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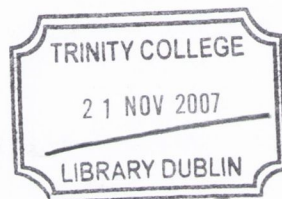
QUANTIFYING THE ENVIRONMENTAL DRIVERS OF TREE PHENOLOGY

Thesis submitted for the degree of Doctor of Philosophy

2007

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THESIS
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Declaration

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Abstract

The prediction of growth onset is particularly complicated, because this phenophase is the end-point of a series of poorly understood physiological changes that are not directly observable, occurring while the bud is dormant. However, it is becoming more and more important to understand how tree growth responds to changes in environmental conditions, and predict how species will be impacted by global warming. In order to gain more understanding of the environmental control of dormancy, ecophysiological studies are necessary. The objectives of this study were to (i) quantify the effects of temperature, photoperiod, light intensity and nutrient deficiency on the percentage and timing of budburst of *Betula pubescens*, *Fagus sylvatica*, *Salix aurita*, *Salix x smithiana* and *Tilia cordata* in a series of experiments, (ii) identify mathematical relationships describing the response of the timing of budburst to these environmental factors, (iii) use the information gained during the experimental study to develop a mechanistic model of budburst. The experiments highlighted the presence of a common mechanism controlling dormancy, based mostly on temperature and a photoperiod signal. The degree to which the two triggers contributed to the progress of dormancy varied among species. In *F. sylvatica* and *B. pubescens* daylength could substitute for chilling completely or almost completely, while *S. aurita*, *S. x smithiana* and *T. cordata* had a stricter requirement for chilling. The phenological differences observed among species might be related to differences in their ecology and life strategy. Dominant, long lived species such as *T. cordata* and *F. sylvatica* showed low rates of budburst, high chilling requirements and responsiveness to light intensity, while opportunistic, pioneer species such as *B. pubescens*, *S. aurita* and *S. x smithiana* had high rates of budburst, low chilling requirements and were not affected by light intensity. In addition, *B. pubescens* and *S. x smithiana* were more responsive to high forcing temperatures than *T. cordata* and *F. sylvatica*. These results suggest that the timing of growth onset in *B. pubescens* and *S. x smithiana* is regulated through a less conservative mechanism than in *T. cordata* and *F. sylvatica*, and that these species trade a higher risk of frost damage for the opportunity of vigorous growth at the beginning of spring, when more light is available.

B. pubescens was selected for a more comprehensive study that would facilitate the development of a physiologically based model of budburst. As this species was found to be particularly sensitive to photoperiod, an attempt was made at incorporating photoperiod into the Unified model (Chuine, 2000) and developing a model which could account for the experimental data and increase the understanding of the environmental control of dormancy. The effect of photoperiod was integrated through a photoperiod-dependent change in the rate of forcing. The model was applied to experimental and field observations and compared with the Unified model. Both models showed high internal validities when calibrated on the field observations, but only the UnPhot model showed high internal validity when applied to the experiments in which photoperiod was manipulated. This suggests that the integration of photoperiod enabled the model to explain a portion of variance unaccounted for by temperature based models. However, when calibrated on field observations and used to predict external field observations, both models showed a low external validity. It is concluded that the model needs to be calibrated on a large dataset containing both experimental and field observations before its framework can be validated (or falsified).

“Il sentiero nel bosco era piano, ombroso e solitario, e camminando si calmò. C'erano fiori sul margine, modesti ma graziosi: primule, miosotis, qualche fiorellino bianco di fragola ed Amelia se ne sentiva attratta.

Non é strano sentirsi attratti dai fiori, ma lei se ne sentiva attratta in modo strano: Amelia si conosceva bene, e sapeva che quel modo era strano. Anche se comune a molti e molte, e non tutti con sangue di larice nelle vene”

“The path through the woods was flat, shadowy and lonely, and while walking she calmed down. There were flowers on the sides, modest but pretty: primroses, forgetmenots, a few white strawberry flowers and Amelia felt attracted to them.

