrum* 'Victoria' and found that 19% of buds had initiated flowers by 15 August, and 86% by 29 August. In a similar study, Vestrheim (1972) investigated the time of floral initiation in *R. nigrum* 'Bang Up', 'Boskoop', 'Brodtorp', 'Silvergieter' and 'Wellington XXX' and discovered all cultivars except 'Bang Up' had initiated flowers by 15 August. Wilson and Adam (1966), however, reported that *R. nigrum* cultivars had initiated floral primordia nine days earlier than reported by Nasr and Wareing (1961) and Vestrheim (1972). These discrepancies may be explained by the theory that early flowering cultivars initiate flowers earlier than later flowering cultivars (Vestrheim, 1972). Further experimentation investigated the timing of floral initiation at three different heights from the base – bottom (nodes 0-6), middle (7-12) or top (13-18) (Nasr and Wareing, 1961). Flowers were not initiated in buds 0-6, and by 22 August circa. 50% of the buds in the middle section had initiated flowers compared to 30% of buds in the top section. All buds in the top and middle sections contained flower initials by 5 September (Nasr and Wareing, 1961). The authors suggested that lower buds failed to initiate flowers because they had entered into a state of dormancy prior to 22 August.

The aim of the experiment reported here was to investigate the timing and rate of floral initiation and development in early, mid, and late flowering *Ribes nigrum* cultivars, and to determine the effect of bud position.

7.2. Materials and Methods

Plant material consisted of two-year old softwood cuttings of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', as described in Chapter 2. The plants were delivered to the University of Reading's Experimental Field Site on 2 July 2003, tied onto supporting wires to keep the pots upright and irrigated automatically with Avoncrop's Soft Fruit Mix 2 (6:11:31 N:P:K) nutrient solution four times a day for a total of one hour until 28 September 2004.

Spider mites were observed in the centre of the buds on 11 April 2005 and plants were originally treated with an overhead spray of bifenthrin (Talstar®) at a rate of $0.4mlL^{-1}$. Mites were again observed the following week and plants were treated with thiachloroprid (Calypso®) at a rate of $1mlL^{-1}$ on 22 April 2005 and 29 April 2005. A follow-up spray of abamectin (Dynamec®) on 12 May 2005 at a rate of $0.3mlL^{-1}$ successfully eradicated the mites.

Using a pair of sharpened secateurs, cutting material containing the current year's growth was taken from 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' and cuttings were labeled and placed into beakers of water. Buds at nodes 2, 4, 6 and 10 from the base of the budstick were dissected by slicing away the bud scales and leaves, and then exposing the apex at the base of the bud. Samples were viewed using an M400 Polyvar dissecting microscope. Number of leaves, flower stage and number of flowers were recorded. The first dissection was carried out on 25 May 2004 and subsequent dissections conducted at regular intervals.

Data relating to the stage of flower development over time was analysed in two parts. Initially, data encompassing 25 May to 16 July 2005 were analysed, then data from 16 July to the termination of the experiment were analysed.

Statistical analyses were conducted using Genstat V's ANOVA to determine differences between cultivars, bud position and dissection date.

7.3. Results

7.3.1. Flower Production

The effects of dissection date and bud position were highly significant (P<0.001; P=0.002), but the interaction was not (P=0.961). Significantly fewer flowers were produced by buds at node two compared to nodes higher up the stem (Figure 7.1).

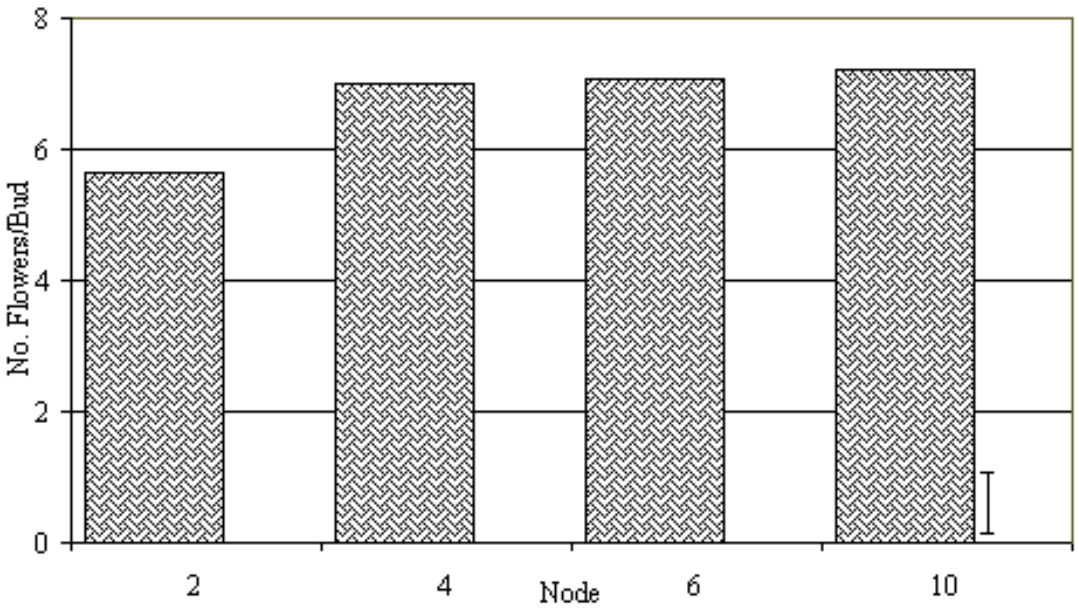


Figure 7.1. ***R. nigrum*** **'Ben Gairn' – effect of bud position on flower production. Data pooled across bud dissection dates. Error bar represents L.S.D. (P<0.05), d.f. = 461**

There was a large degree of variation within the results, but in general the number of flowers produced by each bud increased between 21 September 2004 and 16 February 2005, after which there was a significant decrease (Figure 7.2). Prior to flower emergence, there was a significant increase in the number of flowers produced from 16 February 2005 until 29 April 2005, after which there was again a significant decrease.

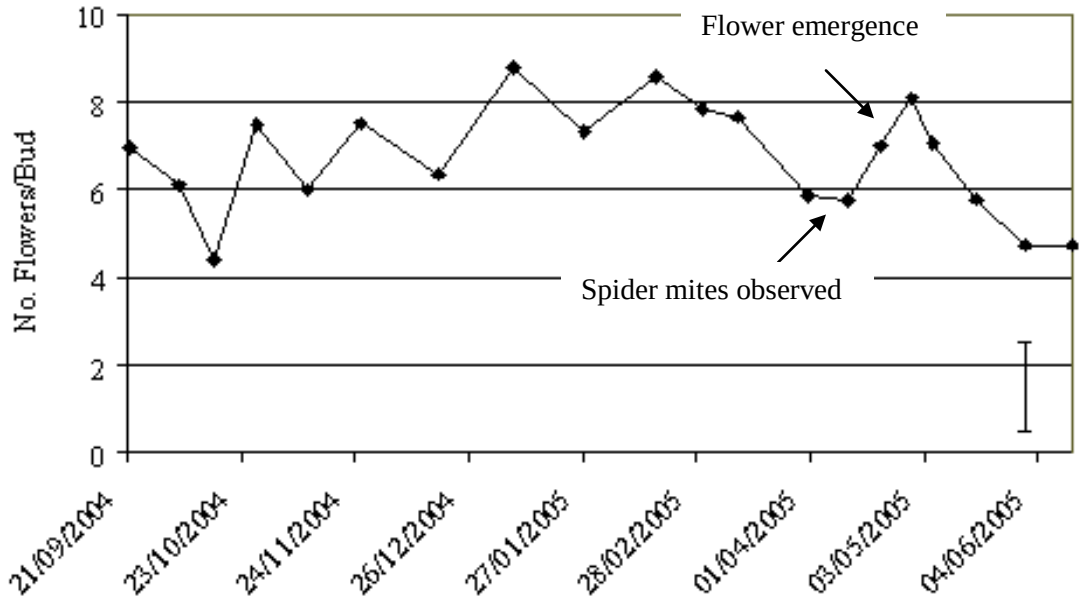


Figure 7.2. ***R. nigrum*** **'Ben Gairn' – effect of dissection date on flower production. Data pooled across bud positions. Error bar represents L.S.D. (P<0.05), d.f. = 461**

For 'Ben Hope', the effects of dissection date and bud position were highly significant (P<0.001; P=0.005), but the interaction was not (P=0.961). Buds at node 2 produced significantly fewer flowers (P<0.001) than those higher up (Figure 7.3).

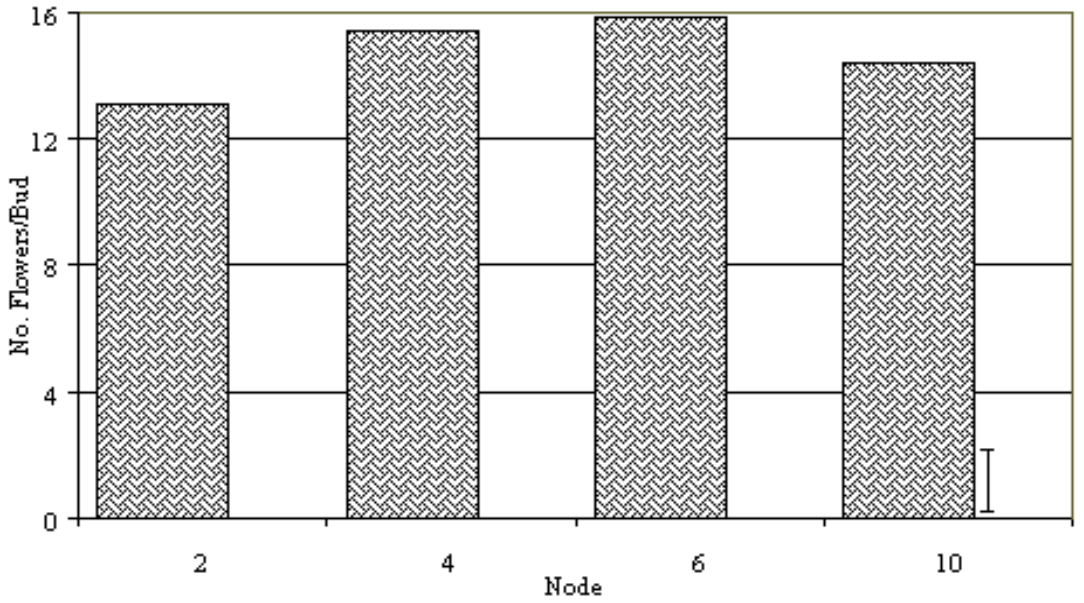


Figure 7.3. ***R. nigrum*** **'Ben Hope' – effect of dissection date on flower production. Data pooled across bud dissection dates. Error bar represents L.S.D. (P<0.05), d.f. = 455**

There was a large degree of variation within the results, but in general the number of flowers produced by each bud significantly increased between 21 September 2004 and 27 January 2005 (Figure 7.4).

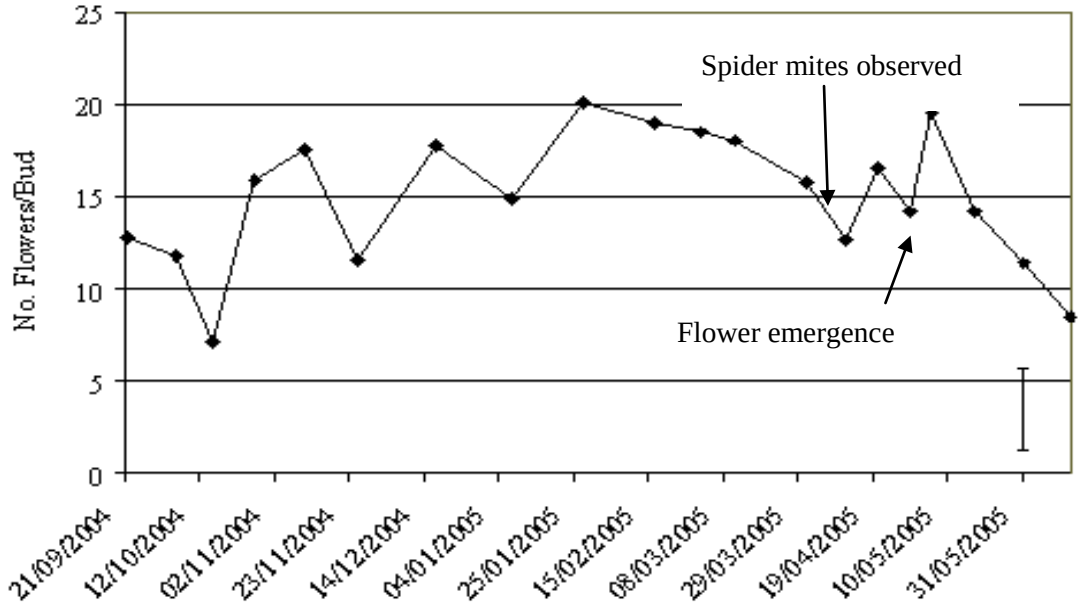


Figure 7.4. ***R. nigrum*** **'Ben Hope' – effect of dissection date on flower production. Data pooled across bud positions Error bar represents L.S.D. (P<0.05), d.f. = 455**

Between 27 January 2005 and 11 April 2005, the number of flowers present in each bud significantly decreased. Prior to flower emergence, and until 5 May 2005, an increase in flower number was observed, but this was short lived and decreased after 5 May 2005.

As with the other cultivars, the effects of bud position and dissection date were highly significant (P<0.001) for 'Ben Tirran', but the interaction was not significant (P=0.972). Buds at node 2 produced significantly fewer flowers than those at higher bud positions. Again, there was a large degree of variation within the results, but in general the number of flowers present in each bud increased to a maximum on 31 March 2005, after which there was a significant decrease (Figure 7.5).

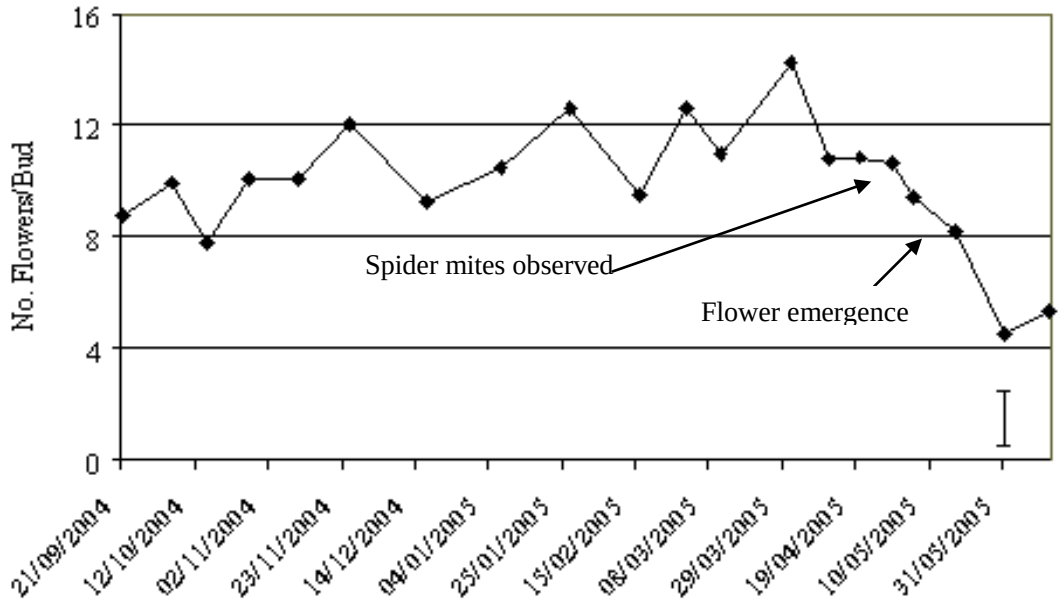


Figure 7.5. ***R.nigrum*** **'Ben Tirran' – effect of dissection date on flower number. Data pooled across bud positions. Error bar represents L.S.D. (P<0.05), d.f. = 457**

The extent of flower death, recorded from 20 April 2005, is depicted in Figure 7.6. Of the 8.74 flowers present in 'Ben Gairn' buds, only 3.81 (43.6%) were alive and only 41.5% (8.21 out of a possible 9.8) of 'Ben Hope's' flowers were alive. Similarly, of 'Ben Tirran's' 11.46 flowers, an average of 6.8 (59.5%) were alive in each bud.

