

RESEARCH PAPER

An approach to the determination of winter chill requirements for different *Ribes* cultivars

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Blackcurrant; climate change; dormancy; winter chilling; woody plants.

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ABSTRACT

Winter chilling is critical for flowering and fruiting of many temperate fruits, with evidence that blackcurrant (*Ribes nigrum* L.) cropping has been adversely affected by warm winters. Accurate models of chill accumulation in blackcurrant are required so that breeding strategies can be formulated for the generation of new cultivars with resilience to future climates. Existing models for chill accumulation have largely been derived from statistical correlation; here we report the derivation of improved models for blackcurrant using controlled environment treatments. Hardwood cuttings from a diverse set of cultivars were exposed to constant or varying chilling temperatures and the effects on bud break after transfer to a warm, permissive environment evaluated. The impact of different combinations of temperature and chilling periods were described in terms of their overall 'Effectiveness' (*E*). Clear genotypic differences were found, with excessive chilling often inhibiting bud break. There was a significant interaction between observed chilling response and the period of low temperature exposure. A number of chilling models to explain observed interactions between chilling temperature and time of exposure on bud break were compared; the most effective involved an optimal response to increasing chill accumulation. The effects of varying temperatures during chilling on bud break were complex, with warm temperature breaks substantially inhibiting bud development and cooler temperature breaks tending to enhance bud burst. The relevance of these models to generic studies of endodormancy is discussed, together with their potential application to the development of phenotyping screens for future breeding using diverse blackcurrant germplasm.

INTRODUCTION

Woody perennial plants in temperate regions enter a dormant period during the winter months, as an adaptive strategy to avoid unfavourable conditions. During this period, all visible growth in any plant structure containing a meristem is suspended (Lang 1987), and the breaking of endodormancy in the spring prior to bud break requires sustained exposure to low near-freezing temperatures before active growth can resume (Howe *et al.* 1999). The amount of chilling received by woody plants over the dormant period and its role in the fulfilment of a chilling requirement, which is itself dependent on species, cultivar and even bud position (Faust *et al.* 1995), can have profound effects on subsequent development; these include erratic bud break, reduced quality and lower yields (Atkinson *et al.* 2004), and these phenomena have been identified in a range of perennial fruit species in both Europe and North America.

The European climate has shown significant changes in the past 100 years, with an increase in average temperature of ca. 1 °C, in itself greater than the global average (Solomon *et al.* 2007). Projected changes in temperatures up to 2080 suggest that they could increase in Northern Europe by up to 2.5 °C (Nakicenovic & Swart 2000), with the increases proportion-

ately greater in the winter in northern latitudes (Solomon *et al.* 2007). Overall, climate modelling shows that cold episodes will decrease significantly in the future, daily temperature minima will increase faster than daily maxima, leading to a decrease in the diurnal range, and frost days will decrease in most areas, with a comparable increase in the length of growing season (Solomon *et al.* 2007).

A study of UK historical data by Sunley *et al.* (2006) using a range of models for assessment of winter temperatures has indicated that levels of winter chilling have declined over the past 50 years in all locations studied in the UK, with a simultaneous reduction in the occurrence and severity of spring frost events. Baldocchi & Wong (2008) estimated that accumulated winter chill is decreasing in the fruit-growing areas of California at a rate of ca. 40 h per decade, while within Europe climate-induced advances in phenology for a range of crops, including woody fruit species, averaged 1.1–1.3 days per decade over the period 1951–2004 (Estrella *et al.* 2007).

The potential effects of a changing climate, especially the impact of a reduction in winter temperatures, are of increasing concern to the European soft fruit industry. In the UK, particular concern surrounds the ability of blackcurrant (*Ribes nigrum* L.) to produce consistent crops, with reports from growers of older cultivars such as 'Ben Lomond' in

southern parts of the UK suggesting that reduction in chilling hours experienced in recent winters had harmed yields and fruit quality (Harding 2007). A closer examination of historical climate and cropping data by Atkinson *et al.* (2004) showed that blackcurrant as a fruit crop is potentially at risk in parts of Europe due to the lack of winter chilling forecast in projections of future climatic conditions (Solomon *et al.* 2007). For these reasons, and also because of its relative ease of handling and short juvenile period, we have therefore used blackcurrant as a model crop to derive and test a new methodology for the derivation of chilling models that might be incorporated more widely into ecophysiological models of development in woody species (see *e.g.* Hänninen *et al.* 2007).

Evidence on the chilling requirement of different fruit species or cultivars can be obtained either through statistical analysis of correlations between flowering behaviour and seasonal temperatures in the field, or by using controlled environment studies involving exposure of plants or cuttings to controlled temperatures and using the resulting data to derive a chill model. Unfortunately, it has been shown that field data simply do not contain sufficient information for parameterisation of the different processes involved in the necessary ecophysiological models (*e.g.* Hunter & Lechowicz 1992; Hänninen *et al.* 2007), therefore the present study emphasises the experimental approach. In the specific case of blackcurrant, Hoyle (1960) conducted early studies of chilling, using potted plants of *cv.* Boskoop Giant. She demonstrated that both cold treatments and long days are effective in inducing bud break in blackcurrant, and that increasing amounts of chilling up to 15 weeks at 3.3 °C progressively decreased time to bud break in short days, while bud break was delayed by inadequate chilling. Similarly, Heide (1974, 1993) found in controlled environment studies with beech (*Fagus sylvatica* L.) that dormancy was controlled by both temperature and photoperiod, although other woody species such as hornbeam (*Carpinus* spp.) may be unaffected by daylength.

The timing of bud burst can be predicted using defined temperature data and feeding these into so-called chilling models that describe both the response of the physiological processes that lead to satisfaction of the dormancy requirement and the bud break process itself. Considerable uncertainty persists regarding which chilling model can best be applied to berry fruit crops. As reviewed in Sunley *et al.* (2006), the most common models that have been used to date include:

- 1 Simple temperature accumulation models, of which the most usual is the number of chilling hours below a threshold of 7.2 °C (although the number of hours between 0 and 7.2 °C has been found to be more suitable for some species).
- 2 Because the effectiveness of chilling varies with temperature, various more sophisticated temperature weighting models have been proposed, of which the most widely used has been the 'Utah model' (Richardson *et al.* 1974), based on peach bud development, where optimum chilling is achieved at 6 °C and other temperatures have a weighting relative to this. A related approach has been to assume that effectiveness increases exponentially with decreasing temperatures (Bidabé 1967; Lantin 1973, 1977).
- 3 Further sophistication can be introduced using sequential dormancy models that include both a chill accumulation phase

and a warming (or anti-chill) accumulation for the bud break or ecodormancy phase (see Cesaraccio *et al.* 2004, 2006).