To feel attracted to flowers is not a weird thing, but she was attracted to them in a strange way. She knew herself well and that was a strange way. Even though it was shared by many and not all of them with larch's blood in their veins”

Primo Levi

(Lilith)



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Background

In temperate and seasonal regions, woody perennials have evolved control mechanisms of their growth cycle that tune them with yearly variations in temperature and precipitation. An important adaptation allowing them to survive the winter season is dormancy, whereby vegetative activity is suspended until favourable growth condition resume in spring. In late summer, trees enter dormancy and develop dormant buds, mainly as a response to an extension in night length, corresponding to a shortening photoperiod (Wareing, 1956; Vaartaja, 1959; Håbjørg, 1972). This state of “suspended growth” is released by low temperatures during the winter (Perry, 1971; Sarvas, 1974; Murray *et al.*, 1989; Battey, 2000). After dormancy release, growth occurs in response to warm temperatures (Perry, 1971; Sarvas, 1974; Cannell, 1989).

The key role of temperature as a driver of these phenological events has long been recognized (Doorembos, 1953; Vegis, 1964; Richardson *et al.*, 1974; Chuine *et al.*, 2003). However the cellular mechanisms regulating the transition from a dormant to a non-dormant state are still unclear. Despite the many studies conducted on this subject (for a review, see Arora *et al.*, 2003) there are still no explanations available as to how chilling temperatures promote dormancy release in winter, nor is it clear how different environmental factors interact in the regulation of dormancy and control the timing of growth onset.

It is becoming more and more important to understand how tree growth responds to changes in temperature. Yearly variations in tree phenology are responsible for large differences in forest productivity (Lieth, 1970, 1997; Kellomäki and Kolström 1994). Also, the timing of phenological events is a potential bio-indicator summarising climate trends and variations, and their impact on biological systems (Parmesan and Yohe, 2003). The global warming that is predicted to affect us in the coming decades will probably have heavy impacts on ecosystems and affect plant development (IPCC, 2001). Already an increasing number of studies report changes in plant and animal life cycles from a wide range of regions across the world (Schwartz and Reiter, 2000; Beaubien and Freeland, 2000; Fitter and Fitter, 2002; Penuelas and Filella, 2003; Parmesan and Yohe, 2003).

Through the analysis of phenological records, Menzel and Fabian (1999) concluded that in Europe the growing season has extended by 10.8 days since the early 1960s, due mainly to an advance in spring phenophases. In Britain, the mean flowering dates of 385 plant species advanced by 4.5 days during the 1990s compared with the means of the period 1954–1990 (Fitter and Fitter, 2002). All these phenological changes are highly correlated with temperature changes occurring during the months that precede them (Fitter and Fitter, 2002; Chuine and Yiou, 2004; Wolfe *et al.*, 2005; Donnelly *et al.*, 2004), which has prompted a new interest in predicting how they will be affected by climate change. For example, earlier budburst due to global warming could lead to extensive frost damage (Murray *et al.*, 1989; Hänninen, 1991, 1995; Kramer, 1994a, 2000).

The study of the regulation of phenological events has also many more immediate applications in horticulture and agriculture. The quantification of the chilling requirements of temperate fruit species is important for the selection of cultivars to be grown in climate conditions different from those affecting their natural ranges (Balandier *et al.*, 1993; Fuchigami and Wisniewski, 1997) and the modelling of growth onset provides important information for the management of the canopy (Lang, 1989; Austin *et al.*, 2002).

Despite the interest in the prediction of growth onset, this phenophase is one of the most difficult to model, because it is the end-point of a series of physiological changes that are not directly observable and occur while the bud is dormant (Lang *et al.*, 1987, Hänninen, 1990). Researchers investigating the physiology of dormancy now believe that dormancy is a complex, dynamic phenomenon, involving many parallel and concomitant pathways and that it can be influenced by conditions both internal and external to the plant (van der Shoot, 1996; Ross, 1996; Arora *et al.*, 2003; Horvath *et al.*, 2003). The complexity of these mechanisms makes it difficult to develop widely applicable models of budburst. Many attempts at this task have been made during recent years (see, for example, Robertson, 1968; Sarvas, 1974; Landsberg, 1974; Richardson *et al.*, 1974; Kobayashi and Fuchigami 1983a; Kobayashi and Fuchigami 1983b; Cannell and Smith, 1983; Hunter and Lechowicz, 1992; Kramer, 1994a, 1994b; Hänninen, 1995; Chuine, 2000). These phenological models predict the timing of budburst and flowering of tree species by the input of environmental variables such as temperature and day length (Sarvas, 1974; Landsberg, 1974; Richardson *et al.*, 1974; Hänninen, 1995; Chuine, 2000). However, models will be only as accurate as our understanding of the complicated environmental interactions through which dormancy