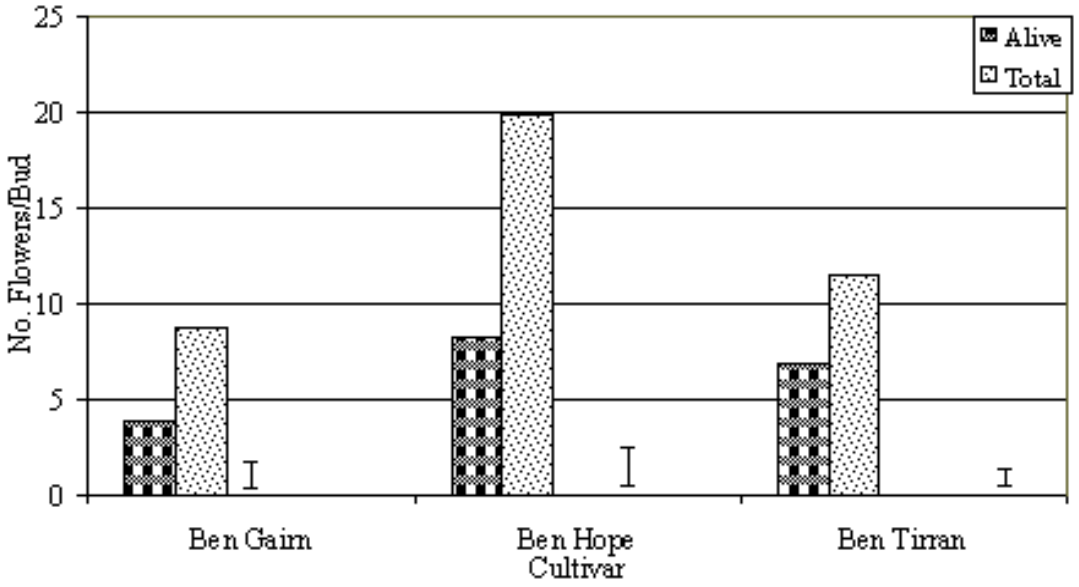
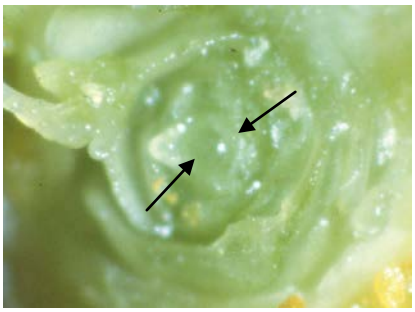


Figure 7.6. ***R. nigrum*** **– comparison of bud flower composition. Data pooled across all bud positions. Error bars represent L.S.D. (P<0.05), d.f. = 192, 191 and 191 respectively.**

7.3.2. Floral Development

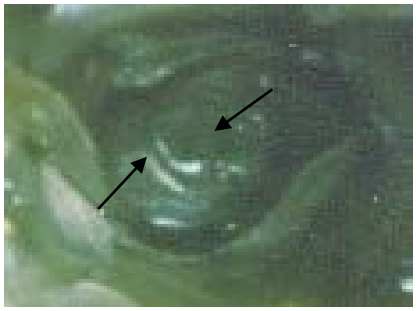
Stages of floral development were rated on a scale of 0 – 13 (Plates 7.2a-g, Table 7.1).

a



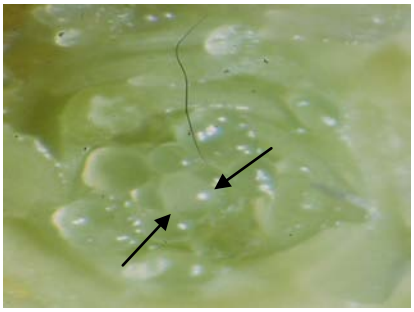
Stage 0. Flat, round vegetative apex

b



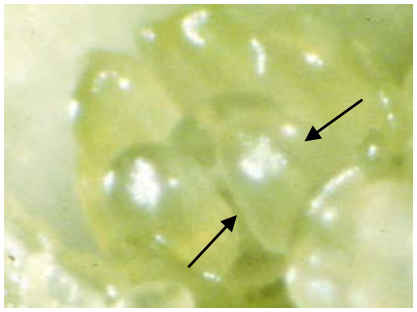
Stage 1. Apex raised

c



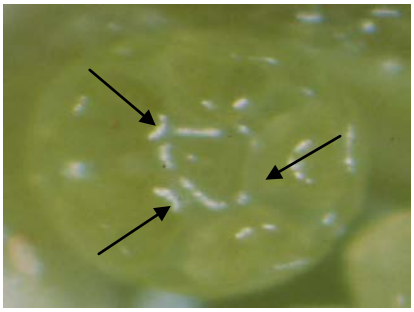
Stage 3. Individual umbletts formed

d



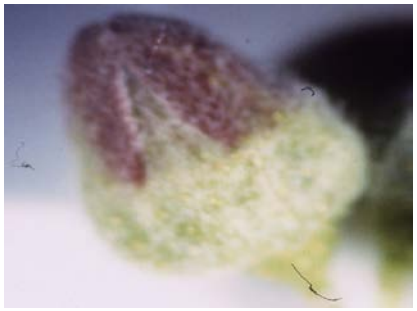
Stage 4. Formation of anthers

e



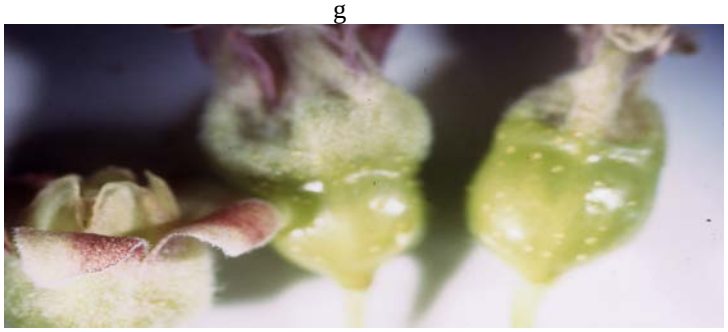
Stage 5. Style discernable

f



Stage 8. Flowers hairy and pink

g



Stage 10. Flowers open

Stage 11. Petals touching, ovary enlarged

Stage 12. Petals dying, ovary swollen

Plate 7.2. *R. nigrum* floral development stages

Table 7.1. Definition of flower stages

Stage	Definition
0	Vegetative, apex flat
1	Apex slightly raised
2	Apex dividing
3	Individual umblettes discernable
4	Formation of 5 anthers, all touching at the tip
5	Flowers swell and anthers separate, circular style, slightly raised in flower centre
6	Petals present at base of anthers
7	Anthers enclosed by petals, slightly raised style triangular in shape. Individual flowers just visible to the naked eye.
8	Flowers partly covered by hairs, tinged pink, anthers swollen and touching, style elongating.
9	Flowers enlarged, covered completely by hairs, style elongated. Flowers emerge
10	Flowers open, pollen visible on anthers
11	Petal tips touching and pointing upwards, ovary swollen, contains green, immature seeds, style dying
12	Petals dead and brown, ovary further enlarged, containing immature seeds, style dead
13	Seeds maturing

No flowers were present, regardless of cultivar or bud position, until 3 August 2004. The effects of cultivar and dissection date were highly significant (P<0.001; P<0.001; P<0.001), as were the interactions. Data were further divided by cultivar and re-analysed.

Ben Gairn

The effect of dissection date was highly significant (P<0.001) but bud position and the interaction were not (P=0.059; P=0.185). Flower development progressed steadily (Figure 7.7) and developed from Stage 2/3 on 3 August to Stage 5 by 10 September where they remained until 25 November.

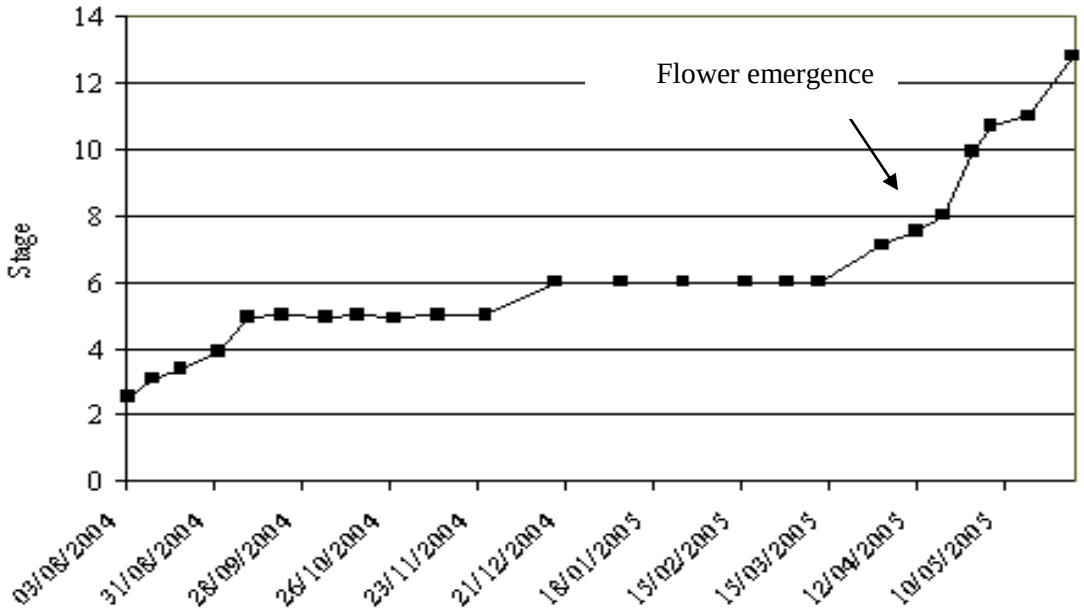


Figure 7.7. ***R. nigrum*** **'Ben Gairn' – floral development in relation to time. Data pooled across all bud positions.**

Buds then progressed to Stage 6 where they over-wintered. Floral development began again on 11 March and progressed rapidly.

Ben Hope

Flower stage was highly dependant on dissection date (P<0.001), but bud position and the interaction between these factors were insignificant (P=0.751; P=1). Flowers had developed from Stage 3 to Stage 5 by 21 September then further progressed to Stage 6 between 25 November and 17 December, where they over-wintered. Development began again on 11 March and progressed rapidly (Figure 7.8).

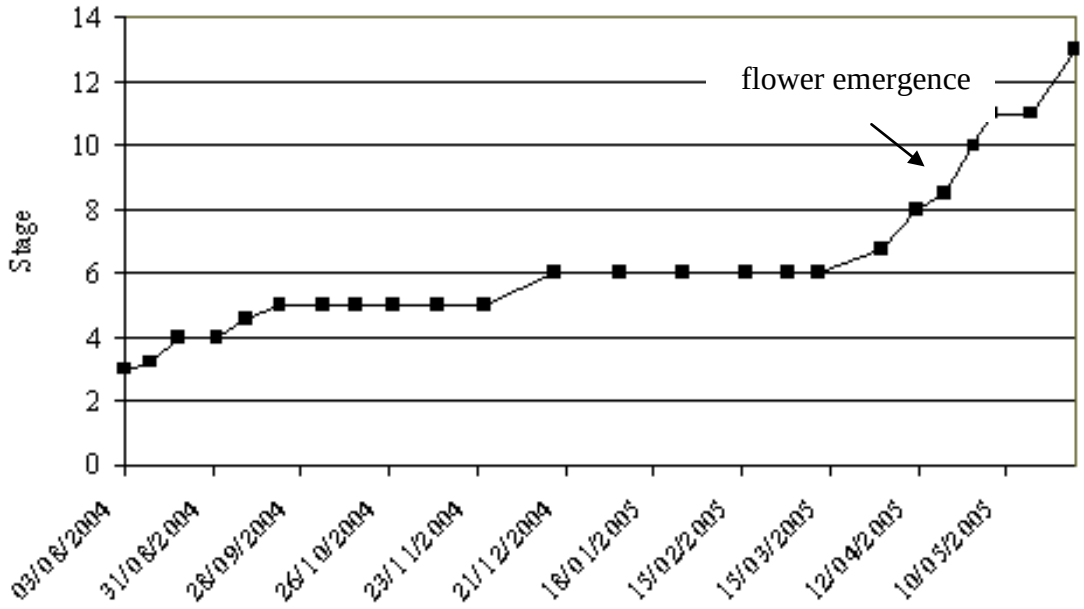


Figure 7.8. ***R. nigrum*** **'Ben Hope' – floral development in relation to time. Data pooled across all bud positions.**

Ben Tirran

As with the other cultivars, the effect of dissection date was highly significant (P<0.001) but bud position and the interaction were not (P=0.966; P=1). Flowers developed from Stage 3 to Stage 5 by 10 September and progressed to Stage 6 between 25 November and 17 December, where they over-wintered. Development began again on 20 April and progressed rapidly (Figure 7.9).