Sunley *et al.* (2006) demonstrated that, unfortunately, none of these models adequately describe existing data on dormancy break and flowering in soft fruit species. The most relevant for cultivars of *Rubus* and *Ribes* were found to be the <7.2 °C and the Lantin exponential models. The problems arise partly because the existing temperature accumulation models are unable to account for the effects of intermittent high (non-chilling) temperatures during the dormant period that are common in maritime climates such as the UK. There was also evidence for differential responses of different cultivars. More recently, Rose & Cameron (2009) used regression analysis to determine optimum chill temperatures for three UK-bred blackcurrant cultivars on the basis of treatment at continuous controlled temperatures, with optima ranging from 1 h at 2.2 °C for *cv.* Ben Hope to 1 h at -3.4 °C for *cv.* Ben Tirran.

In view of the uncertainty that exists, this paper therefore attempts to derive more accurate chill models for *Ribes* on the basis of controlled temperature treatments, as a guide for the development of a high-throughput phenotyping screen for diverse *Ribes* germplasm in the context of a breeding programme. We also aim to determine whether (and if so, how much) sequences of temperature treatments have different effects on the chilling response than continuous exposure to the equivalent average temperature. In addition, we investigate and describe the variability in responses among a range of diverse cultivars, which will provide the basis for future breeding efforts in blackcurrant.

MATERIAL AND METHODS

Plant material

Dormant shoots of blackcurrant (*Ribes nigrum* L.) with *ca.* 12 buds were harvested in October 2007 and 2008 from field plots at SCRI, Invergowrie, Dundee, UK (grid ref. NO 337 304 GB Grid), from 4 to 5-year-old blackcurrant plants. The 20 cultivars used in year 1 and the 13 in year 2 were chosen to represent genotypes from as wide a range as possible of blackcurrant climatic origins; they are listed in the supporting information (Table S1). After all remaining leaves were removed, the shoots were transferred to pots of moist sand, covered with a sealed transparent plastic bag, and then placed in controlled environment chambers (Snijders Scientific, Tilburg, Netherlands) for defined period and temperature regimes, as outlined below. While in the chilling cabinets at their assigned temperatures, a timer controlled the artificial lighting within the cabinets to simulate winter daylight hours on a 8 h/16 h day/night cycle, thus allowing us to simulate natural conditions as closely as possible. After completion of the chilling treatment, the bases of the shoots were placed in water and then transferred to a lit greenhouse at *ca.* 20 °C. Bud burst was recorded at intervals using the following scale:

- 0 = no bud burst
- 1 = initial signs of bud swelling
- 2 = visible leaves on the bud
- 3 = grape stage
- 4 = flowers open

Four replicate shoots were used for each treatment combination, and bud status was recorded for each of the topmost

12 buds on each shoot at each recording date. These data were transferred to an Excel spreadsheet and all subsequent calculations were performed in this spreadsheet and corrected for any missing values, so that results are expressed as a percentage of live buds.

Year 1

In the autumn of 2007, cuttings of 20 cultivars were harvested on 18 October and transferred to the cabinets by 19 October. Cuttings were exposed to one of the temperatures, -5°C , 0°C , 5°C or 10°C , for one of the periods 5, 9, 13, 17 or 21 weeks (Table 1), before being moved to the glasshouse. Recordings of bud burst were after 10, 17, 24, 31, 38 and 45 days.

Year 2

In the autumn of 2008, cuttings of 13 cultivars were harvested in the week beginning 2 October and were transferred to the cabinets by 6 October (see Table S1 for details). Additional temperature treatments involving exposure to various temperature sequences (see Table 1) were incorporated to determine whether the order of exposure to sequences of temperatures affected the response in comparison with continuous exposure to the equivalent average temperature. Cuttings were exposed to either a 6-week or a 12-week chilling period, with each period split into three equal parts at one of the treatment temperatures (-4°C , 0°C , 4°C or 8°C). The temperatures used in the second year were slightly modified on the basis of the results obtained in year 1 to represent better the range of temperatures for blackcurrant chilling. Bud burst recordings were taken after 1 week in the glasshouse at 20°C and thereafter at fortnightly intervals. The control treatments were maintained in the glasshouse throughout.

Data analysis and derivation of 'effectiveness'

Statistical analysis was in IBM SPSS (version 20, IBM, Armonk, New York) and curve fitting used the default spline function in Minitab 15 (Minitab Inc.).

Model 1

The approach used was to define an 'effectiveness' (E) that describes the success of different combinations of temperature and chilling periods at satisfying the chilling requirement. An E of 100 for any bud stage implies 100% of buds reaching that stage (e.g. 100% of flowers open for bud-stage 4). As a first approach (Model 1, which corresponds approximately to a conventional chilling unit model), we hypothesised that satisfaction of the chilling requirement is non-linearly related to the mean temperature of the chilling treatment (cf. Richardson *et al.* 1974) and estimated the chilling effects of the different constant chilling temperatures used in year 1 by calculating an overall treatment effectiveness (E) for each (constant) temperature, T , so that Model 1 is given as

$$E = K_T * N(\text{for } 0 \leq E \leq 100; \text{ otherwise } E = 100) \quad (1)$$

where K_T is a 'temperature factor' (constrained to be positive) representing the effectiveness of any temperature, T , and N is the number of days' chilling. The overall effectiveness increases linearly with increasing chilling time at a rate that depends on K_T , until effectiveness is complete ($E = 100$) and then remains at this value with any additional chilling. For convenience in comparing responses of different cultivars or environmental regimes, we henceforth express the value of K_T as a relative value, K_{Tr} , by expressing K_T relative to the value at 0°C (K_0) according to $K_{Tr} = K_T/K_0$, so that $K_{0r} = 1.0$, and equation (1) becomes $E = K_0 K_{Tr} N$.

The K_T parameters were estimated for any bud stage, by minimising across all the constant treatment combinations, the sum of squares of the deviations between the calculated E and the observed percentage of living buds reaching that stage. This was achieved using the generalised reduced gradient method available in the Solver function in Microsoft Excel:Mac 2011. The Solver add-in (see <http://www.solver.com> for further details) provides a powerful tool for fitting equations using the least squares method. Multiple initial values were tested to ensure that a global minimum had been achieved.

Table 1. The temperature sequences ($^{\circ}\text{C}$) that were used in each year, both for the chilling period and the subsequent warming period; C represents the control treatment (maintained at ca. 20°C).