is regulated, and ultimately of the cellular mechanisms involved in this transition (Arora *et al.*, 2003). As long as these mechanisms are unknown, the only way to facilitate the development of predictive models and gain information on the impact of environmental changes on tree phenology, will be to investigate plant responses through ecophysiological studies at a macroscopic level.

Aims of the study

The main aims of this study were to quantify the effect of temperature, photoperiod, light intensity and nutrient deficiency on spring phenology of trees, and integrate this information into a mechanistic model of budburst. In order to evaluate the effects of these factors on phenology without any confounding effects due to genotypic variation, our approach involved the vegetative propagation of the plant material used for the experiments. The 5 tree species (*Betula pubescens*, *Fagus sylvatica*, *Salix aurita*, *Salix x smithiana* and *Tilia cordata*) under study were vegetatively propagated from the clones growing in the International Phenological Gardens (IPGs).

The specific objectives were to:

1. Quantify the effect of temperature, photoperiod, light intensity and nutrient deficiency on the percentage and timing of budburst of *Betula pubescens*, *Fagus sylvatica*, *Salix aurita*, *Salix x smithiana* and *Tilia cordata*.
2. Identify suitable mathematical relationships describing the response of the timing of budburst in relation to the environmental factors under study.
3. Integrate the information obtained from (1) and (2) into a mechanistic model of budburst.

Thesis structure

The present work is divided into two parts: an experimental study, investigating the response of the timing and percentage of budburst in *Betula pubescens*, *Fagus sylvatica*, *Salix aurita*, *Salix x smithiana* and *Tilia cordata* to the variation in the levels of different environmental factors (temperature, photoperiod, light intensity and nutrient availability) on spring phenology, and a modelling study, that focused only on one species, *Betula pubescens*, and was based on the information obtained during the experimental study.

The first chapter starts with a comprehensive literature review, given as an introduction to the experimental study. The main concepts regarding bud dormancy and its environmental regulation are introduced, and a brief outline of the theories on dormancy breaking is presented. The physiology and ecology of each of the species studied is briefly presented. The methods section illustrates the methods used in the experimental study. Details of the techniques of vegetative propagation used for the 5 tree species are given, and the experimental designs and the statistical methods adopted for the data analysis are described. The results section is concerned with the description of the results and their analysis with the Analysis of Variance (ANOVA). The first part of this section focuses on overall environmental effects and differences among species, the second part concentrates on the effects of environmental factors in each species. The relationships between the environmental variables and the timing and percentage of budburst are shown by plots and fitted with curves. The following section discusses the results, firstly focusing on the overall effects and the differences among species, then discussing in detail the effects observed in each of the species. In the final part of the discussion, the interactions controlling budburst in the investigated tree species are summarized, and the most important factors triggering phenological development are divided into external (photoperiod and temperature) and internal (physiological state of the bud). The implications of the findings of the experimental study for the selection of phenological indicators of climate change are discussed and the ecological significance of the observed responses is considered.

The second chapter is concerned with the integration of the information obtained from the experimental study into a model of budburst. This part starts with a general introduction to mathematical models with a highlight on mechanistic models, and then focuses on

phenological models. Some of the most widely used phenological models are described. Details are given on the Unified model (Chuine, 2000), a model of budburst accounting for most phenological models due to the generality of its functions and the elevated number of parameters defining it. Finally the aims of the study are described. The methods section describes the integration of the effects of photoperiod into the Unified model, based on the findings of the experimental study. A new model, called UnPhot model, is then introduced through a description of its assumptions and their formalization. The following section describes the results of the application of the UnPhot and Unified models to experimental and field observations. The parameter values and the responses to temperature and photoperiod fitted by the two models are presented and analyzed with statistical tests and graphical methods. The internal and external validity of the models is tested. The discussion section deals with the implications of the results of the statistical tests and attempts to explain the reasons for the low external validity of the two models. The final section provides a general summary of the main findings of the experimental and modelling studies and suggests directions for future studies.