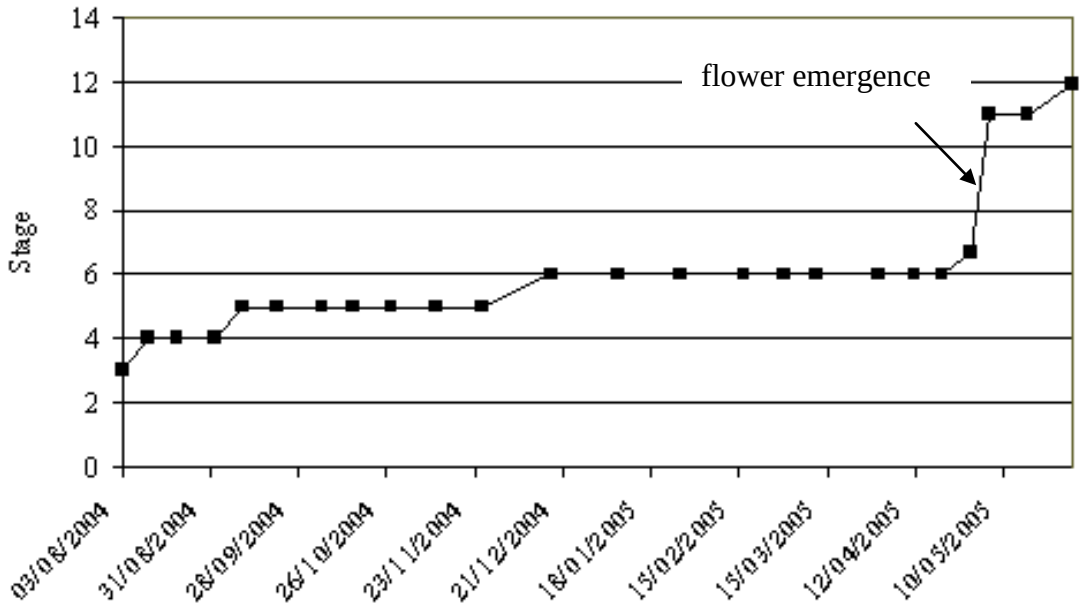


Figure 7.9. ***R. nigrum*** **'Ben Tirran' – floral development in relation to time. Data pooled across all bud positions.**

7.4. Discussion

The aim of this experiment was to determine the date of floral initiation in 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', and to monitor the development of the flowers. All buds remained in a vegetative state until July 16 and between this and August 3, I was otherwise indisposed. During this period, flowers were initiated and developed to Stage 3. The date of flower initiation, therefore, was not recorded to the desired accuracy.

7.4.1. Flower Production

Unexpectedly, high rates of flower abortion were observed towards the end of the experiment. In general, each *Ribes nigrum* bud has been reported to express between 6.6 and 9.6 flowers (Brennan, 1996) which is consistent with initial data, but considerably higher than the final number of berries produced by the cultivars. To ensure adequate pollination and fruit set, it is not uncommon for crops to over-produce flowers, then abort a proportion of the available flowers (Ito and Kikuzawa, 2003). This is unlikely to explain the reduction in reported in this experiment however, as the number of flowers produced by 'Ben Gairn' was within the normal limits and the degree of abortion was too high (Brennan, 1996).

Flower abortion was reported in *Rubus chamaemorus* (Jean and Lapointe, 2001) and *Vaccinium vinifera* (Lavee, 1985) in response to drought and nutrient stress (Jean and Lapointe, 2001). In this experiment, however, the plants were regularly irrigated and did not

visually appear to be suffering from nutrient stress. Alternatively, flowers may have aborted due to unfavourable growing conditions, in that the plants were maintained in pots and hence the root systems were more constricted than if they had been grown under field conditions. It is more likely, however, that the high levels of dead flowers were due to the infestation of spider mite, which were mainly observed in the centre of the buds. Although the mites were chemically treated, control was not achieved instantly and the mites were not eradicated, until 20 May 2005. Mite damage to newly-developed flowers has resulted in flower death of *Fragaria ananassa* (Grasselly, 1995), *V. vinifera* (Roberto *et al*., 2001), *Quercus* spp. (Scutareanu and Roques, 1993) and *Baptisia australis* (Evans *et al*., 1989). Flower death as an indirect result of mite infestation has been reported, whereby presence of mites was found to alter the cold-hardiness temperature of *Ribes nigrum* cultivars by as much as 10ºC, resulting in flower death at relatively high temperatures (Carter and Hummer, 1999).

For all cultivars, there was a significant decrease in the total number of flowers (alive and dead) produced by each bud in the months immediately prior to flower emergence. This reduction, however, is likely to have been a result of flower abortion due to the spider mite infestation. The flowers that aborted became desiccated, appeared brown in colour and detached from the floral apex at the slightest touch. It is likely that some flowers became detached from the apex and were present in the buds as individual flowers. On dissection, the presence of such flowers would not have been easily discernable in the bud and hence were not counted. The reduction in flower number after emergence is likely to have been due to the flowers becoming detached from the buds, and although great care was taken, it is inevitable that some flowers would have become detached from the buds during transit from the Experimental Field Site to the main University Campus.

7.4.2. Floral Development

Although the exact date of flower initiation was not determined, buds had developed from Stage 0 on 16 July to Stage 3 by 3 August, which is considerably earlier than reported for other fruit species e.g. *Rubus ideaus* 'Lloyd George', 4 September (Robertson, 1957), *Rubus occidentalis*, 16 October (Robertson, 1957) and *R. ideaus*, mid-September (Mathers, 1952). The date of *Ribes nigrum* flower initiation had previously been reported to be 1 August (Nasr and Wareing, 1961) and 15 August (Vestrheim, 1972). Germplasm exchange and current breeding objectives e.g. low chilling requirement, may have altered the timing of floral initiation of modern-day *R. nigrum*, compared to the previous research that relied on older

cultivars. Alternatively, the timing of flower initiation may be dependant on environmental parameters and during the past 50 years, global temperature has increased by an average of 1ºC (Landsberg, 1974; Lavee, 1985; Booij *et al*., 1992). Flower formation of *Fragaria ananassa* was advanced after exposure to elevated temperatures (Pietila *et al*., 2002; LeMiere *et al*., 1996) therefore the earlier bud burst reported in this experiment may be due to the temperature differential between the current climate and that when the previous research was conducted.

Floral development of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' progressed slowly from late September and stopped between mid-December and March/April. Suspension of flower development over winter has been reported for several species, but differences in the duration of stoppage are evident. *Rubus occidentalis* 'Cumberland' ceased developing early October, *Rubus ursinus loganobaccus* and *Rubus ideaus* 'Lloyd George' late October, and *Rubus fruticosus* 'Ashton Cross' and 'Himalayan Giant' mid-November (Robertson, 1957). Flower initiation of *R. ideaus* 'Malling Promise' and 'Ranere' stopped in December, as in this experiment, but re-commenced in January and February respectively (Williams, 1959; Waldo, 1933). The predominant factor controlling suspension of *R. ideaus* flower formation was found to be low temperature (Williams, 1959). Although not recorded in this experiment, *Ribes nigrum* flower development may have been prolonged in comparison to previous research due to the increase in global temperature, as described above. In spring, re-commencement of *Vaccinium vinifera* (Lavee, 1985), *Actinidia chinensis* (Linsley-Noakes and Allan, 1987), *R. nigrum* (Vestrheim, 1975) and *Anethum graveolens* (Booij *et al*., 1992) floral development was advanced by exposure to warmer temperatures. In particular, March/April temperatures appeared to be most important for this process (Vestrheim, 1975). Colder winter temperatures may therefore be associated with suspension of flower development and warmer spring temperatures with advancement of development.

Initially it appeared as if *Ribes nigrum* buds would over-winter at Stage 5, but all cultivars had progressed to Stage 6 by 17 December 2004. It is relatively difficult to compare this with previous research, due to the very different scales employed by authors; however, translation of other scales into that employed in this Chapter was attempted. *Rubus ideaus* 'Malling Promise' flowers were well-defined and appeared to over-winter in a mature stage, corresponding to Stage 7 or 8 (Williams, 1959). In contrast, *R. ideaus* 'Ranere' were immature and had reached approximately Stage 2 when growth ceased over winter (Waldo, 1933). *Actinidia chinenesis* over-wintered in Stage 1 (Linsley-Noakes and Allan, 1987),

Rubus fructicosus 'Himalayan Giant' and 'Ashton Cross' at Stage 2, *R. ideaus* 'Lloyd George' at Stage 4, *Rubus occidentalis* x *R. ideaus* at Stage 6 and *Rubus ursinus loganobaccus* at Stage 7 (Robertson, 1957). The over-wintering stage of *Anethum graveolens* was found to be dependant on autumn temperature, with initiation and development being accelerated by exposure to 12ºC compared to 8ºC (Booij *et al*., 1992). Therefore, the warmer the autumn temperature, the more advanced the over-wintering floral stage, which supports the theory of Williams (1959) that flower cessation over winter was a reaction to low temperature.

Flower initiation and development of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' was found to be independent of bud position. In contradiction to this, bud position affected flower initiation in *Ribes nigrum* (Nasr and Wareing, 1961), *Rubus ideaus* (Waldo, 1933; Mathers, 1952; Williams, 1959), *Rubus ursinus loganobacus* and *Rubus fructinosus* (Robertson, 1957). These authors reported a time-lag in development, whereby flowers formed firstly in buds closest to the apical bud, followed by those in the middle section of the stem, and basal buds lastly. Positional differences have been suggested to be a result of apical dominance and flower initiation was deemed to start when lateral buds were released from correlative inhibition (Waldo, 1933; Williams, 1959; Nasr and Wareing, 1961a).

Although in general 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' behaved similarly, at several stages minor differences were apparent. 'Ben Gairn' buds were marginally less developed by 3 August, 'Ben Hope' entered Stage 5 11 days later and 'Ben Tirran' remained in an over-wintering state for 31 days longer than the other cultivars. By the end of the experiment, 'Ben Tirran' had reached Stage 12 whereas 'Ben Gairn' and 'Ben Hope' were at Stage 13. More significant differences were observed in previous *Ribes nigrum* research, whereby flower initiation was delayed in 'Bang Up' compared to 'Boskoop', 'Silvergeiter', 'Wellington XXX' and 'Brodtorp' (Vestrheim, 1972). *Rubus ideaus* cultivar differences were far more apparent, with the date of flower initiation varying from 30 August for 'Ranere' and 'Lloyd George' to 8 February for 'Latham' and 'Chief' (Waldo, 1933).

It had been reported that early flowering *Ribes nigrum* and *Vaccinium vinifera* cultivars initiated flowers earlier than later flowering cultivars (Vestrheim, 1972; Lavee, 1985). In this experiment, however, flower initiation for all cultivars occurred between 16 July and 3 August. Differences between cultivars, however, were observed in the spring, when the early and mid flowering 'Ben Gairn' and 'Ben Hope' re-commenced flower development

before the later flowering 'Ben Tirran'. Floral development progressed rapidly after spring bud burst, and this is when cultivar differences were most apparent. Little difference was observed between 'Ben Gairn' and 'Ben Hope', but this would be expected as these cultivars bud burst at similar times (Atwood, 2004). 'Ben Tirran' on the other hands, bursts bud circa. 4 weeks later than the other cultivars, and a similar delay in the re-commencement of flower development would be expected.

The lack of effect of bud position and cultivar-specificity, especially during the stage changes, may be due to the extended time-periods between observations. If dissections were carried out are more frequent intervals, subtle differences between bud position and cultivars may become apparent.

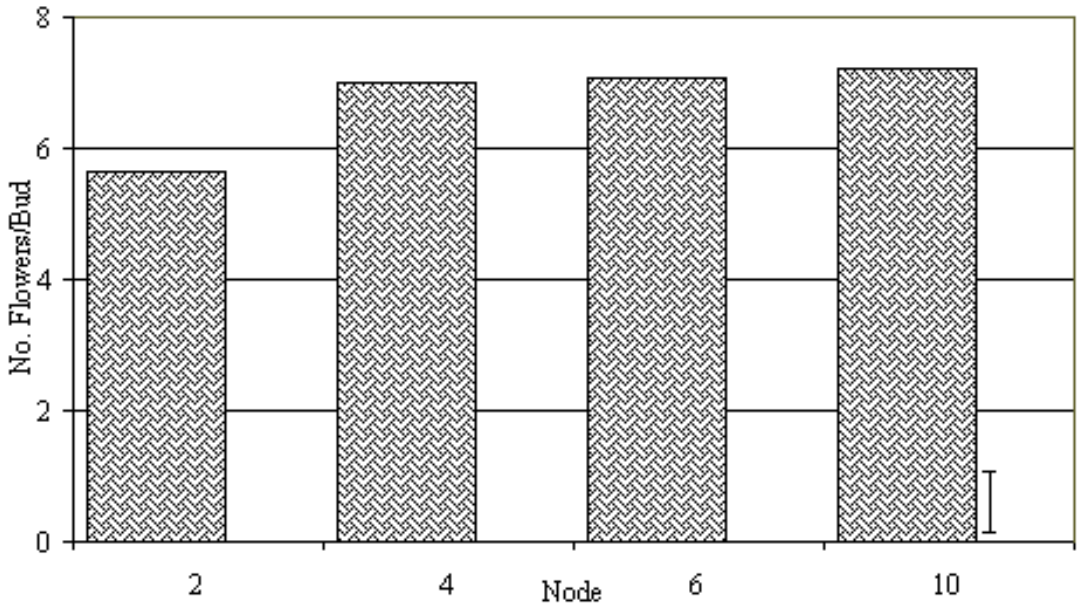
Ideally, this experiment should be repeated, in the absence of spider mites, firstly to clarify the date of flower initiation, but also to determine the extent of variation between years and to . When repeating this experiment, however, it is important to bear in mind that crop management techniques may affect the timing and rate of flower development e.g. summer pruning advanced *Prunus avium* flower initiation by 31 days (Guimond *et al*., 1998). Williams (1959) stated that discrepancies in the timing of *Rubus ideaus* flower initiation compared to earlier literature were due to climatic differences. Similarly, differences in the date of *Rubus fructicosus* flower initiation in Oregon and Maryland were explained in terms of temperature differences (Waldo, 1933). Year to year variations were observed for *R. ideaus*, whereby floral initiation began on 5 October in 1932 and 25 September the following year (Waldo, 1933).

7.5. Conclusions

Flowers were initiated in *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' between 16 July and 3 August 2004. Flower development progressed slowly until 17 December and the buds over-wintered in Stage 6 then developed rapidly from March/April. Flower initiation was unaffected by bud position, and only slight cultivar differences were apparent, but further experimentation at more frequent intervals may record differences in both parameters. Repetition would also allow a more detailed record of flower abortion. Care should be taken to ensure the plants are kept pest and disease free to ensure infestation does not contaminate the results. The controlling factor in flower initiation and development appeared to be

temperature, and future research should investigate the effects of proposed climate change scenarios on the timing and rate of floral development.

Plate 7.1. Scanning electron microscope image of *Prunus persica* 'Redhaven' flower initiation Stage 0/1.
(after Engin and Iqbal, 2004) 146



Chapter Eight.
General Discussion, Conclusions and Future Work

In a survey of commercial blackcurrant growers, most commented that in recent years spring bud burst had become delayed and irregular and that the flowering period was prolonged, with late flowering cultivars being more affected than earlier flowering cultivars (Atkinson *et al*., 2004). Such observations were not restricted to the UK, and similar findings were reported by growers in Australia (Westmore, 2000). It was also noted by most growers that the incidence of spring frosts had decreased substantially in the past decade and that the winters were milder. To confirm this observation, annual chill units, calculated for four geographical locations using the <7ºC, 0-7ºC, Lantin and Utah models, decreased considerably between 1950 and 2002 (Atkinson *et al*., 2004). There has been concern within the blackcurrant industry that the physiological symptoms the growers reported were directly related to the UK climate, in particular milder winters, and that climate change would further exacerbate the situation.

Like many temperate-zone crops, *Ribes nigrum* requires a period of winter chilling to break dormancy and ensure synchronised bud burst and flowering the following spring. Despite being a commercially important crop, the optimum chilling temperature for overcoming dormancy in modern-day *R. nigrum* cultivars was unknown, and the primary objective of this thesis aimed to address this. The main objective of this research was to construct chill unit models for the cultivars 'Ben Gairn', 'Ben Hope' and 'Ben Tirran'. In addition, although the effects of proposed climate change scenarios on *R. nigrum* have been rigorously modelled (Atkinson *et al*., 2004), no experimental evidence was available to substantiate the results. Therefore, in this research, the extent of the physiological responses to climate change scenarios were observed and short-term solutions to alleviating the effects and inducing premature bud burst were investigated. Lastly, exposure to warm spring temperatures were found to have a detrimental effect on flower production, yet the timing and rate of modern-day *R. nigrum* flower initiation and development has not been established.