Year	Sequence	Chilling period (d)	Mean temperature	Warming period (d)
Year 1	-5°C	35, 63, 91, 119, 147	-5°C	10, 17, 24, 31, 38, 45
	0°C	35, 63, 91, 119, 147	0°C	10, 17, 24, 31, 38, 45
	5°C	35, 63, 91, 119, 147	5°C	10, 17, 24, 31, 38, 45
	10°C	35, 63, 91, 119, 147	10°C	10, 17, 24, 31, 38, 45
Year 2	$-4, -4, -4$	42, 84	-4°C	10, 24, 38, 52
	0, 0, 0	42, 84	0°C	10, 24, 38, 52
	4, 0, -4	42, 84	0°C	10, 24, 38, 52
	$-4, 0, 4$	42, 84	0°C	10, 24, 38, 52
	4, 4, 4	42, 84	4°C	10, 24, 38, 52
	8, 4, 0	42, 84	4°C	10, 24, 38, 52
	0, 4, 8	42, 84	4°C	10, 24, 38, 52
	$-4, 8, 8$	42, 84	4°C	10, 24, 38, 52
	8, 8, -4	42, 84	4°C	10, 24, 38, 52
	8, 8, 8	42, 84	8°C	10, 24, 38, 52
	C, C, C	42, 84	ca. 20°C	10, 24, 38, 52

Model 2

Because our observations suggested that excess chilling could sometimes lead to inhibition of bud burst and flowering, a response that cannot be simulated with equation (1), we tested various empirical modifications of this model to simulate this type of inhibitory response. The inhibition by supra-optimal chilling was most suitably modelled by assuming a simple polynomial (quadratic) response:

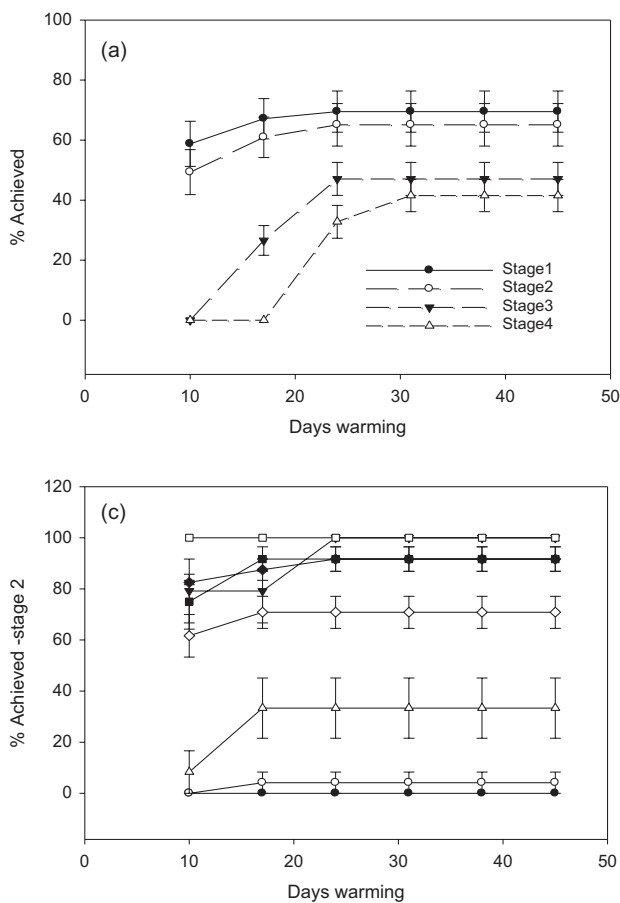
$$E = (K_T * N) + b(K_T * N)^2 = (K_0 K_{Tr} * N) + b(K_0 K_{Tr} * N)^2 \quad (2)$$

where b is a constant. Again parameters were estimated using the Solver function in Excel.

RESULTS

Effect of time at permissive temperature

Following any chilling treatment, the stage of bud development reached a stable value after 28–42 days at permissive temperature (20 °C), remaining constant thereafter (Fig. 1).



Similar trends were obtained after other treatment periods. All cultivars studied showed rather similar trends, in both year 1 and in year 2 (data not shown), although with differing final levels for any chilling treatment (see Fig. 1c and d for representative cultivars and bud stages). To ensure maximal development we only report bud development after 45 days' warming (52 days for year 2) in all subsequent analyses. As expected, the percentage reaching later bud stages (grape or flowers open) was smaller than for the earlier stages of bud burst, since the scoring of the earlier stages included all those buds reaching a later stage. Similar patterns of response were found in year 2. It should be noted that the very limited bud development in some cultivars (especially 'Amos Black' and 'Andega') reflected supra-optimal chilling with the severe chilling treatment used for this particular dataset, as shown below. Note that four-way ANOVAs for any bud stage indicated that all factors, and their interactions, were highly significant ($P < 0.001$).

Effect of chilling temperature and time on bud development

The effect of different periods and chill temperatures on the average attainment of each of the four standard bud stages is

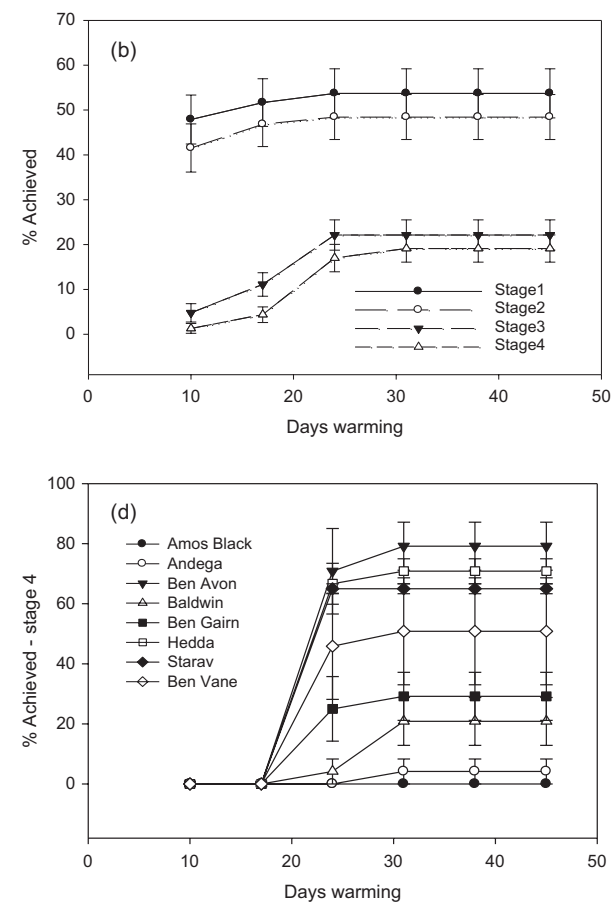


Fig. 1. Response to varying time, in a permissive warming environment, of the percentage of buds reaching each developmental stage in year 1. Averaged data in (a) and (b) are for shoots removed from cabinets after 147 days' chilling at -5 °C (a), and at 5 °C (b), where bars represent \pm standard error of mean ($n = 20$ genotypes). The variation between some contrasting cultivars after treatment at -5 °C for 147 days followed by different warming periods is also shown for achievement of bud-stage 2 (c) and bud-stage 4 (d), where error bars are \pm standard error of mean percentage achievement of any bud stage ($n = 4$ shoots).

presented in Fig. 2 (average of all cultivars in year 1), when measured after the standard 45 days in a permissive environment. When averaged across all the cultivars studied, bud development generally increased both with time of chilling and with lower temperatures, with the pattern for the different bud stages being similar in form, although with differing magnitudes. There was, however, some evidence, particularly for the initial bud-swelling stage, that the response decreased slightly after very long periods at the lowest temperature, suggesting that excessive exposures to chilling temperatures can lead to a reduction in the effect.