**EFFECTS OF TEMPERATURE, PHOTOPERIOD, LIGHT INTENSITY
AND NUTRIENTS ON BUD DEVELOPMENT IN *BETULA*
PUBESCENS, *FAGUS SYLVATICA*, *SALIX AURITA*, *SALIX X*
SMITHIANA AND *TILIA CORDATA***

INTRODUCTION

1. Dormancy in woody species

1.1. Dormancy: an important adaptation for the regulation of growth

Trees have been able to colonize the cool and temperate zones of the world through the evolution of features enabling them to cope with the seasonal fluctuations in temperature and precipitation occurring at mid-high latitudes. Among these adaptations are the synchronization of growth with favourable seasonal conditions and the ability to stop growth and become dormant during unfavourable conditions. Dormancy involves the suspension of visible growth in any plant structure containing a meristem (Lang *et al.*, 1987) and is one of the most dramatic adaptations that plants have evolved to regulate and orchestrate their developmental processes (Leopold, 1996). The term “dormancy” encompasses a series of events which span from the long rest during the winter months, to the interval between growth flushes during the vegetative season and can be induced by a range of causes, either environmental or internal to the plant.

Bud dormancy is thought to have evolved 400 millions years ago, in the first land plants, as a control mechanism to adjust growth and branching to light and nutrient availability. Axillary branching, apical dominance and flushing growth evolved in response to these conditions. Dormancy thus had a morphogenetic function, and only later, when plants had to adapt to seasonal climates, it became a mechanism to survive an unfavourable season Viéumont and Crabbé (2000). In many tropical trees that grow in year-round favourable conditions with constant moisture and warmth, dormancy still has only a morphogenetic function. Their growth follows a periodical pattern that occurs through the accumulation of discrete growth cycle, interrupted by short quiescent phases (Okubo, 2000). The same behaviour is also typical of temperate trees that alternate periods of rapid shoot elongation with periods of little or no growth during the favourable season, a phenomenon called flushing growth (Crabbé and Barnola, 1996). In both cases growth periodicity is due to the interplay between adjoining meristematic and apical domains (Crabbé, 1994) and it does not depend on external environmental conditions. Similarly, the growth arrest experienced by lateral buds under the inhibiting influence of an apical meristem is due to internal

causes. Apical dominance is in fact a result of the high auxin production of the leading meristems that diverts most of the nutrient supply, preventing the growth of lateral buds (Crabbé, 1994).

With the evolution of dormant seeds the influences inhibiting growth finally shifted from internal correlative inhibition to external environmental triggers (Leopold, 1996). These mechanisms linking environmental signals to dormancy onset and release were subsequently maintained by trees as a survival strategy against the unfavourable season, allowing them to first colonise upland areas in tropical regions and later to adapt to temperate climates (Viéumont and Crabbé, 2000).

1.2 The complex interaction of environmental and internal triggers of dormancy

The absence of growth during dormancy is a complex phenomenon, consisting of several intra- and extra- meristematic subprocesses. Organogenesis, internodal elongation and leaf expansion interact strongly, and any limiting factor for any of these subprocesses can ultimately inhibit growth as a whole (Crabbé and Barnola, 1996). Shortened internodes result in an accumulation of leaf primordia at the apex, and their combined effect is to produce heteroblasty and modify the leaves into cataphylls and produce dormant buds. If the conditions leading to dormancy persist, the growth will be completely stopped and the cells of the meristem cease dividing (Crabbé and Barnola, 1996). These events occur in the summer, between subsequent growth flushes and are induced by correlative effects within the plant. Summer correlative dormancy is quickly overcome by a new cycle of growth unless particular environmental conditions trigger the passage to longer, environmentally regulated dormant phases, such as winter dormancy (Crabbé, 1994). On release from this dormancy by appropriate environmental triggers (such as exposure to chilling temperatures, in the case of winter dormancy) the bud meristem recovers its growth ability (Battey, 2000).