8.1. Specific Responses due to Cultivar

The cultivars used throughout these experiments showed differential responses, particularly with respect to temperature. Differences based on cultivars have been reported for other crop species (Weinberger, 1956; Plancher, 1983a; Young and Werner, 1985). Variations in

chilling requirements within *Ribes nigrum* can be anticipated, as cultivars are placed into one of three groups – 'Ben Gairn' is the earliest flowering cultivar, 'Ben Tirran' the latest and 'Ben Hope' intermediate. In addition, the breeding histories of the cultivars are extremely complex, with germplasm introduced not only from different UK cultivars, but from Scandanavian and Russian cultivars, as well as from different species. The concept of cultivar-specific response, however, has implications for management regimes, for example, premature defoliation on 25 September successfully advanced bud burst of 'Ben Gairn' and 'Ben Tirran', but had no effect on 'Ben Hope'. It also has implications for the application of chill unit models, which were determined to be specific to each cultivar. Although models now exist for the three studied cultivars, the chilling requirements of the remaining commercial cultivars are unknown. The models presented in this thesis may be broadly applicable to a range of cultivars, but insufficient field data has hampered testing of this theory.

8.2. Chill Unit Models

When chill unit models were constructed for 'Ben Gairn', 'Ben Hope' and 'Ben Tirran', both extremes of the temperature range utilised in Experiment 1 (-3.4ºC and 8.9ºC) were found to contribute significantly to the chilling requirement of the cultivars and the optimum chilling temperature was found to be -3.4ºC. This was considerably lower than reported for other species e.g. *Prunus avium* 'Sunburst', 'Summit' and 'Stella', 3.6°C, 3.2°C and 3.1°C respectively (Mahmood, 1999) and *Malus domestica* 'Starkrimson Delicious', 6.1ºC (Shaltout and Unrath, 1983). This result should perhaps have been expected, as the optimum chilling temperature of *R. nigrum* 'Silvergeiter' and 'Meitgo' was -3ºC (Plancher, 1984) and Lantin (1977) predicted that as the chilling temperature decreased to -7ºC, a positive effect on *R. nigrum* plant growth would be observed. In addition, recent breeding objectives involved the introduction of Scandanavian germplasm with the aim of increasing the chilling requirement of the crop, which would be expected to alter the optimum chilling temperature.

As the optimum temperature for 'Ben Gairn', 'Ben Hope' and 'Ben Tirran' was determined to be -3.4ºC, and this was the lowest temperature tested, it was decided to extend the experiment in the subsequent year, to determine the effects of lower temperatures (-10.1ºC) on all cultivars (Experiment 3). A sub-set of plants were placed in the 1.5ºC cold store that was utilised in Experiment 1 to determine any differences between the sets of plants. When results from Experiment 3 were incorporated into the original data and modelled, however,

very different results were produced. Most noticeably, the optimum chilling temperatures of 'Ben Gairn', 'Ben Hope' increased to 1ºC and 1.7ºC respectively, whilst 'Ben Tirran' decreased to -4.3ºC.

Although every attempt was made to use similar size and age of plant material in both experiments, and to maintain the plants in identical conditions, the cultivar responses to the chilling regimes in Experiment 3 does cause concern. Pre-chilling conditions, such as irrigation cycling, pest and disease occurrence, and provenance have been reported to affect a plant's chilling response (Plancher, 1984; Erez and Couvillon, 1987; Brennan, R., *Pers. comm.*). The *Puccinia* infestation, although controlled by chemical application, may have altered the chilling response of the plants, although no published reports on the effects of disease on the chilling response are available. Given the uncertainly surrounding the results of Experiment 3, and the suspicion that the data was suspect, the GSK/Fraser (2) model should be discarded in preference of the original GSK/Fraser model.

8.2.2. Validation of the Chill Unit Models

In order to be commercially applicable, chill unit models must be able to predict the date of chill satisfaction over a range of geographical positions and years. In order to establish the accuracy of both models, they were validated against field data collected for Norfolk and Gloucester in 2002/2003 and 2003/2004 and compared against the <7ºC, 0-7ºC, Lantin and Utah models. Based on the available data, incorporating results from Experiment 3 did not affect the accuracy of the 'Ben Gairn' model, but reduced the accuracy of the 'Ben Hope' and 'Ben Tirran' models which further calls into question the legitimacy of constructing models based on successive years' experiments.

For all three cultivars, the GSK/Fraser model was either the most or one of the most accurate models. The Lantin, GSK/Fraser, and GSK/Fraser (2) models were equally accurate for 'Ben Gairn' and the 0-7ºC was as accurate for 'Ben Tirran'. As reported previously (Shaltout and Unrath, 1983; Erez and Couvillon, 1987; Atkinson *et al*., 2004) the Utah model failed to accurately predict chilling satisfaction of the cultivars tested. Atkinson *et al*. (2004) concluded that species-specific models would increase the accuracy of chill unit models and be of more benefit to growers. The accuracy of the GSK/Fraser model, ±1 day for 'Ben Gairn' and ±3 days for 'Ben Hope' and 'Ben Tirran' was comparable to the accuracy of

previous chill unit model experiments (Kobayashi *et al*., 1982; Cannell and Smith, 1983; Andersen, 1992).

The decreased accuracy of the GSK/Fraser (2) model compared with the original GSK/Fraser model further supports the theory that the results of Experiment 3 are suspect, as discussed previously. Until more conclusive experimental evidence regarding the effect of temperatures below -3.4ºC on the physiology of *R. nigrum* is available, use of the GSK/Fraser (2) model should be discontinued in favour of the more accurate GSK/Fraser model

It was concluded that 'Ben Gairn' required 2384 chill hours, 'Ben Hope' 2604 and 'Ben Tirran' 1211 hours of chilling. As 'Ben Tirran's chilling requirement is satisfied three months later than 'Ben Gairn' and 'Ben Hope's (Atwood, 2004), it was originally thought that 'Ben Tirran' had the highest chilling requirement of all three cultivars tested (Saunders, R. *Pers. comm*.). According to the GSK/Fraser model, however, 'Ben Tirran' required less than half the number of chilling hours of the other tested cultivars. If the model is extended out-with the experimental temperature range, temperatures above 8ºC could negate previously accumulated chill units. A relatively warm winter's day, therefore, may negate 'Ben Tirran's chilling, whilst significantly contributing to 'Ben Gairn' and 'Ben Hope's.

8.2.3. Budsticks v Whole Plants

Research into chill unit models has often been hampered by inadequate facilities, in that the number of plants/trees that can be placed into a cold store or forcing house is often limited. A similar constraint was obvious throughout this thesis, where replication number was dependant on space. In an attempt to maximise the available data, it has been common practice to utilise budsticks (unrooted cuttings) in dormancy experiments. The obvious advantage in using such material is the considerably reduced space required for relatively large replications. When the bud burst behaviours of 'Ben Gairn' and 'Ben Hope' budsticks were compared to that of whole plants, however, the levels of bud burst were much lower in budsticks, which has also been reported by previous authors (Snelgar *et al*., 1997; Mahmood *et al*., 2000a). Lower bud burst may have been an acceptable trade-off had the behaviour of the budsticks mirrored that of whole plants, but no such correlation was observed. Budsticks of 'Ben Tirran' behaved in a similar manner to whole plants, but experiments cannot be conducted using budsticks of some cultivars and whole plants of others.

This has been a contentious subject area, and while some authors reported a strong correlation between the behaviour of budsticks and whole plants (Mahmood *et al*., 2000a), others disputed this (Lantin, 1973; Chandler and Tufts, 1993). Previous *Ribes nigrum* research concluded that the cutting of a stem to produce a budstick altered the chilling requirement (Lantin, 1973; Plancher, 1983b). In addition, removal of the root system not only affected the ability to uptake nutrients, but budsticks often initiated roots from the cut stem, which diverted energy away from bud bursting. The inhibitory effect of apical dominance may be exacerbated on removal of the root system, as root-derived phytohormones may have a role to play in bud development, for example cytokinins have been implicated, although whether they need to be regenerated via. the root system remains contentious (Taiz and Zeiger, 2002). The full force of the inhibitory effect of the apical bud could account for the lower bud burst of budsticks, as could lack of nutrients as a result of having no root system (McPherson *et al*., 1995).

8.3. The Role of Temperature in Dormancy Induction/Release

As mentioned previously, processes controlling dormancy induction and release are poorly understood, but are likely to be regulated by temperature. On exposure to stress events, e.g. chilling, accumulation of dehydrins (glycine-rich proteins) has been recorded (Rowland *et al*., 2004). The role of dehydrins in dormancy control, however, has not been established, and instead they are thought to provide a degree of cold-hardiness (Arora *et al*., 1997). Dormant pea buds expressed the gene PsDRM1, production of which was stimulated by exposure to cold temperatures (Stafstrom, 2002). High concentrations of ABA, associated with dormancy induction, are reported to enhance the expression of PsDRM1 (Stafstrom, 2002). However, no further corroborating evidence relating expression of PsDRM1 with dormancy has been found.

Photoperiod-induced dormancy in *Betula* spp. was characterized by the closing of plasmodesmata entrances via. production of 1,3-beta-D-glucan-containing sphincter complexes (Rinne and Schoot, 2004). Cell walls were also sealed, thus preventing nutrient transfer and cell-cell signalling (Jian *et al*., 1997; Rinne and Schoot, 2004). The concentration of 1,3-beta-D-glucanase increased in spring (Krabel *et al*., 1993) and the enzyme degraded the sphincter complexes, thus renewing cell to cell signalling (Rinne and Schoot, 2004). This enzyme was also implicated in seed dormancy release (Luebner-Metzger, 2003).

During release from dormancy, water is transported into the bud and a reversal in respiratory mechanism from an energy-consuming to an energy-producing system was observed (Faust and Wang, 1993). Production of DNA, RNA and proteins increased and bud starch concentration decreased as a result of conversion to sugars for energy release (Faust and Wang, 1993; Lang, 1989). The role of hormones is a hotly disputed subject, with initial workers suggesting a definite hormonal control and others disputing this (Mielke and Dennis, 1975; Powell, 1987; Masai *et al*., 1993). Dormancy release has been associated with decreased levels of ABA and increased levels of gibberellins, cytokinins and indole acetic acid (IAA) (Edwards, 1985; Olsen *et al*., 1997; Bondock *et al*., 1995; Piola *et al*., 1998; Masai *et al*., 1993). Exogenous application of IAA, gibberellic acid (GA_3) and zeatin (Edwards, 1985; Nadel *et al*., 1991; Taylor *et al*., 1995; Gu and Read, 2004) resulted in release of dormancy, but application of ABA delayed bud burst (Taylor *et al*., 1995; Baldwin *et al*., 2000). The fate of applied hormones, however, is unknown, therefore any correlation between hormone application and bud bust must be made with caution. Alternatively, bud burst may be regulated by genetic control and hormone concentration may be a result of, rather than the cause of, bud burst. The concentration of KNAP2 was reported to reach a maximum prior to bud burst and then decrease rapidly, followed by an increase in GA (Brunel *et al*., 2002; Horvath *et al*., 2003).

Research into this topic is on-going, and with the invention of new technology and investigative techniques, the mechanisms behind dormancy induction and release will be identified.

8.4. The Physiological Effects of Climate Change

Exposure to increasing winter temperatures (from -3.4ºC to 8.9ºC) was detrimental to the timing of bud burst, final bud burst and flower production, which has been reported for several crop species (Cannell and Smith, 1983; Couvillon and Erez, 1985; Mahmood, 1999). Once a dormant state has been entered, the ability of a plant to develop is limited, even if placed in favourable environmental conditions, until a certain proportion of the chilling requirement has been satisfied (Heins *et al*., 2000). The detrimental effects of increasing winter temperatures, therefore, suggest that the chilling requirements of the cultivars are not being achieved. Following the thinking of Faust and Wang (1993), if buds haven't received enough winter chilling and hence dormancy was not properly released, the reversal of the respiratory system from energy-consuming to energy-producing may not have been

completed. Protein, DNA and RNA production would not proceed as normal, which would affect the bud-breaking capabilities of the buds. Alternatively, lack of winter chilling could inhibit production of PsDRM1, which could result in poor bud burst (Stafstrom, 2002). Similarly, 1,3-beta-D-glucanase accumulation may be inhibited, which would prevent degradation of the sphincter complexes, thus preventing cell to cell signalling and normal growth and development (Rinne and Schoot, 2004).

Plants were placed in simulated climate change scenarios on 1 February 2005 after exposure to natural chilling. By this time, 'Ben Gairn' and 'Ben Hope' had received 100% of the required chilling respectively, but 'Ben Tirran' had received only 53%. In accordance with Cannell and Smith (1986), Murray *et al*. (1989) and Mahmood *et al*. (2000b), warmer spring temperatures advanced vegetative and floral bud burst of 'Ben Gairn' and 'Ben Hope'. This supports the theory that the post-chilling heat requirement is increasingly satisfied as the ambient temperature is increased (Cannell and Smith, 1986; Atkinson *et al*., 2004). Negative effects on final bud burst, extension growth, flower and fruit production were observed under the elevated temperature regimes, as described in Chapter 5. This response is similar to that reported by *Ribes nigrum* growers (Atkinson *et al*., 2004), yet contrary to the theory of plant development that as temperature increases, developmental rates increase (Heins *et al*., 2000). Under the warmer temperature regimes, subsequent plant growth may have been constrained by low light levels or short days (Bisgrove and Hadley, 2002), but it is unclear whether these factors would result in an overall decrease in growth parameters in relation to control plants. Growth may have been affected by availability of nutrients, and although the plants were hand-watered when required and supplied with nutrients, the concentration may have been too low for the rapidly growing plants. This is an important consideration for growers, in that crop management techniques may have to be modified in response to the changing climate. The reduction in flower number under the simulated climate change scenarios may explain the reduced fruit production when plants were exposed to insufficient chilling (Chapter 3).

8.5. Alleviating the Effects of Climate Change

Although the primary aim of the *Ribes nigrum* breeding process has been to produce cultivars with lower chilling requirements, such crops may take over 10 years to become commercially available. Growers are already reporting the effects of lack of winter chilling (Atkinson *et al*., 2004; Westmore, 2004), hence short term measures to ensure the crop remains viable in the UK must be implemented. Crop management regimes have been employed in tropical

countries to overcome the problem of insufficient chilling, and such methods may prove valuable in this country (Delap, 1966; Terblanche *et al*., 1979; Dennis, 1987; Edwards, 1987; George and Nissen, 1992).