The averaged data, however, disguise the large differences between genotypes, with some cultivars showing very marked loss of ability to reach any given bud stage after longer periods at the lower temperatures (Fig 3, Figs S1 and S2). Cultivars such as 'Amos Black', 'Baldwin' and 'Andega' show marked inhibition with time, with almost all bud development being inhibited by 147 days chilling (Fig. 3). In contrast, others such as 'Ben Gairn', 'Hedda' and 'Ben Klibreck', showed increasing bud development with increasing chill.

As well as differences in the form of the response curve with increasing chilling time, there were also clear differences in chilling requirement (Figs S1 and S2). Some cultivars such as 'Ben Dorain' responding optimally and consistently to the lowest temperatures with an increasingly weak response as the chilling temperature increased, while others showed a relatively rapid early response to low temperatures (e.g. 'Baldwin'), but with the effect wearing off with time. Others such as 'Ben Gairn' showed a very slow response, although with

the optimal temperature being 0 °C rather than -5 °C; 'Ben Starav' also appeared most responsive at 0 °C. For other cultivars such as 'Amos Black', long exposures to temperatures as high as 5 °C were optimal (although lower temperatures were optimal for shorter chilling periods). See data for specific details of the performance of different cultivars.

Overall effectiveness

Results of fitting the observed effectiveness to all the chilling periods are illustrated in Fig. 4, with some fitted temperature coefficients (K_{Tr}) and other parameters summarised in Table S2. Although the simple linear accumulation of temperature (Equation 1) explains a substantial proportion of the total variance (Fig. 4a), this cannot explain any supra-optimal chilling. The quadratic function used in equation (2) is much more suitable, leading to a substantial reduction in the error sum of squares (SS); it can simulate the differing optimum chilling periods for different temperatures and effectively accounts for the reduction in effectiveness after extended periods at the lower temperatures.

The temperature response functions derived for these data depend on the method used for their calculation (Table S2, Fig. 5). Interestingly the results are very similar for different bud stages, as shown by comparison of bud-stages 2 and 4 in Fig. 5.

The best fitting model (model 2) suggests a greater responsiveness to the lowest temperatures than do the other models. This arises because it takes account of the rapid response to

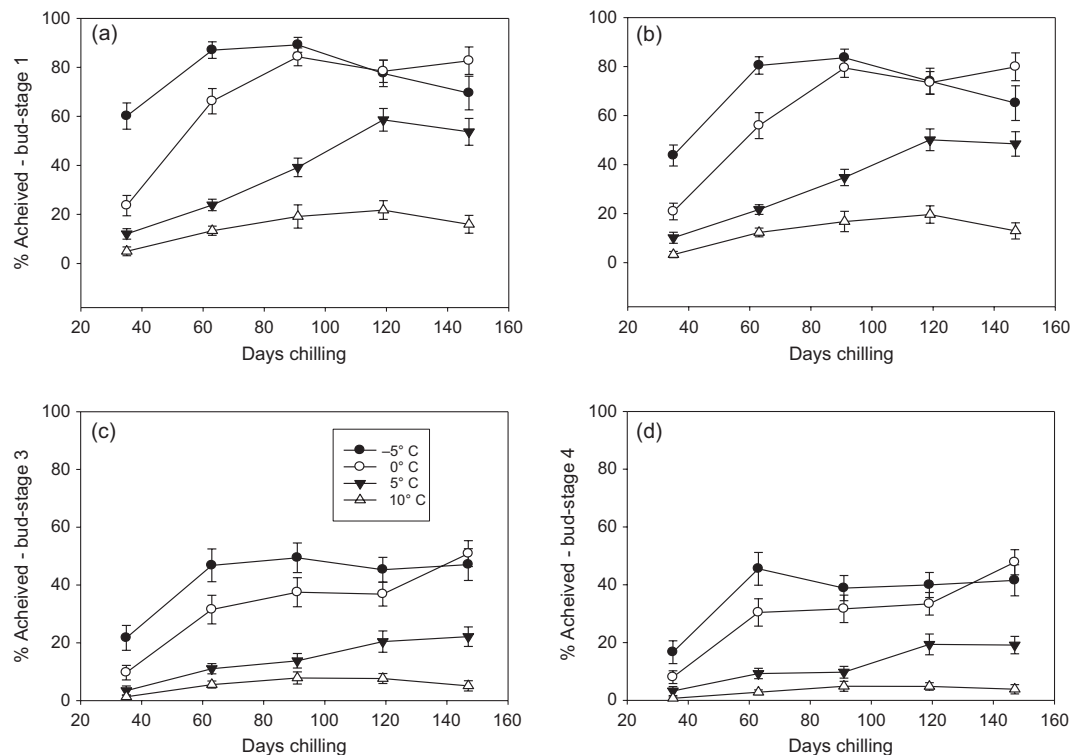


Fig. 2. The average across all 20 cultivars in year 1 of the percentage of buds reaching each of the four bud stages as a function of chilling temperature and time of chilling, all measured after 45 days' warming: a: bud-stage 1, b: bud-stage 2, c: bud-stage 3 and d: bud-stage 4. Values are the means \pm standard error of the means (n = 20 genotypes).