The fact that treatments that change the context of meristematic domains (such as defoliation) also modify the course and the depth of environmentally regulated dormancy (Bailly and Mauget, 1989) suggests that it depends on the chain of correlative events preceding dormancy, as well as environmental influences. The effect that the tree

correlative history has on bud dormancy provides also an explanation for the different behaviour of buds on the same tree or the same twig on growth resumption.

1.3 Different types of dormancy

In order to differentiate the different types of dormancy Lang *et al.* (1987) proposed a classification based on the cause of growth arrest and its position relative to the dormant meristem. His categories correspond to the three types of dormancy already identified by Doorembos (1953), Samish (1954) and Romberger (1963). They reflect the view of dormancy evolution and development proposed by Crabbé and Barnola (1996) and are consistent with the idea that environmental control is superimposed upon an endogenous cycle of growth that includes a resting phase (Battey, 2000).

When growth inactivity is a direct consequence of environmental conditions, it is defined as “ecodormancy” (Lang *et al.*, 1987). This type of dormancy corresponds to the state defined by Doorembos (1953) as “imposed dormancy” and the state defined as “quiescence” by Samish (1954), Romberger (1963) and Chuine (2000). “Endodormancy” (Lang *et al.*, 1987) or “dormancy” (Romberger, 1963; Landsberg, 1974; Hänninen, 1990; Chuine, 2000) is caused by factors within the dormant bud and cannot be easily released by favourable environmental conditions. It occurs during the winter resting phase in temperate woody species. During deep rest, buds can be induced to budburst only once they have been exposed to a period of chilling temperatures breaking the internal blocks causing endodormancy. They then become receptive to favourable environmental conditions and start to grow as soon as warm weather allows it. Finally, “paradormancy” is the temporary arrest of growth due to causes within the plant but outside the dormant tissue (Lang *et al.*, 1987). Only when these extra-bud influences are removed, growth resumes. It is the equivalent to the state described as “correlative dormancy” by Romberger (1963).

1.4 The growth cycle of temperate trees

In many temperate trees, the main responsible trigger for growth cessation and dormancy onset is shortening daylength (Junttila, 1980; Rinne *et al.*, 1994; Thomas and Vince-Prue, 1997; Welling *et al.*, 1997) or increasing night length (Vaartaja, 1959; Hänninen, 1995) in late summer. The duration of the period of short days necessary to induce dormancy has

been found to vary from species to species (Downs and Borthwick, 1956). Dormancy is subsequently broken by the exposure to chilling temperatures (Roemberger, 1963; Perry, 1971; Sarvas, 1974; Battey, 2000), although freezing temperatures have also been shown to release buds from dormancy (Rinne *et al.*, 1997), as well as sublethal heat stress (Shirazi and Fuchigami, 1995; Wisniewski *et al.*, 1997). Chilling is a vaguely defined concept, typically referring to temperatures below about 10°C (Battey, 2000). However, different studies differ as to the temperature range considered active for dormancy release (Sarvas, 1974; Richardson *et al.*, 1974; Mahmood *et al.*, 2000), and differences also exist among species as to the chilling requirement necessary for dormancy release (Heide, 1993a; Murray *et al.*, 1989).

After a variable duration of exposure to chilling temperatures, endo-dormancy is broken and the bud enters quiescence. At this stage, the exposure to warm temperatures is the main trigger leading to budburst, even though in some species long days have been shown to reduce the time to budburst (Heide, 1993a; Myking and Heide, 1995) or be required as a condition for budburst (*Fagus sylvatica*) (Heide, 1993b). Additional chilling during this period has been shown to decrease the amount of exposure to warm temperatures before bud burst (Murray *et al.*, 1989; Heide 1993a; Skuterud and Dietrichson 1994; Myking and Heide, 1995).