8.5.1. Post-Harvest Nitrogen Application

Post-harvest nitrogen application had no effect in the following spring on either time to first bud burst or final bud burst of 'Ben Gairn', 'Ben Hope' and 'Ben Tirran'. This perhaps should not have been surprising, as the treatment was applied late in the season and it is doubtful whether the plants were active enough to take up the excess nitrogen. This is further substantiated by the lack of effect of treatment on the date of senescence – nitrogen application often extends the growing season and delays senescence, as reported for *Prunus persica* 'Flordaprince' (George and Nissen, 1992) and *Malus domestica* 'Lord Lambourne' (Hill-Cottingham and Williams, 1967).

The beneficial effect of nitrogen application, however, has proved not only to be species and cultivar specific, but efficacy may depend on the form of nitrogen applied (Delap, 1966; Delap, 1967; Hill-Cottingham and Williams, 1967; Terblanche *et al*., 1979; George and Nissen, 1992). In this experiment, nitrogen was added to the irrigation system, but may have been washed away by heavy rains. Late season nitrogen fertiliser application in the field may also be ineffective if followed by heavy rains.

8.5.2. Premature Defoliation

More success was gained by subjecting the plants to manual premature defoliation and beneficial effects on time to bud burst and final bud burst were recorded. Previous research with *Ribes nigrum* reported no beneficial effects of premature defoliation (Plancher, 1983b; Westmore, 2004), but both authors defoliated the plants relatively close to the natural defoliation date, which may have been too late for the plants to benefit. The subsequent advancement of bud burst after leaf removal suggested that leaves were directly involved with the process of dormancy. The results of this experiment further support the theory that a dormancy promoter is transported from the leaves to the bud.

Although the optimum treatment involved defoliation on 1 September, severe tip-die back was recorded for all plants in this treatment. A similar result was reported by previous authors and may have been a result of the plants not gaining cold-hardiness (Corke and

Wilson, 1963; Westwood, 1978). Defoliation on 25 September did not damage the plants, but was less effective and did not affect 'Ben Hope'. As a note of caution, the effects of defoliation on yield were not recorded in this experiment, and care must be taken to ensure such treatment does not substantially reduce yields as reported by Anderson and Seeley (1992).

8.5.3. Dormancy Breaking Chemicals

Dormancy-breaking chemicals have been used to promote bud burst of temperate-zone fruit crops grown in tropical countries where year-round high temperatures do not satisfy chilling requirements. Traditional chemicals, however, are not registered for use in the UK and must undergo rigorous and expensive registration processes before application to crops. Chemical adjuvants, that have undergone registration and are typically used to aid pesticide/herbicide penetration, have successfully advanced *Ribes nigrum* bud burst, although field experiments have been relatively crude and non-scientific (Saunders, R., *Pers. comm*.). The mode of action of the adjuvants is unknown and the degradation pathway of the active ingredient, alkylphenyl hydroxypolyoxyethylene, has not been identified.

Three adjuvants, with similar active ingredients, were applied to 'Ben Tirran' budsticks at concentrations of 2%, 1% or 0.5%, and although the final bud burst of the treated budsticks was extremely low, the fact that treated plants did bud burst whereas the control plants did not was in itself a significant result. Success was gained in the field when Abacus (active ingredients alkylphenyl hydroxypolyoxyethylene, esterified rape seed oil and natural fatty acids) was applied to Ben Tirran plants at concentrations between 5% and 10% (Saunders, R., *Pers. comm*.), which suggests the concentrations used in this experiment were too low. Alternatively, lack of bud burst could be attributed to the use of budsticks (as described previously) or the chilling deficit of the budsticks may have been too high for any treatment other than chilling to overcome.

8.5.4. Extended Photoperiodic Regimes

Extending the photoperiod from 8-hours to 17-hours substituted for insufficient chilling and had a dramatic effect on both time to first bud burst and final bud burst for all cultivars, as reported by previous authors (Olmsted, 1951; Wareing, 1954a,b; Hoyle, 1960; Heide, 1973). However, the bud burst behaviour of budsticks may not represent that of whole plants and

interpretation of the results should be conducted with caution. Although this treatment had beneficial effects on bud burst, the practical applications are limited.

8.5.5. Practical Applications of Altering Crop Management Practices

There has been great interest in these experiments amongst the GSK growers. Treatments such as nitrogen application and dormancy breaking chemicals are relatively easy to apply and rely on chemicals that are approved for use on *Ribes nigrum*. Similarly, treatment timings coincide with the 'quiet' period of growers, and can be implemented without relying on casual labour or the sacrifice of an equally important job. The growers were however, more interested in the results of the defoliation experiment, although there were slight concerns over the results of chemical leaf removal. In particular, the significant advancement of 'Ben Tirran' bud burst was of interest, as this is one of the cultivars the growers are having most bud burst problems with. The danger in advancing bud burst by such large margins, however, is that late spring frosts may damage the newly expanding buds and negatively affect yield. The effect of insufficient winter chilling, however, could reduce yields to a similar level, however, and hence the risk may be worth taking.

Although the photoperiodic experiment produced the desired results, application of this treatment is likely to be extremely limited. The crop is grown in the field and permission might have to be granted to erect floodlights, or something similar, in fields. Pollution, in terms of light and noise from generators, might incur financial penalties and ultimately, the financial cost of running lights are unlikely to out-weigh the benefits, especially considering other treatments, e.g. premature defoliation and chemical application, can be conducted relatively easily and cheaply.

8.6. Flower Initiation

Unlike the results of Vestrheim (1972), this experiment did not discover any differences between cultivars in the date of flower initiation. Flower initiation commenced between 16 July and 3 August, significantly earlier than reported previously for *Ribes nigrum* (Nasr and Wareing, 1961b; Wilson and Adam, 1966; Vestrheim, 1972). The results of this experiment did not support the hypothesis that cultivars that flower early in the season initiated flowers earlier than those that flower later in the season (Vestrheim, 1972), nor that initiation was dependant on bud position (Nasr and Wareing, 1961b). The date of flower initiation, however, was not determined with the desired level of accuracy and lack of plant

material limited the number of dissections that were conducted. More frequent examination may reveal differences between cultivars and bud positions.

8.7. The Future of the *Ribes nigrum* Industry in the United Kingdom

Although climate change is set to have a major impact on the agricultural and horticultural world, changes can and are being made to minimise this. Conventional breeding programmes, both in the UK and New Zealand, are in the process of breeding low chill requirement cultivars to enable *Ribes nigrum* to benefit from the warmer winters. Many of the common UK cultivars, however, have not performed well in New Zealand, and vice versa (Westwood, G., *Pers. Comm.*), although germplasm exchange between the UK and New Zealand breeding programmes is ongoing (Atkinson *et al*., 2004). One of the main disadvantages in breeding low chilling into the crop, however, is that buds will burst relatively early and may be susceptible to frost damage, which was a common problem for 'Ben Lomond'. Although under the predicted climate change scenarios, the number of frosts experienced in the UK will decrease, their severity and unpredictability may increase (Bisgrove and Hadley, 2002).

Meanwhile, commercial growers have been particularly pro-active in terms of experimenting with techniques to help alleviate the effects of warmer winters, e.g. application of dormancy breaking chemicals. Although more research is required, initial results of altering management techniques proved promising and growers have discussed the possibility of implementing field trials using such techniques as described in this thesis. Several growers now have mini weather stations in their fields and are recording, amongst other data, field temperature, which will provide comprehensive data on alterations in winter temperature.

Throughout the duration of this PhD, commercial growers have been thoroughly interested in the results, and are keen to apply not only the management techniques, but also the chill unit models. This is perhaps the greatest hurdle that had to be overcome – creating models that would predict chilling satisfaction was only the beginning, and the models had to be accurate and grower-friendly. Unlike previous models, the GSK/Fraser model is relatively simple to use and depends on one equation per cultivar, to relate hourly field temperature to chill units. To further aid the growers, a software programme was designed, which not only calculates the number of chilling hours that have been received, but automatically calculates the additional number of hours of chilling required (Figure 8.1).

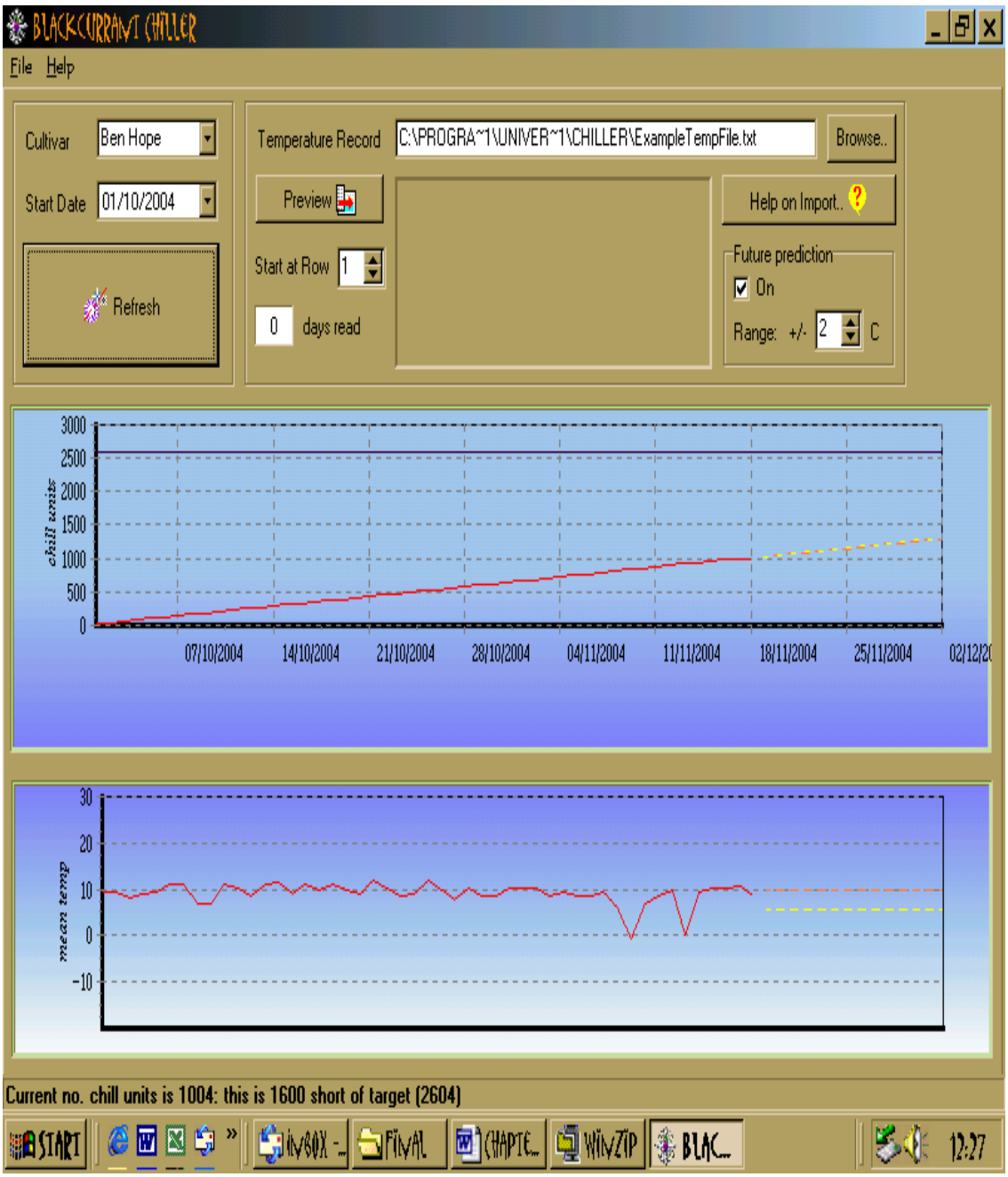


Figure 8.1. Screen shot of the GSK/Fraser Chill Unit Model software

(Harwood, 2005)

Growers simply have to import the temperature data, select the cultivar ('Ben Gairn', 'Ben Hope' or 'Ben Tirran') and the programme calculates how many chill units have accumulated, and how many more are required. The starting date for chill accumulation can be altered but is currently set at 1 October. In addition, the programme predicts, based on temperature data from the previous 7 days, temperature and chill unit accumulation over the next three weeks. This programme is available, free of charge, to all GSK growers.

GlaxoSmithKline and its growers are extremely proud of Ribena's British roots and are determined to keep the majority of the production in the UK. The pro-active attitude of the growers, breeders and GSK will ensure the future of *Ribes nigrum* and Ribena production remains in the UK.

Conclusions

Initially, the optimum temperature for maximising bud burst of *Ribes nigrum* 'Ben Gairn', 'Ben Hope' and 'Ben Tirran was found to be -3.4ºC. The results of a successive experiment, aimed at further refining this temperature, were determined to be suspect and should be regarded with caution. When validated against field data, the GSK/Fraser model was found to be as predictive or more so than other available models, and predicted chilling satisfaction within 1 day ('Ben Gairn') and 3 days ('Ben Hope' and 'Ben Tirran'). In addition, the GSK/Fraser model was much easier to implement than other models e.g. Lantin and Utah, and predictive software has increased its user-friendliness. The decreased accuracy of the GSK/Fraser (2) model further substantiated the theory that results from Experiment 3 were suspect, and until further experimentation can resolve this, the use of the original GSK/Fraser model should be preferential.

Insufficient winter chilling and elevated spring temperatures, as predicted under the climate change scenarios, were found to advance vegetative and floral bud burst, but detrimental effects on growth and reproductive development were apparent. Attempts to manipulate the crop management regime, in order to alleviate the effects of insufficient chilling, had mixed results, with beneficial effects being recorded for premature defoliation and dormancy breaking chemical application. The date of flower initiation was observed to be between 16 July and 3 August, with little development occurring over winter and rapid development after vegetative bud burst. Contrary to previous research, no effect of cultivar or bud position was apparent, although this may have been affected by the interval between dissection dates.

Future Work

Many questions regarding the physiology of *R. nigrum* in relation to temperature were answered. However, many questions still remain…

Results from the first year's experiment suggested temperatures below -3.4°C and above 8.9°C significantly contributed to chilling, although this was not substantiated in a subsequent experiment. Future work should concentrate on widening the temperature range and recording the crop response.

Although the chill unit models were validated against field data, the available information was limited. The models should be further validated against a more diverse geographical area using data from several consecutive years.

Little work has concentrated on defining the start date of chill accumulation. In order to maximise the predictive power of chill unit models, a physiological indicator of the start of chilling would be beneficial.

Mixed results were obtained when altering the crop management techniques to force and promote premature bud burst. Premature defoliation was found to be an effective treatment, but future work must concentrate on altering the timing of defoliation to prevent physiological damage.

Results from the dormancy-breaking experiment suggested the chemicals Abacus and Activator 90 were effective at forcing bud burst. As well as determining the optimum concentration for maximising bud burst, the maximum chilling deficit that can be overcome by such applications should be determined in order for the crop to fully benefit.

The bud dissections provided new and thorough data on the timing of flower initiation and development. Knowledge of the effect of proposed climate change scenarios on these processes would be beneficial.

Perhaps the most important future work should concentrate on encouraging growers to monitor their crops and record physiological occurrences, e.g. date of bud burst, final bud burst, date of flower, yield and winter field temperature. These data could provide a wealth of information that could relate crop performance to winter chilling and could be invaluable in monitoring the effects of climate change.

References

Abbott, D.L. (1969). The role of budscales in the morphogenesis and dormancy of the apple fruit bud. In: Physiology of Tree Crops, Second Long Ashton Symposium. (Luckwill, L. C. and Cutting, C.V. eds.) Academic Press, London. pp65-82.