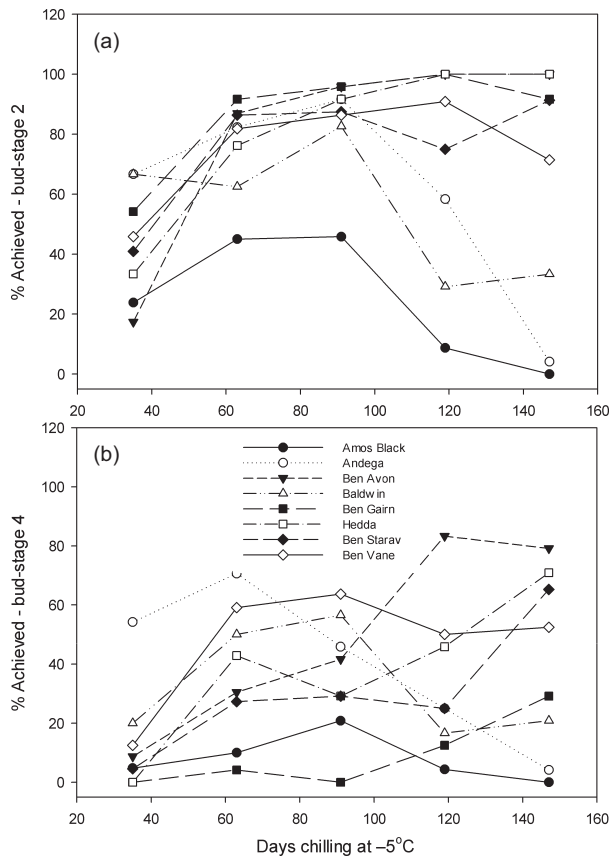


Fig. 3. Effect on representative contrasting cultivars of differing periods of chill at -5°C (recorded after a 45-day warming period) on a: percentage attainment of bud-stage 2 and b: percentage attainment of bud-stage 4. Error bars are omitted for clarity, but are similar to those in Fig. 1c and d.

low temperatures, which can then be lost with longer exposures. In all cases, however, there is a suggestion that temperatures below 0°C are optimal. The final curves on these graphs indicate the temperature sensitivity of chilling (average of all cultivars) if only data for the longest treatment period (147 days) are used; the resulting suggestion that lower temperatures are less effective than 0°C is largely an artefact of the very long chilling period and is biased somewhat by the supra-optimal chilling applied for some cultivars in this case.

This effect is illustrated further in Fig. 6, which shows that the poor fit across all data of a single effectiveness function may be at least partially explained by the change in relative effectiveness as the length of the chilling period changes. The cultivars that are most responsive to the lowest chilling temperatures for short chilling periods become relatively unresponsive after longer periods of chilling, while those that are unresponsive to low temperatures in short chilling periods become more responsive after longer chill periods.

Effect of varying temperature sequences

As for the first-year experiment, ANOVAs for any bud stage across all experimental treatments showed highly significant

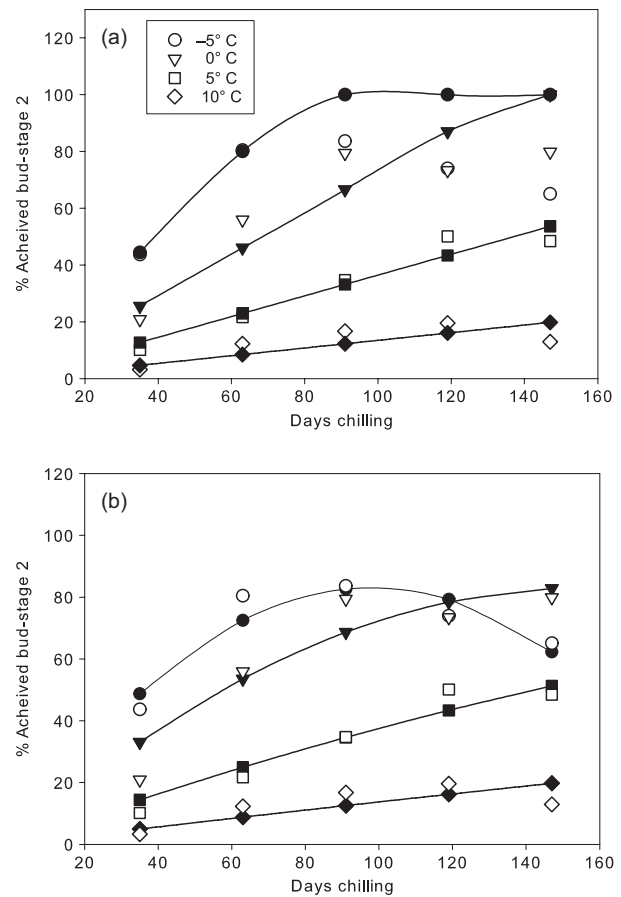


Fig. 4. Achievement of bud-stage 2 (%) averaged across all cultivars in year 1 (for samples incubated at -5°C , 0°C , 5°C and 10°C), where open symbols and dashed lines represent average actual achievement of bud-stage 2 (shown in each graph) and solid symbols and lines represent the modelled values of E for a: model 1, b: model 2.

main effects and interactions for all factors. For an average temperature of 0°C over the chilling treatment, the varying temperature regimes generally resulted in consistently more advanced buds than did a constant temperature, whether measured after treatments of 42 or 84 days (Fig. 7). This suggested that exposure to -4°C outweighed the effect of an equivalent period of exposure to $+4^{\circ}\text{C}$. Nevertheless the effect of varying temperatures was marginally larger for the decreasing temperature sequence (*i.e.* when the final temperature was -4°C) for the 84-day chilling period, but marginally smaller for the decreasing temperature sequence with the 42-day chilling period.

When the average temperature was maintained at 4°C , the results were more variable. Both regimes centred on 4°C ($+4, 0, -4$ and $-4, 0, 4^{\circ}\text{C}$) were less effective than the continuous 4°C for the 42-day chilling treatment, but the $+4, 0, -4^{\circ}\text{C}$ regime was at least as good or better than continuous for the 84-day chilling treatment. On the other hand, after 42 days' chilling, both the regimes that included a -4°C treatment were more effective than the continuous treatment, but only the $-4, +8, +8^{\circ}\text{C}$ sequence tended to be more effective than the continuous treatment for the 84-day chilling treatment. In all cases the $0, 4, 8^{\circ}\text{C}$ sequence was the least effective of the 4°C averages.