Despite the fact that temperature is the main factor in dormancy transition (Romberger, 1963; Sarvas, 1974; Seeley, 1996), the succession of dormancy events can be altered, delayed or exacerbated at any stage by the effect and the interplay of a number of environmental conditions (van der Schoot, 1996; Besford *et al.*, 1996).

This is due to the reliance of plants on more than one environmental signal for the start and progression of winter rest. Crabbé and Barnola (1996) pointed out that as plants do not possess a specific receptor for temperature and have to rely on the rate of enzymatic reactions for the perception of temperature, this factor alone does not provide a clear-cut signal. In addition, temperature can fluctuate widely around the same time of the year and change suddenly, making the start of the cold season unpredictable on a sole temperature basis. For this reason, plants have evolved physiological mechanisms linking different environmental cues to dormancy induction and release. The complicated interactions between external and internal factors, and the changing response of the buds mark the

progress of dormancy and determine the timing of budburst in the spring (van der Schoot, 1996; Horvath *et al.*, 2003).

2. Environmental control of dormancy

2.1 Temperature

Temperature is the main factor in the transition of winter dormancy (Romberger, 1963; Sarvas, 1974; Kramer, 1994b; Seeley, 1996; Chuine *et al.*, 2003). However, its effects can vary according to the physiological state of the bud and the response may differ among species (Crabbé and Barnola, 1996). This changing effect is caused by the lack of a specific receptor for temperature, which makes its perception depend on the dynamic physiological state of the bud (Crabbé and Barnola, 1996).

2.1.1 Dormancy Induction

During photoperiodic dormancy induction low constant temperatures or low night temperatures have been generally found to increase growth incapability and hasten endodormancy (Downs and Borthwick, 1956; Lavender *et al.*, 1968; Håbjørg, 1972; Junttila, 1980; Heide, 2003; Arora *et al.*, 2003, Mølmann *et al.*, 2005). One possible mechanism by which temperature can affect photoperiodism is by altering the phytochrome perception of day length (Mølmann *et al.*, 2005), a phenomenon that has already been shown for photoperiodic induction of flowering in *Arabidopsis thaliana* (Halliday *et al.*, 2003).

Experiments testing the effects of temperature in *Picea abies* suggest that temperature can indeed alter the critical photoperiod inducing growth cessation (Heide, 1974). Heide (2003) suggests that the temperature at the timing of growth cessation and dormancy onset also affects the depth of the following dormancy: *Betula pendula*, *Betula pubescens* and *Alnus glutinosa* induced to dormancy at higher temperatures showed increased chilling requirement and later growth resumption.

2.1.2 Dormancy release

Chilling temperature

The effect of temperature changes dramatically after full dormancy induction: chilling temperatures are in fact the main trigger for dormancy release (Perry, 1971; Sarvas, 1974; Cannell and Smith, 1983; Battey, 2000) and are needed to release the metabolic blocks that prevent bud growth (Crabbé, 1994). The concept of “chilling temperature” is not clearly defined and a consistent response to chilling has not been detected in dormant plants. Different studies differ as to the active chilling temperature range, the optimal chilling temperature and the duration of the exposure to chilling temperatures that is required to restore growth capability. Such differences have not only been recorded among species, but also within the same species. Some studies even report of particular environmental conditions or combinations of factors that trigger dormancy release in unchilled plants (Kawase, 1961; Rageau *et al.*, 1998).

Sarvas (1974) quantified the effects of a range of chilling temperatures on dormant seeds and seedlings of *B. pubescens* and found that temperatures above 10°C had no effect on dormancy release. His results however contrasted with those of Myking and Heide (1995), that later found that exposure to an artificial winter temperature of 12°C was still able to break dormancy in the same species. On the other hand, in a study on *B. pubescens* by Kawase (1961) birch seedlings given long photoperiod (18 h) after dormancy induction at 10 h for 4 weeks were able to resume growth even if receiving a day/night temperature of 21/15.5°C. Experiments on peach (*Prunus persica*) by Erez and Lavee (1971) suggest an optimal temperature for bud dormancy release of 6°C, while for the same species, Richardson *et al.* (1974) indicates more vaguely optimal temperatures between 2.5 and 9.1°C, with temperatures up to 15°C having an effect, even if considered only minimal. Studies and reviews differ as to the upper and lower thresholds of the chilling temperatures active in breaking dormancy. The chilling range generally considered active in most tree species has been indicated alternatively as -5-10°C (Samish, 1954), 0-10°C (Battey, 2000), 0-7°C (Arora *et al.*, 2003).