Aerts, R., Cornelissen, J.H.C., Dorrepaal, E., van Logtestijn, R.S.P., Callaghan, T.V. (2004). Effects of experimentally imposed climate scenarios on flowering phenology and flower production of subarctic bog species. *Global Change Biology*, **10**, 1599-1609.

Allan, P. (1999). Measuring winter chilling in areas with mild winters. *Deciduous Fruit Grower*, 49, 1-10.

Anderson, J.L., Richardson, E.A. and Kesner, C.D. (1986). Validation of chill unit and flower bud phenology models for 'Montmorency' sour cherry. *Acta Horticulturae*, **184**, 71-78.

Anderson, J.L. and Seeley, S.D. (1992). Modeling strategy in pomology: development of the Utah Models. *Acta Horticulturae,* **313**, 297-306.

Anderson, T.B. (1992). A simulation study in dynamic "Utah" models. *Acta Horticulturae,* **313**, 315-323.

Anon. (1973). *Blackcurrants*. 2nd Edition. Stephen Austin and Sons, Ltd., Hertford, U.K., pp 98.

Anon. (2004). International Agro Additive Specialties. www.iaas.nl

Arora, R., Wisniewski, M.E. and Rowland, L.J. (1997). Low temperature-induced expression of dehydrins in deciduous fruit crops and their relation to cold acclimation and/or dormancy. *Acta Horticulturae,* **411**, 175-182.

Artf. A.M., Walker, M.D., Gurevitch, J., Alatalo, J.M., Bret-harte, M.S., Dale, M., Diemer, M., Gugerli, F., Hnery, G.H.R., Jones, M.H., Hollister, R.D., Jonsdottir, I.S., Laine, K., Levesque, E., Marion,G.M., Molau, U., Molgaard, P., Nordenhall. U., Raszhiven, V., Robinson, C.H., Starr, G., Stenstrom, A., Stenstrom, M., Totland, O., Turner, P.L. Walker, L.J., Webber, P.J., Welker, J.M. and Wookey, P.A. (1999). Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. *Ecological Monographs*, **69**, 491-511.

Ashcroft, G.L., Richardson, E.A. and Seeley, S.D. (1977). A statistical method of determining chill unit and growing degree hour requirements for deciduous fruit trees. *HortScience*, **12**, 347-348.

Atkins, T.A. and Morgan, E.R. (1990). Modelling the effects of possible climate change scenarios on the phenology of New Zealand fruit crops. *Acta Horticulturae*, **276**, 201-208.

Atkinson, C.J., Sunley, R.J., Jones, H.G., Brennan, R. and Darby, P. (2004). DEFRA Desk study in winter chill in fruit.

Aussenac, G. (2002). Ecology and ecophysiology of circum-Mediterranean first in the context of climate change. *Annals of Forest Science*, **59**, 823-832.

Badeck, F.W., Bondeau, A., Bottcher, K., Doktor, D., Lucht, W., Schaber, J. and Sitch, S. (2004). Responses of spring phenology to climate change. *New Phytologist*, **162**, 295-309.

Bailey, J.S. and Rossi, A.W. (1965). Effect of fall chilling, forcing temperature and day length on the growth and flowering of Catskill strawberry plants. *Proceedings of the American Society for Horticultural Science*, **87**, 245-251.

Baldwin, B.D., Bandara, M.S. and Tanini, K.K. (2000). Role of bud scales and phytohormones in the maintenance and release of endo-dormancy in field-grown Saskatoon berry floral buds. *Acta Horticulturae*, **520**, 209-218.

Barba, N.W. and Melo-Abreu, J.P. de. (2002). Validation of rest completion models in peach trees in two regions of Portugal. *Acta Horticulturae*, **592**, 445-449.

Battey, N. H. (2000). Aspects of seasonality. *Journal of Experimental Biology*, **51**, 1769-1780.

Bautista, A.D., Vargas, G. and Colmenares, J.C. (1987). Effects of ethephon on bud brak and fertility of three grape cultivars. *Acta Horticulturae*, **199**, 100.

Bigras, F.J. (1996). Conifer Bud Dormancy and Stress Resistance : A Forestry Perspective. In Lang, G.A. ed. *Plant Dormancy : Physiology, Biochemistry and Molecular Biology*. CAB International, Oxon, U.K., pp 400.

Bindi, M, Gozzini, B and Orlandini, S. (1993). The combined effects of climate changes and increases in atmospheric CO_2 concentration on the potential growth of three grapevine varieties. *Acta Horticulturae*, **388**, 43-52.

Bondock, M.A., Abdel-Hamid, N. and El-Wakeel, H.F. (1995). Bud break, fruit maturity and yield of "Anna" apple as influenced by the plant bioregulator "Thidiazuran". *Annals of Agricultural Science (Cairo)*, **40**, 343-358.

Booij, R., Meurs, E.J.J., Thiel, F. and Boekestein, A. (1992). Cryo-scanning electron microscopy of the apex of celeriac (*Apium graveolens* L. var *rapaceum* (Mill)DC) during initiation of the inflorescence. *Scientia Horticulturae*, **51**, 309-320.

Brennan, R.M. (1996). Currants and gooseberries. In: *Fruit Breeding, Volume II: Vine and Small Fruit Crops*. (Janick, J. and Moore, J. Eds.). John Wiley and Sons, Inc., U.K., pp 477.

Brookes, R.M. (1942). Climate in relation to deciduous fruit production in California II. Effect of the warm winter of 1940-41 on apricot, plum and prune varieties in Northern California. *Journal of the American Society for Horticultural Science*, **39**, 209-211.

Brown, D.S. (1952). Climate in relation to deciduous fruit production in California. IV. Effect of the mild winter of 1950-51 on deciduous fruits in Northern California. *Journal of the American Society for Horticultural Science*, **77**, 111-118.

Brown, D.S. (1960). The relation of temperature to the growth of Apricot flower buds. *American Society for Horticultural Science*, **75**, 138-147.

Brunel, N., Leduc, N., Poupard, P., Simoneau, P., Mauget, J.C. and Viemont, J.D. (2002). KNAP2, a class I KN1-like gene is a negative marker of bud growth potential in apple trees (*Malus domestica* [L.] Borkh.). *Journal of Experimental Biology*, **53**, 2143-2149.

Campbell, N.A. (1996). *Biology*. 4th Edition. Benjamin and Cummings, San Fransisco, U.S.A. pp 1206.

Campbell, N.A. and Reece, J.B. (2002). *Biology*. 6th Edition. Benjamin and Cummings, San Fransisco, U.S.A., pp1247.

Campbell, R.K. and Sugano, A.I. (1975). Phenology of bud burst in Douglas-Fir related to provenance, photoperiod, chilling and flushing temperature. *Botanical Gazette*, **136**, 290-298.

Cannell, M.G.R. (1989). Chilling, thermal time and the date of flowering of trees. In; *Manipulation of fruiting*, (ed. Wright, C.J.), Butterworths, London, pp99-113.

Cannell, M.G.R. and Smith, R.I. (1983) Thermal time, chill days and prediction of budburst in *Picea Sitchensis*. *Journal of Applied Ecology*, **20**, 951-963.

Cannell, M.G.R. and Smith, R.I. (1986). Climatic warming, spring budburst and frost damage on trees. *Journal of Applied Ecology*, **23**, 177-191.

Carew, J.G. and Battey, N.H. (2000). Short cut to raspberry crops. HDC News, April.

Carew, J.G., Hadley, P. and Darby, J. (1999). The effect of temperature on the vegetative growth and reproductive development of the primocane fruiting raspberry 'Autumn Bliss'. *Acta Horticulturae*, **505**, 185-190.

Carew, J.G., Mahmood, K., Darby, J., Hadley, P and Battey, N.H. (2001). The effects of low temperatures on the vegetative growth and flowering of the primocane fruiting raspberry 'Autumn Bliss'. *Journal of Horticultural Science and Biotechnology*, **76**, 264-270.

Carter, J. and Hummer, K.E. (1999). Gooseberry mite infestation decreases the cold hardiness of dormant black currant flower buds. *HortScience*, **34**, 218-220.

Cathey, H.M. and Borthwick, H.A. (1957). Photoreversibility of floral initiation in chrysanthemum. *Botanical Gazette*, **119**, 71-76

Chandler, W.H. and Tufts, W.P. (1993). Influence of the rest period on opening of buds of fruit trees in spring and on development of flower buds of peach trees. *American Society for Horticultural Science*, **30**, 180-186.

Clapham, A.R., Tutun, T.G. and Warburg, A.F. (1995). *Excursion Flora of the Brittish Isles*. 3rd Edition. Cambridge University Press, Cambridge, U.K., pp 499.

Corke, A.T.K. and Wilson, D. (1963). The effect of premature defoliation on the cropping of black currant bushes. *Annual Report, Long Ashton Agricultural and Horticultural Research Station 1963*, 71-73.

Couvillon, G.A. and Erez, A. (1985). Influence of prolonged exposure to chilling temperatures on bud break and heat requirement for bloom of several fruit species. *Journal of the American Society for Horticultural Science*, **110**, 47-50.

Crabbé, J. and Barnola, P. (1996). A new conceptual approach to bud dormancy in woody plants. In: *Plant Dormancy: Physiology, Biochemistry and Molecular Biology*. (Ed. Lang, G.A.). CAB International, Oxon., U.K., pp 400.

Crisosto, C.H., Lombard, P.B. and Fuchigami, L.H. (1987). Effect of fall ethephon and hand defoliation on dormant bud ethylene levels, bloom delay and yield components of 'Redhaven' peach. *Acta Horticulturae*, **201**, 203-211.

Cullen, J. (1997). *The identification of flowering plant families*. 4th Edition. Cambridge University Press, Cambridge, U.K., pp 215.

Cumming, S.G. and Burton, P.J. (1996). Phenology-mediated effects of climatic change on some simulated British Columbia forests. *Climatic Change*, **34**, 213-222.

Daie, J., Campbell., W.F. and Seeley, S.D. (1981). Temperature-stress induced production of abscisic acid and dihydrophaseic acid in warm and cool season crops. *Journal of the American Society for Horticultural Science*, **106**, 11-13.

Del Real-Laborde, J.I., Anderson, J.L. and Seeley, S.D. (1990). An apple tree dormancy model for subtropical conditions. *Acta Horticulturae*, **276**, 183-191.

Delap, A.V. (1966). The response of young apple trees of differing nitrogen status to a urea spray in autumn. *Annual Report of the East Malling Research Station 1966*, 139-143.

Delap, A.V. (1967). The effect of supplying nitrate at different seasons on the growth, blossoming and nitrogen content of young apple trees in sand culture. *Journal of Horticultural Science*, **42**, 149-167.

Dennis, F.J. Jnr. (1987). Producing temperature-zone fruits at low latitudes – an overview. *Hortscience*, **22**, 1226-1227.

Eagles, C.F. and Wareing, P.F. (1964). The role of growth substances in the regulation of bud dormancy. *Physiologia Plantarum*, **17**, 697-709.

Ebert, A., Bender, R.J., Petri, J.L. and Braga, H.J. (1986). First experiences with chill-unit models in Southern Brazil. *Acta Horticulturae*, **184**, 79-86.

Edwards, G.R. (1987). Producing temperate-zone fruit at low altitudes: avoiding rest and the chilling requirement. *Hortscience*, **22**, 1236-1248.

Engin, H. and Iqbal, N. (2004). Examination of flower bud initiation and differentiation in 'Redhaven' peach by using scanning electron microscope. *Pakistan Journal of Biological Science,* **7**, 1824-1826.

Erez, A. (2000). Bud dormancy: a suggestion for the control mechanism and its evolution. In: *Dormancy in plants: from whole plant behaviour to cellular control*. (Viement, J.D. and Crabbe, J. Eds). Cabi Publising, Oxon, U.K, pp 400.

Erez, A. and Couvillon, G.A. (1987). Characterization of the influence of moderate temperatures on rest completion in peach. *Journal of the American Society for Horticultural Science*, **112**, 677-680.

Erez, A., Couvillon, G.A. and Hendershott, C.H. (1979). Quantitative chilling enhancement and negation in peach buds by high temperatures in a daily cycle. *Journal of the American Society for Horticultural Science*, **104**, 536-540.

Erez, A. and Yablowitz, Z. (1997). Effect of dormancy breaking agents with Armobreak in the peach. *Acta Horticulturae*, **441**, 183-190.

Erez, A., Yablowitz, Z. and Korcinski, R. (1998). Greenhouse peach growing. *Acta Horticulturae*, 465, 593-600.

Faust, M. and Wang, S.Y. (1993). Biochemical events associated with resumption of growth in temperate zone fruit trees. *Acta Horticulturae*, **329**, 257-263.

Ferguson, I.B., Lurie, S. and Bowen, J.H. (1994). Protein synthesis and breakdown during heat shock of cultured pear (*Pyrus communis* L.) cells. *Plant Physiology*, **104**, 1429-1437.

Finetto, G.A. (1993). The effect of hydrogen cyanamide on breaking endo-dormancy of mid-chilling apple cultivars in Yemen A.R. during two years. *Acta Horticulturae*, **329**, 268-270.

Frisby, J.W. and Seeley, S.D. (1993). Chilling of endodormant peach propagules: III. Budbreak and subsequent growth of physiologically dwarfed to near normal seedlings. *Journal of the American Society for Horticultural Science*, *118*, 258-262.

Fuchigami, L.H. and Nee, C. (1987). Degree growth stage model and rest-breaking mechanisms in temperate woody perennials. *HortScience*, **22**, 863-845

George, A.P., Broadley, R.H., Nissen, R.J. and Ward, G. (2002). Effects of new rest-breaking chemicals on flowering, shoot production and yield of subtropical tree crops. *Acta Horticulturae*, **575**, 835-840.

George, A.P. and Nissen, R.J. (1992). Effects of water stress, nitrogen and paclobutrazol on flowering, yield and fruit quality of the low-chill peach cultivar, 'Flordaprince'. *Scientia Horticulturae*, **49**, 197-209.

George, A. P. and Nissen, R.J. (2002). Effects of drought on fruit set, yield and quality of custard apple (*Annona* spp. hybrid) 'African Pride' plants. *Journal of Horticultural Science and Biotechnology*, **77**, 418-427.

Ghariani, K. and Stebbins, R.L. (1994). Chilling requirements of apple and pear cultivars. *Fruit Varieties Journal*, **48**, 215-222.

Gianfagna, T.J. and Mehlenbacher, S.A. (1985). Importance of heat requirement for bud break and time of flowering in apple. *HortScience*, **20**, 909-911.

Gough, R.E. and Abbott, J.D. (1984). Fertilize carefully for better blueberries. *American Fruit Grower*, **104**, 65.

Green, A. (1971). Soft Fruits. In: *The biochemistry of fruits and their products.* Volume II. (Hulme, A.C. Ed.). Academic Press, London, U.K, pp 620.

Griffiths, M. (1997). *The New RHS Dictionary – Index of Garden Plants*. 2nd Edition. The MacMillan Press Ltd, Basingstoke, U.K., pp 1234

Gross, Y. and Kigel, J. (1994). Differential sensitivity to high temperature of stages in the reproductive development of common bean (*Phaseolus vulgaris* L.). *Field Crops Research*, **36**, 201-212.