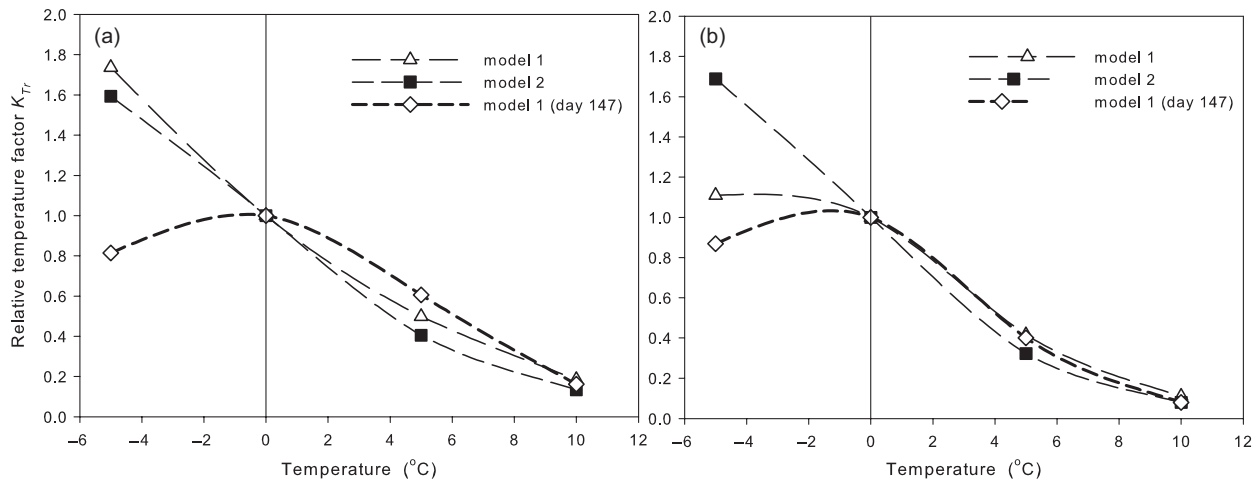


Fig. 5. Estimated values of K_{Tr} at the four experimental temperatures in year 1, fitted across all cultivars and sample dates using different methods of calculation (a) for bud-stage 2, and (b) for bud-stage 4; the lines joining the points represent spline functions derived using Sigmaplot12. The two first lines show the fits of models 1 and 2 using all the data, and the third line indicates the temperature coefficients fitted just to the final percentage buds reaching stage 2 (a) or stage 4 (b) after 147 days' treatment (using model 1).

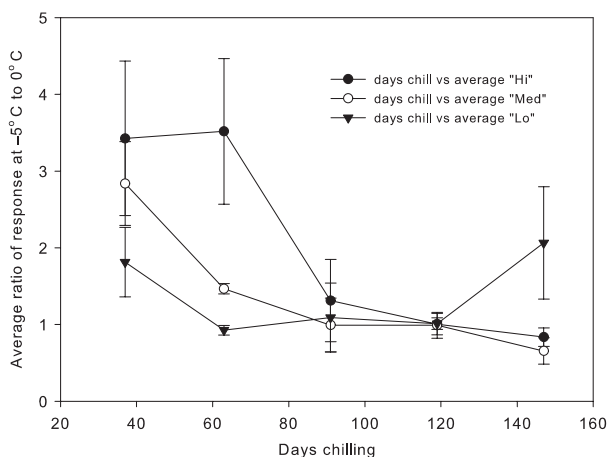


Fig. 6. Average response at $-5\text{ }^{\circ}\text{C}$ relative to that at $0\text{ }^{\circ}\text{C}$ for different periods of chilling for year 1, with cultivars grouped by response after 63 days' chilling. 'Hi' represents those cultivars with the greatest response to $-5\text{ }^{\circ}\text{C}$ (9559-6, Ben Avon, B1824, Ben Gairn, Ben Starav and Ben Tirran), 'Lo' represents those cultivars least responsive to $-5\text{ }^{\circ}\text{C}$ (9134-7, 9521-2, Baldwin, Ben Klibreck, Pilot Mamkin and Ben Vane) and 'Med' represents the remaining cultivars ($n = 8$). Points represent means \pm standard error of the mean.

As expected, the continuous $-4\text{ }^{\circ}\text{C}$ treatment was most effective and the continuous $+8\text{ }^{\circ}\text{C}$ and ambient control treatments were least effective overall (Table S3). Although the general trends shown by the continuous temperature regimes in the year 2 experiment were similar to the results from year 1 (data not shown), the results were not directly comparable between years because of the differing temperatures and chilling/warming periods used.

DISCUSSION

The use of excised blackcurrant shoots provides a practical model system to investigate the winter chilling responses of

woody plants. The germplasm used in these experiments was chosen to reflect as far as possible the range of variation present in this section of the *Ribes* genus, including cultivars from diverse geographical locations: cultivars from Scotland and from further south (e.g. 'Baldwin', 'Andega') and further north (e.g. 'Brödtorp' and 'Pilot Aleksandr Mamkin'). Different Scottish cultivars are known to differ in cropping season and presumably also in chilling requirement: 'Ben Gairn' is a very early-ripening cultivar, whereas 'Ben Tirran' is the latest ripening available. Furthermore, 'Brödtorp' is an accession from the Finnish wild, while EMR B1834 is a BC_5 from an original cross between blackcurrant and gooseberry (*Ribes grossularia* L.). The commercial cultivars commonly grown in the UK at the present time (the 'Ben' series bred in Scotland) are represented, including types such as 'Ben Lomond' that have previously been identified as at risk from declining levels of winter chilling (Rose & Cameron 2009). The data show clearly that chilling requirement and chilling functions vary with blackcurrant cultivars, so that there is considerable diversity within the available gene pool for this trait that can be utilised by breeders into the future.

The temperature effectiveness functions found for this study generally more closely approximate the exponential function of Bidabé (1967) and Lantin (1973) than the more conventional chill units where the temperature factor is assumed constant over a range of temperatures (Sunley *et al.* 2006). For all the genotypes used in this study, the effectiveness of chilling increases as temperature falls to $0\text{ }^{\circ}\text{C}$ and in most cases even below (especially 'Ben Gairn', 'Ben Starav' and 'Ben Tirran'). An important result from the present data, however, is the demonstration that the most appropriate temperature function depends on the model used for temperature accumulation over time (Fig. 5). Furthermore, there is clearly also an interaction between the temperature response function that is appropriate for any cultivar and the length of low-temperature exposure (Fig. 6). The ability to separate germplasm on the basis of optimal chilling temperature can enable the selection of appropriate cultivars for specific