Chilling duration

The effect of chilling is cumulative: a certain amount of exposure to chilling temperatures has to be fulfilled before budbreak is possible. Thus, chilling exposure has also a

quantitative component to it. After dormancy release, warm temperatures are able to stimulate growth in buds, which burst upon the accumulation of a certain heat requirement (Perry, 1971; Sarvas, 1974; Hänninen, 1990; Battey, 2000). However, if additional chilling is received during this period, the heat requirement for budbreak is reduced until a minimum, after which further chilling does not elicit any effects (Nienstaedt, 1966; Landsberg, 1974; Couvillon and Erez, 1985; Heide, 1993a; Myking and Heide, 1995). Experimental studies have shown that the decrease of the thermal requirements upon exposure to increasing chilling is exponential (Cannell and Smith, 1983; Murray *et al.*, 1989).

Chilling negation

A further complication to the effect of temperature during dormancy is the action of high temperatures (21 and 24°C) applied for short periods during chilling. Erez *et al.* (1979) have shown that such temperatures negated the effect of chilling, and that the amount of the chilling negation depended on the length of the cycle during which high/chilling temperatures were applied. The fact that the effect of chilling applied continuously for 4-9 days was not cancelled by high temperatures suggested that high temperatures negated only chilling occurring during the 20-40 hours prior to the warm spell (Couvillon and Erez, 1985). Hänninen and Pelkonen (1989) found that intermittent warm periods during chilling had a different effect according to their timing, increasing the portion of seedlings for which dormancy was released if they were received in the early (first 3 weeks) stages of chilling exposure and decreasing it if they were received later (after 4-7 weeks of chilling).

Sub-lethal stress

Whereas the main trigger for dormancy release has been traditionally considered to be chilling temperature, a few studies have shown that also freezing temperatures (Rinne *et al.*, 1997) and sub-lethal heat stress (Shirazi and Fuchigami, 1995; Wisniewski *et al.*, 1997) can release endodormancy. Rinne *et al.* (1997) tested the effect of freezing temperatures interruptions (-4.6°C to -20°C) during chilling on seedling of *B. pubescens* and *B. pendula* and found that both percentage and speed of budburst were increased by freezing temperatures, the lower the temperature, the more so. Shirazi and Fuchigami (1995) obtained the same dormancy releasing effect after exposing plants of *Cornus sericea* to a temperature of 47°C for one hour. The exposure to sub-lethal heat stress caused slight

electrolyte leakage and ethylene production, and, in addition to breaking dormancy, decreased the amount of thermal time to budburst (Wisniewski *et al.*, 1997). Wisniewski *et al.* (1997) hypothesised that the mechanism through which extreme heat and cold act on dormancy release is analogous to the effect that dormancy breaking oxidizing chemicals such as hydrogen cyanamide exert on fruit tree buds. Such a hypothesis is supported by the finding that dormancy release coincides with the upregulation of the antioxidant machinery of the plant (Wang and Faust, 1988) and that both dormancy breaking chemicals and extreme temperatures stimulate the production of peroxide scavenging system enzymes (Rowland and Arora, 1997). The biochemical events resulting from these sub-lethal stresses are however the result of a shock treatment and are conceivably different from those through which chilling releases the metabolic blocks of dormancy (Arora *et al.*, 2003).

2.1.3 Quiescence

Similar to dormancy release, temperature is the main factor affecting bud development during quiescence (Romberger, 1963; Sarvas, 1974; Landsberg, 1974; Cannell and Smith, 1983) and acts cumulatively (Richardson *et al.*, 1974; Seeley, 1996; Cannell, 1989; Hänninen, 1990). On the other hand, its effects are the opposite of those it elicits during dormancy: temperatures in the chilling range that during dormancy promote bud development, during quiescence delay development. Sarvas (1974) provided conclusive experimental evidence for a logistic relationship between temperature and the rate of development of flower buds of several tree species. These findings were later supported by Campbell (1978), and are consistent with the hypothesis that during quiescence bud growth capability is restored and responds to temperature similarly to during the growing season (Sarvas, 1974).