Gu, S.J. and Read, P.E. (2004). The influence of plant growth regulators included in the forcing solution on bud break of dormant grapevines (*Vitis* spp.). *Acta Horticulturae*, 640, 225-234.

Guerriero, P., Scalabrelli, G and Grazzini, G. (1990). Chilling effect on inhibition removal in kiwifruit dormant lateral buds. *Acta Horticulturae*, **282**, 79-86.

Guttridge, C.G., Anderson, M.M., Thompson, P.A. and Wood, C.A. (1961). Post-harvest defoliation of strawberry plantations. *Journal of Horticultural Science*, **36**, 93-101.

Guttridge, C.G. and Mason, D.T. (1966). Effects of post-harvest defoliation of strawberry plants on truss initiation, crown branching and yield. *Horticultural Research*, **6**, 22-32.

Heide, O.M. (1974a). Growth and dormancy in Norway spruce ecotypes (*Picea abies*) I. Interaction of photoperiod and temperature. *Physiologia Plantarum*, **30**, 1-12.

Heide, O.M. (1974b). Growth and dormancy in Norway spruce ecotypes II. After-effects of photoperiod and temperature on growth and development in subsequent years. *Physiologia Plantarum*, **31**, 131-139.

Heide, O.M. (1993a). Dormancy release in beech buds (*Fagus sylvatica*) requires both chilling and long days. *Physiologia Plantarum*, **89**, 187-191.

Heide, O.M. (1993b). Daylength and thermal time responses of bud burst during dormancy release in some northern deciduous trees. *Physiologia Plantarum*, **88**, 531-540.

Heins, R.D., Liu, B. and Runkle, E.S. (2000). Regulation of crop growth and development based on environmental factors. *Acta Horticulturae*, **514**, 13-24.

Hermano, F.G., Ben-ek, J.F., Vargas, T.T. and Edwards,G.R. (1987). Apples and peaches in tropical highlands of the Philippines. *Acta Horticulturae*, **199**, 63.

Higuchi, H., Utsunomiya, N. and Sakuratani, T. (1998). High temperature effects on cherimoya fruit set, growth and development under greenhouse conditions. *Scientia Horticulturae*, **77**, 23-31.

Holloway, P.S., Stushnoff, C. and Wildung, D.K. (1983). Chilling and budbreak in Lingonberries, *Vaccinium vitis-idaea* L. *Journal of the American Society for Horticultural Science*, **108**, 88-90.

Hopkins, W.G. (1999). *Introduction to plant physiology*. 2nd Edition. John Wiley and Sons Inc., New York., U.S.A., pp 576.

Horridge, J.S. and Cockshull, K.E. (1974). Flower initiation and development in the glasshouse rose. *Scientia Horticulturae*, **2**, 273-284

Horvath, D.P., Anderson, J.V., Chao, W.S. and Foley, M.E. (2003). Knowing when to grow: signals regulating bud dormancy. *Trends in Plant Science*, **8**, 534-540.

Hough, M. (2002). Winter chill and blackcurrants. Meteorological Office Report.

Hoyle, D. A. (1960). Some effects of temperature and day-length on the breaking of winter dormancy in black currant. *Journal of Horticultural Science*, **35**, 229-238.

Hulme, M. and Jenkins, G.J. (1998). Climate change scenarios for the U.K.: Scientific report. UKCIP Technical Report No. 1, Climatic Research Unit, Norwich, U.K., pp 80.

Hulme, M., Jenkins, G.J., Lu, X., Turnpenny, J.R., Mithcell, T.D., Jones, R.G., Lowe, J., Murphy, J.M., Hassell, D., Boorman, P., McDonald, R. and Hill, S. (2002). Climate change scenarios for the United Kingdom : The UKCIP02 Scientific Report, Tyndall Centre for Climate Change Research, School of Environmental Sciences, University of East Anglia, Norwich, U.K. pp 120.

Hummer, K.E. and Barney, D.L. (2002). Crop reports : Currants. *HortTechnology*, **12**, 377-387.

Inoue, H. And Kataoka, I. (1992). Effects of nutrition and temperature on flower bud differentiation of satsuma mandarin. *Journal of the Japanese Scociety for Horticultural Science*, **60**, 771-776.

Itai, C. And Benzioni, A. (1974). Regulation of plant response to high temperature. In : Mechanisms of regulation of plant growht (Eds. Bieleski, R.L., Ferguson, A.R. and Cresswell, M.M.), The Royal Society of New Zea land, Wellington, New Zealand, 477-482.

Ito, E. And Kikuzawa, K. (2003). Reduction of geitonogamy : flower abscission for departure of pollinators. *Ecological Research*, **18**, 177-183.

Iwasaki, K. and Weaver, R.J. (1977). Effects of chilling, calcium cyanamide and bud scale removal on bud break, rooting and inhibitor content of buds of 'Zinfandel' grape (*Vitis vinifera* L.). *Journal of the American Society for Horticultural Science*, **102**, 584-587.

Jacobs, J.N., Jacobs, G. And Cook, N.C. (2002). Chilling period influences the progression of bud dormancy more than does chilling temperature in apple and pear shoots. *Journal of Horticultural Science and Biotechnology*, 77, 333-339.

Jean, D. And Lapointe, L. (2001). Limited carbohydrate availability as a potential cause for fruit abortion in *Rubus chamaemorus*. *Physiologia Plantarum*, 112, 379-387.

Johnson, A.M. (1931). *Taxonomy of the flowering plants*. The Century Co., London, U.K., pp. 684.

Jonkers, N., Knepper, T.P. and del Voogt, P. (2001). Aerobic biodegradation studies of nonylphenol ethoxylates in river water using liquid chromatography electrospray tandem mass spectrometry. *Environmental Science and Biotechnology*, **35**, 335-340.

Keep, E. (1995). Currants. In : *Evolution of crop plants*. 2nd Edition. (Smartt, J. and Simmonds, N.W. Eds.). Longman Scientific and Technical, Essex, U.K., pp 531.

Kim, S.Y., Hong, C.B. and Lee, I. (2001). Heat shock stress causes stage-specific male sterility in *Arabidopsis thaliana*. *Journal of Plant Research*, **114**, 301-307.

Kobayashi, K.D. and Fuchigami, L.H. (1983). Modelling temperature effects in breaking rest in red-osier dogwood (*Cornus sericea* L.). *Annals of Botany*, **52**, 205-215.

Kobayashi, K.D., Fuchigami, L.H. and English, M.H. (1982). Modeling temperature requirements for rest development in *Cornus sericea*. *Journal of the American Society for Horticultural Science*, **107**, 914-918.

Krabel, D., Eschrich, W., Wirth, S. and Wolf, G. (1993). Callase-(1,3-beta-D-glucanase) activity during spring reactivation in deciduous trees. *Plant Science*, **93**, 19-23.

Kronenberg, H.G., Wassenaar, L.M. and van de Lindeloof, C.P.J. (1976). Effect of temperature on dormancy in strawberry. *Scientia Horticulturae*, **4**, 361-366.

Kuden, A.B. and Son. L. (1997). Dormancy breaking experiments on apricots. *Acta Horticulturae*, **441**, 153-157.

Landsberg, J.J. (1974). Apple fruit bud development and growth ; analysis and an empirical model. *Annals of Botany*, **158**, 1013-1023.

Lang, G.E. (1989). Dormancy – Models and manipulations of environmental/physiological regulation. In *Manipulation of Fruiting* (Wright, G.J. Ed.). Butterworths, London, U.K., pp 400.

Lang, G.E., Early, J.D., Arroyave, N. J., Darnell, R.L., Martin, G.C. and Stutte, G.W. (1985). Dormancy: Toward a reduced, universal terminology. *HortScience*, **20**, 809-811.

Lang, G.E., Early, J.D., Martin, G.C. and Darnell, R.L. (1987). Endo-, para-, and ecodormancy: Physiological terminology and classification for dormancy research. *HortScience*, **22**, 371 – 377.

Lantin, B. (1973). Cold-temperature requirements of buds of blackcurrants (*Ribes nigrum* L.) and a few redcurrants (*Ribes* sp.). *Annals Amelio. Plantes*, **23**, 27-44.

Lantin, B. (1977)**.** Estimate of cold requirements for releasing dormancy in blackcurrant (*Ribes nigrum* L.) and redcurrant (*Ribes* sp.) buds.

Lavee, S. (1985). *Vitis vinifera*. In: *CRC handbook of flowering* (ed. Halevy, A.H.). CRC Press, Boca Raton, Florida, 456-471.

Lawes, G.S. (1984). Winter temperatures and kiwifruit bud development. *Orchardist of New Zealand*, 57, 110.

Leiten, F. (1992). Effect of winter temperature on continuous culture of Elsanta. *Fruitteelt*, **5**, 28-29.

Leubner-Metzger, G. (2003). Functions and regulation of beta-1,3-glucanases during seed germination, dormancy release and after-ripening. *Seed Science Research*, **13**, 17-34.

Levitt, J. (1980). *Responses of plants to environmental stresses. Volume 1. Chilling, freezing and high temperature stresses*. Academic Press, New York, pp497.

Linsley-Noakes, G.C. and Allan, P. (1987). Effects of winter temperature on flower development in two clones of kiwifruit (*Actinidia deliciosa* (A. Chev.) *Scientia Horticulturae*, **33**, 249-260.

Linsley-Noakes, G.C. and Allan, P. (1994). Comparison of two models for the prediction of rest completion in peaches. *Scientia Horticulturae*, **59**, 107-113.

Lloyd, J. and Firth, D. (1990). Effect of defoliation time on depth of dormancy and bloom time for low-chill peaches. *HortScience*, **25**, 1575-1578.

Lopez-Medina, J. and Moore, J.N. (1999). Chilling enhances cane elongation and flowering in primocane-fruiting blackberries. *HortScience*, **34**, 638-640.

Lyrene, P.M. (1994). Environmental effects on blueberry flower size and shape are minor. *Journal of the American Society for Horticultural Science*, **119**, 1043-1045.

McPherson, H.G., Stanley, C.J. and Warrington, I.J. (1995). The response of bud break and flowering to cool winter temperatures in kiwifruit (*Actinidia deliciosa*). *Journal of Horticultural Science*, **70**, 737-747.

Mahmood, K. (1999). The effect of environmental factors on vegetative and reproductive development in sweet cherry (*Prunus avium* L.). PhD Thesis, University of Reading.

Mahmood, K., Carew, J.G., Hadley, P. and Battey, N.H. (2000a). Chill unit models for the sweet cherry cvs Stella, Sunburst and Summit. *Journal of Horticultural Science and Biotechnology*, **75**, 602-606.

Mahmood, K., Carew, J.G., Hadley, P. and Battey, N.H. (2000b). The effect of chilling and post-chilling temperatures on growth and flowering of sweet cherry (*Prunus avium* L.). *Journal of Horticultural Science and Biotechnology*, **75**, 598-601.

Masia, A., Coluazzi, M and Ramini, A. (1993). Hormonal changes during the rest period in frit buds of *Prunus persica* L Batsch under three different climatic conditions. *Acta Horticulturae,* **329**, 281-283.

Mason, D.T. (1966). Inflorescence initiation in the strawberry. I. Initiation in the field and its modification by post-harvest defoliation. *Horticultural Research*, **6**, 33-44.

Mason, D. (1967). Inflorescence initiation in the strawberry II. Some effects of date and severity of post-harvest defoliation. *Horticultural Research*, **7**, 97-104.

Mathers, B. (1952). A study of fruit-bud development in *Rubus ideas. Journal of Horticultural Science,* **27**, 266-272.

Mielke, E.A. and Dennis, F.G. Jnr. (1975). Hormonal control of flower bud dormancy in sour cherry (*Prunus cerasus* L.). II. Levels of abscisic acid and its water soluble complex. *Journal of the American Society for Horticultural Science,* **100**, 287-290.

Mielke, E.A. and Dennis, F.G. Jnr. (1978). Hormonal control of flower bud dormancy in sour cherry (*Prunus cerasus* L.). III. Effects of leaves, defoliation and temperature on levels of abscisic acid in flower primordia. *Journal of the American Society for Horticultural Science,* **103**, 446-449.

Montgomery-Brown, J. and Reinhard, M. (2003). Occurance and behaviour of alkylphenol polyethoxylates in the enviroment. *Environmental Engineering Science*, **20**, 471-486.

Murray, M.B., Cannell, M.G.R. and Smith, R.I. (1989). Date of budburst of fifteen tree species in Britain following climatic warming. *Journal of Applied Ecology,* **26**, 693-700.

Nadel, B.L., Altman, A., Pleban, S. and Huttermann, A. (1991). In vitro development of mature *Fagus sylvatica* L. buds. I. The effect of medium and plant growth regulators on bud growth and protein profiles. *Journal of Plant Physiology*, **138**, 596-601.

Neumann, D., Nover, L., Parthier, B., Rieger, R., Scharf, K., Wollgiehn, R.W. and Nieden, U.Z. (1989). *Heat shock and other stress response systems of plants*. Ed. Nover, L., Neumann, D and Scharf, K-D. Springer-Verlag, Berlin, pp155.

Nishimoto, N. and Fujisaki, M. (1995). Chilling requirement of buds of some deciduous fruits grown in southern Japan and the means to break dormancy. *Acta Horticulturae*, **395**, 153-160.

Noodèn, L.D. and Weber, J.A. (1978). Environmental and hormonal control of dormancy in terminal buds of plants. In: *Dormancy and developmental arrest* (Clutter, M.E. ed.) Academic Press Inc., London. pp221-268.

Norby, R.J., Hartz-Rubin, J.S. and Verbrugge, M.J. (2003). Phenological responses in maple to experimental atmospheric warming and CO_2 enrichment. *Global Change Biology*, **9**, 1792-1801.

Ofir, M. and Koller, D. (1974). Relationship between thermoinduction and photoinduction of flowering and dormancy in *Hordeum bulbosum* L., a perennial grass. *Australian Journal of Plant Physiology*, **1**, 259-270.

O'Hare, T.J. and Turnbull, C.G.N. (2004). Root growth, cytokinin and shoot dormancy in lychee (*Litchi chinensis* Sonn.). *Scientia Horticulturae*, **102**, 257-266.

Olmsted, C.E. (1951). Experiments on photoperiodism, dormancy, leaf age and abscission in Sugar Maple. *Botanical Gazette*, **112**, 365-393.

Olsen, J.E., Junttila, O. and Mortiz, T. (1997). Long-day induced bud break in *Salix pentandra* is associated with transiently elevated levels of GA1 and gradual incrase in indole-3-acetic acid. *Plant and Cell Physiology*, **38**, 536-540.

Olszyk, D., Wise, C., VanEss, E. and Tingey, D. (1998). Elevated temperature but not elevated CO_2 affects long-term patterns of stem diameter and height of Douglas-fir seedlings. *Canadian Journal of Botany*, 28, 1046-1054.

Olszyk, D., Wise, C., VanEss, E., Apple, M. and Tingey, D. (1998). Phenology and growth of shoots, needles and buds of Douglas-fir seedlings with elevated CO_2 and (or) temperature. *Canadian Journal of Botany*, 76, 1991-2001.