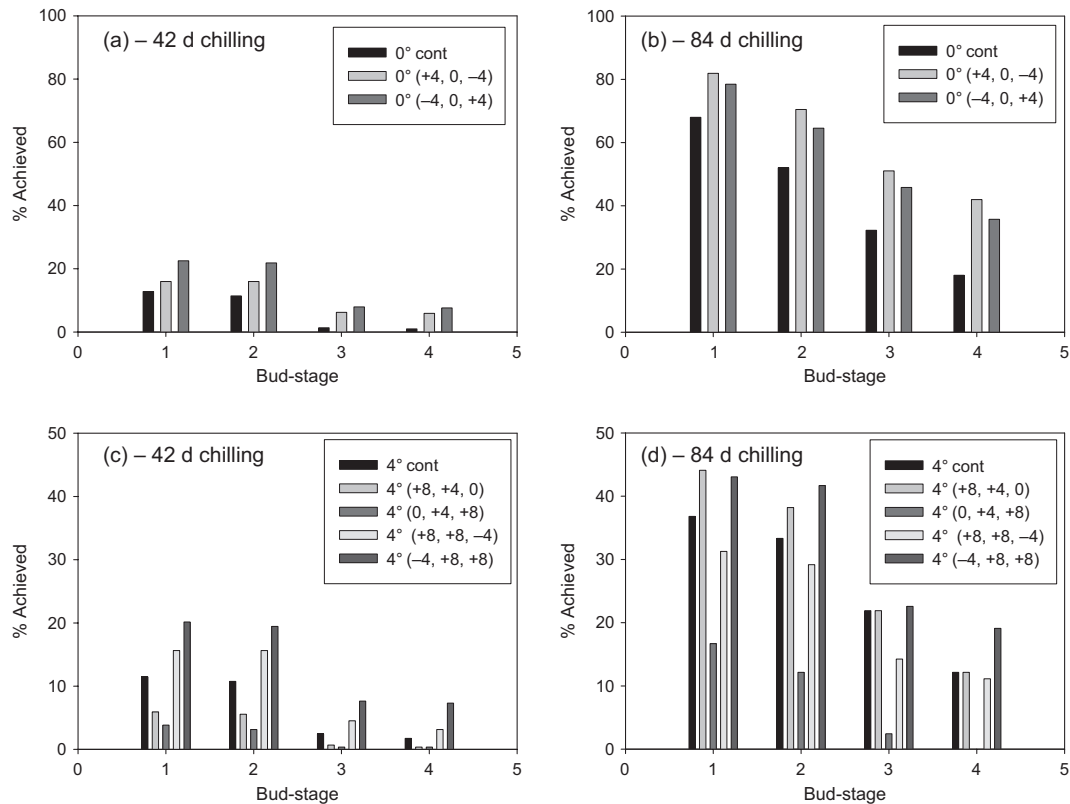


Fig. 7. Temperature responses of the four bud stages for year 2 data averaged across all cultivars: a: after 42 days' chilling at a continuous 0 °C (cont), or at +4, 0, -4 °C or at -4, 0, 4 °C, b: after 84 days' chilling under the same temperature regimes, c: after 42 days' chilling at 4 °C (continuous) or at +8, +4, 0 °C, or at 0, +4, +8 °C, or at +8, +8, 0 °C, or at 0, +8, +8 °C, and d: after 84 days' chilling under the same five temperature regimes.

climatic requirements. Although we have used blackcurrant as a model here, the findings may be equally relevant to other woody species where future chilling levels may pose a threat to their sustainability and productivity.

An important conclusion from these studies, and one that has been little recognised in the past, is that chilling can be supra-optimal, with long exposures to low temperatures inhibiting subsequent bud development at permissive temperature environments. This effect was particularly clear in the cultivars 'Amos Black' and 'Andega' (both from relatively low-chill regions) for which there was a marked inhibition of bud development as exposure to low temperature chilling increased (Fig. 3). Some cultivars, however, did not show any inhibitory effect of excess low temperature exposure. This finding reinforces the need for cultivars to be grown in appropriate areas, so that low chill-requiring cultivars such as 'Andega' are not planted in high chill regions, such as northern UK and Scandinavia.

Winter in northern temperate regions is often characterised by periods of low (chilling) temperatures, often sub-zero, interspersed with warmer spells, and the different response of plants to an alternating temperature regime during the dormancy period has been noted previously by Erez *et al.* (1979), Pérez *et al.* (2008) and others. The former work defined a threshold of upper temperature negation, and found that alternating diurnal temperatures were more efficient than constant temperatures in fulfilling the chilling requirement of peach buds. Erez *et al.* (1988) went further, in the development of a dynamic model to explain these

effects in warm temperate conditions. In the current study, where the temperature variation was more protracted, it was found that varying temperature regimes substantially enhanced the response to a given average temperature (as compared with an equivalent constant temperature) when centred on 0 °C, but had rather more variable results when centred on 4 °C.

We developed and tested a number of empirical chilling models to explain the interaction between chilling temperature and time of exposure to describe (or predict) bud development. The best model involved a quadratic function of time and effectiveness that allowed a decreasing effectiveness as chill accumulated beyond an optimum. The development of effectiveness coefficients for different chilling temperatures was linked to, and based on, the variation in response shown by the different genotypes, so that the most effective temperature for fulfilment of the chilling requirement of low-chill cultivars such as 'Andega' was significantly higher than that for 'Ben Gairn', for example. These differing responses across the genetic base available to breeders are in themselves of key importance when designing future phenotyping screens, both in blackcurrant and also in other vulnerable woody species.

We do not at this time have any good insight into the mechanistic basis of the observed interaction between time and temperature in the decrease of endodormancy in response to chilling. Further work will be necessary to unpick the underlying mechanism, and thereby develop more sophisticated models, but it can be speculated that a simple mechanism to achieve the observed interactions might involve the

combination of a saturating release of endodormancy in response to chilling combined with a linear inhibition of the process. In spite of the empirical nature of the models derived here, the approach described does appear to provide a powerful basis for the development of approaches to phenotyping genotypes for their chilling response. In particular, the use of hardwood cuttings, as employed here, rather than whole plants, will be much more effective for the development of high-throughput screens. Although it is known that there are some differences in response between the two (Mahmood *et al.* 2000; Fraser 2005), our use of cuttings from 4- to 5-year-old plants is likely to have minimised any such effect (Mahmood *et al.* 2000).

The present study was conducted using periods of constant temperature treatment to derive the appropriate temperature factors (K_T). The next step in the development of this approach would be to use smooth temperature functions to fit the observed K_T s: this would allow us to calculate expected effects of natural variation of temperature in the field by integration of equations 1 or 2 over time. In this way, the models proposed here would become directly analogous to the standard 'chilling hour' or 'Utah' types of model.

The data presented in this study provide a clear indication that there is significant diversity in chilling requirement across the genetic base of blackcurrant, as a platform for varietal development. Additionally, using the models developed here, phenotyping strategies can be employed to expose cuttings of potential new cultivars to chilling temperatures and derive predictions of their developmental behaviour based on the models developed here. This work can be aligned with parallel studies to identify genes and linked markers involved in the dormancy–temperature responses to chilling in blackcurrant (Hedley *et al.* 2010), so that potentially useful germplasm can be selected at the earliest possible stage. The breeding of new blackcurrant cultivars is a long-term process, and as a result it is necessary for breeders to introduce traits that may be of future importance for the sustainability of the crop production. The projections regarding climate change in the UK are consistent in their prediction of rises of between 2–3 °C in mean winter temperatures in the main fruit-growing regions by 2050 (UKCP09 2009, medium emissions scenario). As a consequence, the need for production of new cultivars with lower chilling requirements than 'Ben Lomond' or 'Ben Dorain' must be addressed by breeders now, to provide new cultivars suitable for future production.

CONCLUSIONS

1 For blackcurrants, the effectiveness of chilling increases as temperature falls to 0 °C and even below for most cultivars.