Osborne, C.P., Chuine, I., Viner, D. and Woodward, F.I. (2000). Olive phenology as a sensitive indicator of future climatic warming in the Mediterranean. *Plant, Cell and Environment*, **23**, 701-710.

Pennell, D. (2003). The Brogdale horticultural trust national plant collections of bush fruit.

Petrovic, M., Diaz, A., Ventura, F. and Barcela, P. (2001). Simulataneous determination of halogenated derivatives of alkylphenol ethoxylates and their metabolites in sludges, river sediments and surface, drinking and waste waters by liquid chromatography and mass spectrometry. *Annals of Chemistry*, **73**, 5886-5895.

Pietila, M., Kauppi, A., Aflatuni, A. and Karp, K. (2002). The significance of autumn and overwinter cover for strawberry. *Acta Horticulturae*, **567**, 527-530.

Pilate, G., Sotta, B., Maldiney, R., Jacques, M., Sossoountzov, L. and Maginiac, E. (1989). Abscisic acid, indole-3-acetic acid and cytokinin changes in buds of *Pseudotsuga menziesii* during bud quiescence release. *Physiologia Plantarum*, **76**, 100-106.

Piola, F., Label, P., Vergne, P., Aderkas, P. and von Rohr, R. (1998). Effects of endogenous ABA levels and temperature on cedar (*Cedrus libani* Loudon) bud dormancy *in vitro*. *Plant Cell Reports*, **18**, 279-283.

Plancher, B. (1983a). Chilling requirement and bud break temperature of *Ribes nigrum* L. *Gartenbauwissenschaft*, **48**, 210-216.

Plancher, B. (1983b). Chilling requirements of rooted layers, detached shoots and single-noded cuttings of *Ribes nigrum* L. *Gartenbauwissenschaft*, **48**, 248-255.

Plancher, B. (1984). The most effective temperature for the satisfaction of chilling requirement of *Ribes nigrum* L. *Gartenbauwissenschaft*, **49**, 49-52.

Plooy, P., Jacobs, G. and Cook, N.C. (2002). Quantification of bearing habit on the basis of lateral bud growth of seven pear cultivars grown under conditions of inadequate winter chilling in South Africa. *Scientia Horticulturae*, **95**, 185-192.

Powell, L.E. (1987). Hormonal aspects of bud and seed dormancy in temperate-zone woody plants. *HortScience*, **22**, 845-850.

Ramina, A., Colauzzi, M., Masia, A., Pitacco, A., Caruso, T., Messina, R. and Scalabrelli, G. (1995). Hormonal and climatological aspects of dormancy in peach buds. *Acta Horticulturae*, **339**, 35-46.

Rallo, L. and Martin, G.C. (1991). The role of chilling in releasing olive floral buds from dormancy. *Journal of the American Society for Horticultural Science*, **116**, 1058-1062.

Rendle, A.B. (1925). *The Classification of flowering plants*. Volume II. Dicotyledons. Cambridge University Press, London, U.K., pp 625.

Richardson, E.A., Anderson, J.L. and Campbell, R.H. (1986). The omnidata biophenometer (TA45-P): A chill unit and growing degree hour accumulator. *Acta Horticulturae*, **184**, 95-100.

Richardson, E.A., Seeley, S.D. and Walker, D.R. (1974). A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. *Hortscience*, **9**, 331-332.

Richardson, E.A., Seeley, S.D., Walker, D.R., Anderson, J.L. and Ashcroft, G.L. (1975). Pheno-climatography of spring peach bud development. *HortScience*, **10**, 236-237.

Richardson, S.D. (2002). Environmental mass spectrometry: Emerging contaminants and current issues. *Annals of Chemistry*, **74**, 2719-2742.

Rinne, P.L.H. and Schoot, C. van der. (2004). Cell-cell communication as a key factor in dormancy cycling. *Journal of Crop Improvement*, **10**, 113-156.

Roberts, A.N., Tomasovic, B.J. and Fuchigami, L.H. (1974). Intensity of bud dormancy in Douglas-fir and its relation to scale removal and rooting ability. *Physiologia Plantarum*, **31**, 211-216.

Robertson, M. (1957). Further investigations of flower-bud development in the genus *Rubus*. *Journal of Horticultural Science*, **32**, 265-273

Roefolfse, E.W. and Hand, D.W. (1989). The effect of daylength on the development of glasshouse celery. *Journal of Horticultural Science*, **64**, 283-292

Rosa, N. (1986). Temperature and sucker development. *Lighter*, 56, 7-11.

Roychoudhury, A., Chatterjee, R. and Mitra, S.K. (1990). Effect of different doses of nitrogen, phosphorus, potassium, magnesium, calcium and iron on growth and development in chilli. *Indian Cocoa, Arecanut and Spices Journal*, **13**, 96-99.

Salisbury, F.B. and Ross, C.W. (1992). *Plant Physiology*. 4th Edition. Wadsworth Publishing Company, California, pp. 681.

Samish, R.M. (1954). Dormancy in woody plants. *Annual Review of Plant Physiology*, **5**, 183-203.

Sato, S., Peet, M.M. and Gardner, R.G. (2004). Altered flower retention and developmental patterns in nine tomato cultivars under elevated temperature. *Scientia Horticulturae*, **101**, 95-101.

Saure, M.C. (1985). Dormancy release in deciduous fruit trees. *Horticultural Review*, **7**, 239-300.

Saxe, H., Cannell, M.G.R., Johnsen, O., Ryan, M.G. and Vourlitis, G. (2001). Tree and forest functioning in response to global warming. *New Phytologist*, **149**, 369-400.

Scalabrelli, G. and Couvillon, G.A. (1986). The effect of temperature and bud type on rest completion and the GDH°C requirement for budbreak in 'Redhaven' peach. *Journal of the American Society for Horticultural Science*, **111**, 537-540.

Scutareanu, P.P. and Roques, A. (1993). The pest insect fauna on male and female reproductive structures of oaks in Romania. *Journal of Applied Entomology*, 115, 321-328.

Shaltout, A.D. and Unrath, C.R. (1983). Rest completion prediction model for 'Starkrimson Delicious' apples. *Journal of the American Society for Horticultural Science*, **108**, 957-961.

Shirazi, A.M. and Fuchigami, L.H. (1993). Effects of "near-lethal" stress on bud dormancy and stem cold hardiness in red-osier dogwood. *Tree Physiology*, **15**, 275-279.

Siller-Cepeda, J.H., Fuchigami, L.H. and Chen, T.H.H. (1992). Hydrogen cyanamide-induced budbreak and phytotoxicity in 'Redhaven' peach buds. *HortScience*, **27**, 874-876.

Singla, S.L., Pareek, A. and Grover, A. (1996). *High Temperature*. In: Plant Ecophysiology (Ed. Prasa, M.N.V). John Wiley and Sons Inc., 101-127.

Snelgar, W.P., Manson, P.J. and McPherson, H.G. (1997). Evaluating winter chilling of kiwifruit using excised canes. *Journal of Horticultural Science*, **72**, 305-315.

Spiegel-Roy, P. and Alston, F.H. (1979). Chilling and post-dormant heat requirement as selection criteria for late-flowering pears. *Journal of Horticultural Science*, **54**, 115-120.

Sugiura, T. and Yokozawa, Y. (2004). Impact of global warming on environments for apple and Satsuma mandarin production estimated from changes of the annual mean temperature. *Journal of the Japanese Society for Horticultural Science*, **73**, 72-78.

Sussex, I.M. (1978). Dormancy and development. In: *Dormancy and developmental arrest* (Clutter, M.E. ed.) Academic Press Inc., London. pp297-301.

Swartz, H.J. and Powell, L.E. Jnr. (1981). The effect of long chilling requirement on time of bud break in apples. Acta Horticulturae, 120, 173-178.

Swartz, H.J., Geyer, A.S., Powell, L.E. and Lin, S.C. (1984) The role of bud scales in the dormancy of apples. *Journal of the American Society for Horticultural Science*, **109**, 745-749.

Sykes, M.T. and Prentice, I.C. (1995). Boreal forest futures: modeling the controls on tree species range limits and transient responses to climate change. *Water, Air and Soil Pollution*, **82**, 415-428.

Sykes, M.T., Prentice, I.C. and Cramer, W. (1996). A bioclimatic model for the potential distributions of north European tree species under present and future climates. *Journal of Biogeography*, **23**, 203-233.

Taiz, L. and Zeiger, E. (2002). *Plant Physiology*. 3rd Edition. Sinauer Associate, Inc., Massachusetts, pp 690.

Taulavuori, K., Laine, K. and Taulavuori, E. (2002). Artificial deacclimation response of *Vaccinium myrtillus* in mid-winter. *Annals of Botany Fennici*, **39**, 143-147.

Taylor, D.R., Atkey, P.T., Wickenden, M.F. and Crisp, C.M. (1997). A morphological study of flower initiation in strawberry (*Fragaria x ananassa*) using cryo-scanning electron microscopy. *Annals of Applied Biology*, **130**, 141-152.

Taylor, J.S., Robertson, J.M., Harker, K.M., Bhalla, M.K., Daly, E.J. and Pearce, D.W. (1995). Apical dominance in rhizomes of quackgrass, *Elytrigia repens*: the effect of auxin, cytokinins and abscisic acid. *Canadian Journal of Botany*, **73**, 307-314.

Tisne-Agostini, D., Riolacci, S. and Habib, R. (1992). Modelling proportionate bud break in kiwifruit. *Acta Horticulturae*, **313**, 307-313.

Tumara, F., Tanabe, K. and Banno, K. (1992). The effect of chilling treatment on intensity of bud dormancy, respiration and endogenous growth regulators in Japanese Pear 'Nijisseiki'. *Journal of the Japanese Society for Horticultural Science*, 60, 763-769.

Tehranifar, A. and Battey, N.H. (1997). Comparison of the effects of GA3 and chilling on vegetative vigour and fruit set in strawberry. *Acta Horticulturae*, *439*, 627-631.

Tehranifar, A., Le Miere, P. and Battey, N.H. (1998). The effects of lifting date, chilling duration and forcing temperature on vegetative growth and fruit production in the Junebearing strawberry cultivar Elsanta. *Journal of Horticultural Science and Biotechnology*, **73**, 453-460.

Terblanche, J.H., Stassen, P.J.C., Hesebeck, I. And Strydom, D.K. (1979). Effects of autumn nitrogen nutrition and a winter rest-breaking spray on the growth, development and chemical composition of young 'Golden Delicious' apple trees grown in sand culture. *Scientia Horticulturae*, **10**, 37-48.

Thomas, M., Ranson, S.L. and Richardson, J.A. (1966). *Plant Physiology*. 5th Edition. Longman Scientific Publishings, New York, U.S.A., pp 1062.

Thomas, B. and Vince-Prue, D. (1997). *Photoperiodism in plants.* 2nd Edition. Academic Pres Inc., London. pp248.

Thompson, P.A. and Guttridge, C.G. (1960). The role of leaves as inhibitors of flower induction in strawberry. *Annals of Botany*, **24**, 482-490.

Tinklin, I.G. and Schwabe, W.W. (1970). Lateral bud dormancy in blackcurrant (*Ribes nigrum*) L. *Annals of Botany*, **34**, 691-707.

Vestrheim, S. (1972). Flower initiation in black currants in a southern Norway location. *Agricultural University of Norway Report No. 73*, 50, 1-5

Villiers, T.A. (1975). *Dormancy and the survival of plants*. Edward Arnold (Publishers) Limited, London, U.K, pp 72.

Vince-Prue, D. (1975). *Photoperiodism in plants*. McGraw Hill Book Company Ltd, Maidenhead, UK, pp 424.

Wagstaffe, A. and Battey, N.H. (in press). Charaterisation of the thermodormancy response in the everbearing strawberry 'Everest'.

Walser, R.H., Walker, D.R. and Seeley, S.D. (1981). Effect of temperature, fall defoliation and gibberellic acid on the rest period of peach leaf buds. *Journal of the American Society for Horticultural Science*, **106**, 91-94.

Walther, G., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J., Hoegh-Guldberg, O. and Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, **416**, 389-395.

Wang, S.Y. and Faust, M. (1994). Changes in the antioxidant system associated with budbreak in 'Anna' apple (*Malus domestica* Borkh.) buds. *Journal of the American Society for Horticultural Science*, **119**, 735-741.

Wareing, P.F. (1954a). Growth studies in woody species V. Photoperiodism in dormant buds of *Fagus sylvatica* L. *Physiologia Plantarum*, **6**, 693-706.

Wareing, P.F. (1954b). Growth studies in woody species VI. The locus of photoperiodic perception in relation to dormancy. *Physiologia Plantarum*, **7**, 261-277.

Warrag, M.O.A.R. (1983). Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp.) to heat stress. *Dissertation Abstracts International B.*, **44**, 1662.

Weijun, Z. and Leul, M. (1999). Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. *Plant Growth Regulation*, **27**, 99-104.

Weinberger, J.H. (1956). Prolonged dormancy trouble in peaches in the Southeast in relation to winter temperatures. *Proceedings of the American Society for Horticultural Science*, **67**, 107-112

Werner, E. and Young, D.J. (1985). Chill unit and growing degree hour requirements for vegetative bud break in six apple rootstocks. *Journal of the American Society for Horticultural Science*, **110**, 411-413.

Westmore, G. (2004). Winter chilling in blackcurrant. BSc Thesis, University of Tasmania, Australia.

Westwood, M.N. (1978). *Temperate-zone pomology*. W.H. Freeman and Company, San Fransisco, U.S.A., pp 428.

Whalley, D.N. and Cockshull, K.E. (1976). The photoperiodic control of rooting, growth and dormancy in *Cornus alba* L. *Scientia Horticulturae*, **5**, 127-138.

White, J.M., Wainwright, H. and Ireland, C.R. (1999). Endodormancy and paradormancy in the raspberry cultivar 'Glen Clova'. *Acta Horticulturae*, **505**, 199-205.

Wiebel, J., Dounton, W.J.S. and Chacko, E.K. (1992). Influence of applied growth regulators on bud dormancy and growth of mangosteen (*Garcinia mangostama*). *Scientia Horticulturae*, **52**, 27-35.

Williams, M.S., Starman, T.W. and Faust, J.E. (1999). High temperatures reduce postharvest flowering of specialty floral crop species. *HortTechnology*, **9**, 94-98.

Wisniewski, M., Sauter, J., Fughigami, L. and Stephien, V. (1997). Effects of near-lethal heat stress on bud break, heat-shock proteins and ubiquitin in dormant poplar (*Populus nigra* 'Charkowiensis' x. *P. nigra* 'Incrassata'). *Tree Physiology*, **17**, 453-460).

Wright, C.J. (1985). *Ribes*. In: *CRC Handbook of flowering*. Volume IV. (Ed. Halvey, A.H.). CRC Press, Inc., Florida, U.S.A., pp 780.

Yotsuya, T., Ichii, T., Sawano, M., Nakanishi, T. and Ozaki, T. (1984). Effects of bud scales and gibberellins on dormancy of in vitro cultured Japanese pear leaf buds. *Scientia Horticulturae*, **24**, 177-184.

Young, E. and Werner, D.J. (1985). Chill unit and growing degree hour requirements for vegetative bud break in six apple rootstocks. *Journal of the American Society for Horticultural Science*, **110**, 411-413.