REFERENCES

- Atkinson C.J., Sunley R.J., Jones H.G., Brennan R., Darby P. (2004) *Winter chill in fruit*. UK Department for Food, Environment and Rural Affairs, London, Report No. CTC0206.
- Baldocchi D., Wong S. (2008) Accumulated winter chill is decreasing in the fruit growing areas of California. *Climate Change*, **87** (Suppl 1), S153–S166.
- Bidabé B. (1967) Action de la température sur l'évolution des bourgeons de pommier et comparaison de méthodes de contrôle de l'époque de floraison. *Annales de Physiologie Végétale*, **9**, 65–86.
- Cesaraccio C., Spano D., Snyder R., Duce P. (2004) Chilling and forcing model to predict bud-burst of crop and forest species. *Agricultural and Forest Meteorology*, **126**, 1–13.
- Cesaraccio C., Spano D., Snyder R.L., Duce P., Jones H.G. (2006) *Improvement of chilling and forcing model to predict bud-burst*. Paper presented at the 17th Conference on Biometeorology and Aerobiology, San Diego.
- Erez A., Couvillon G.A., 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., Fishman S., Gat Z., Couvillon G.A. (1988) Evaluation of winter climate for breaking bud rest using the dynamic model. *Acta Horticulturae*, **232**, 76–89.
- Estrella N., Sparks T.H., Menzel A. (2007) Trends and temperature response in the phenology of

2 Chilling can be supra-optimal, with long exposures to low temperatures inhibiting subsequent bud development at permissive temperature environments. This implies that the choice of temperature and time is crucial in any screening test.

3 The most effective empirical model to describe the results involved an optimal response to accumulated chill.

4 Chilling requirement and modelled chilling functions vary among blackcurrant cultivars.

5 Varying temperature regimes can substantially modify the bud development responses.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Samples of the range of chilling responses (% achievement of bud-stage 4) found in different cultivars in the year 1 experiment. All records taken after 45 days' warming at permissive temperature.

Figure S2. Samples of the range of chilling responses (% achievement of bud-stage 4) found in different cultivars in the year 1 experiment. All records taken after 45 days' warming at permissive temperature.

Table S1. Blackcurrant germplasm used in experiments in each year.

Table S2. Parameters in equations 1 and 2 estimated for achievement of bud-stage 2 after 45 days' warming, estimated across data for all the cultivars in year 1 (first part of table) and for some representative cultivars (second part of table). Data for all lengths of chilling period are fitted together. The sums of squares (SS) are for 400 data points for the full models and 20 data points for the single cultivars.

Table S3. Effectiveness of varying temperature regimes (year 2), expressed as the percentage achievement of each of the four bud stages. Results are the averages of all cultivars used in year 2.

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- crops in Germany. *Global Change Biology* **13**, 1737–1747.
- Faust M., Liu D., Line M.J., Stutte G.W. (1995) Conversion of bound to free water in endodormant buds of apple is an incremental process. *Acta Hortica*, **395**, 113–118.
- Fraser G.A. (2005) *An analysis of dormancy and chilling of Ribes nigrum L.* PhD thesis, University of Reading, UK, 196 pp.
- Hänninen H., Slaney M., Linder S. (2007) Dormancy release of Norway spruce under climatic warming: testing ecophysiological models of bud burst with a whole-tree chamber experiment. *Tree Physiology*, **27**, 291–300.
- Harding J. (2007) Problem of global warming is at heart of currant affairs. *The Times*, <http://business.timesonline.co.uk/tol/business/columnists/article2317479.ece> (accessed 10 March 2012).
- Hedley P.E., Russell J.R., Jorgensen L., Gordon S., Morris J.A., Hackett C.A., Cardle L., Brennan R.M. (2010) Candidate genes associated with bud dormancy release in blackcurrant (*Ribes nigrum* L.). *BMC Genomics*, **10**, 202.
- Heide O. (1974) Growth and dormancy in Norway spruce (*Picea abies*). I. Interaction of photoperiod and temperature. *Physiologia Plantarum*, **30**, 1–12.
- Heide O. (1993) Dormancy release in beech buds (*Fagus sylvatica*) requires both chilling and long days. *Physiologia Plantarum*, **89**, 187–191.
- Howe G.T., Davis J., Jeknic Z., Chen T.H.H., Frewen B., Bradshaw H.D., Saruul P. (1999) Physiological and genetic approaches to studying endodormancy-related traits in *Populus*. *HortScience*, **34**, 1174–1184.
- Hoyle D.E. (1960) Some effects of temperature and daylength on the breaking of winter dormancy in blackcurrant. *Journal of Horticultural Science*, **35**, 229–238.
- Hunter A.F., Lechowicz M.J. (1992) Predicting the timing of budburst in temperate trees. *Journal of Applied Ecology*, **29**, 597–604.
- Lang G.A. (1987) Dormancy – a new universal terminology. *HortScience*, **22**, 817–820.
- Lantin B. (1973) Cold temperature requirements of buds of blackcurrant (*Ribes nigrum* L.) and of some redcurrants (*Ribes* spp.). *Annales Amélioration des Plantes*, **23**, 27–44.
- Lantin B. (1977) Estimation of the cold requirements to break dormancy in buds of blackcurrant (*Ribes nigrum* L.) and other currants (*Ribes* spp.). *Annales Amélioration des Plantes*, **27**, 435–450.
- Mahmood K., Carew J.G., Hadley P., Battey N.H. (2000) Chill unit models for sweet cherry cvs. Stella, Sunburst and Summit. *Journal of Horticultural Science and Biotechnology*, **75**, 602–606.
- Nakicenovic N., Swart R. (Eds.) (2000) *Intergovernmental Panel on Climate Change special report on emission scenarios*. Cambridge University Press, Cambridge.
- Pérez F.J., Ormeño J.N., Reynaert B., Rubio S. (2008) Use of the dynamic model for the assessment of winter chilling in a temperate and a subtropical climatic zone of Chile. *Chilean Journal of Agricultural Research*, **68**, 198–206.
- Richardson E.A., Seeley S.D., Walker D.R. (1974) A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. *HortScience*, **9**, 331–332.
- Rose G.A., Cameron R.W. (2009) Chill unit models for blackcurrant (*Ribes nigrum* L.) cultivars 'Ben Gairn', 'Ben Hope' and 'Ben Tirran'. *Scientia Horticulturae*, **122**, 654–657.
- Solomon S., Qin D., Manning M., Chen Z., Marquis M., Averyt K.B., Tignor M., Miller H.L. (Eds) (2007) *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
- Sunley R.J., Atkinson C.J., Jones H.G. (2006) Chill unit models and recent changes in the occurrence of winter chill and spring frost in the United Kingdom. *Journal of Horticultural Science and Biotechnology*, **81**, 949–958.
- UKCP09 (2009) *UK Climate Projections*. <http://ukclimateprojections.defra.gov.uk> (accessed 10 March 2012).