The effect of exposure to warm temperatures after dormancy release is cumulative: budburst does not occur immediately upon temperature rising to a certain threshold but after a variable time of exposure to “forcing temperatures” (Richardson *et al.*, 1974; Seeley, 1996; Cannell, 1989). The term “forcing” is employed by horticulturist and budburst modelers and refers to treatments or environmental conditions that are normally able to induce budburst upon dormancy release (see, for example, Sarvas, 1974) or to the

period during which these conditions are active (see, for example, Chuine, 2000 and Kramer, 1994b).

2.2 Photoperiod

2.2.1 Dormancy induction

Day length is an important cue for plants signalling the end of the growing season and the start of winter (Thomas and Vince Prue, 1997). In most of the temperate woody species studied so far, photoperiod affects the duration and the extension of growth and the time at which buds enter dormancy (see Welling *et al.*, 1997; Downs and Borthwick, 1956; Thomas and Vince Prue, 1997; Howe *et al.*, 1995; Håbjørg, 1972). In early studies, four major types of response were identified (Vince-Prue, 1975):

1. Plants that grow indefinitely when exposed to Long Day (LD) condition (for example *Betula pubescens* and *Robinia pseudoacacia*) and enter dormancy rapidly when the day length is shortened.
2. Plants for which dormancy is delayed but not entirely prevented by LD (for example *Acer pseudoplatanus*)
3. Plants for which Short Day (SD) extends the dormant period between successive growth flushes (for example *Pinus sylvestris*).
4. Plants for which the cessation of growth is apparently insensitive to photoperiod. These plants enter dormancy following an endogenous rhythm, like apple tree, pear and cherry.

Photoperiodic ecotypes

The critical daylength (or nightlength) at which dormancy is triggered varies widely among species and also between populations of the same species (Vaartaja, 1954; Downs and Bevington, 1981; Paus *et al.*, 1986; Howe *et al.*, 1996; Thomas and Vince Prue, 1997; Li *et al.*, 2003). Photoperiodic ecotypes have been described for several temperate woody species. For example, northern ecotypes of *Betula pendula* have been shown to have a

longer critical photoperiod and faster height growth in LD-conditions than the southern ecotypes (Li *et al.*, 2003). The critical photoperiod for terminal budset in *Populus trichocarpa* from southern provenance (34°10' and 40°32'N) is 11 hours, as opposed to the 15 hours that have been measured in ecotypes from 53°35'N and 53°50'N (Howe *et al.*, 1995). Leaf abscission in *Salix polaris* from different latitudes (78°N and 69°N) was differently affected by the interaction of temperature and photoperiod, with the more northerly ecotype being more sensitive to high temperature and SD than the southern ecotype.

Ecotypes of Scandinavian trees and shrubs collected from different latitudes also showed differences in their critical daylengths, ranging from an average of 15 hours in ecotypes from 55°N to 23 hours in ecotypes from 70°N (Thomas and Vince-Prue, 1997). It has been shown that in addition to a difference in the sensitivity to day length, different ecotypes also show a difference in the sensitivity to light wavelength. In Norway, two systems for the detection of day length have been shown to co-exist in *Picea abies* populations (Clapham *et al.*, 1998; Mølmann *et al.*, 2003). In southern populations night timekeeping seems to be of primary importance (dark dominance), while northern populations have an increasing requirement for far-red in the day time (light dominance). This difference could be explained with the existence of two different types of phytochrome mediated response, one to photoperiod and the other to light quality (Howe *et al.*, 1996). The increased proportion of far red light towards the end of the day seems to be an important cue to signal the end of the growing season in northern ecotypes which experience very long days during the summer (Mølmann *et al.*, 2003).

Differences in sensitivity to wavelength have been recorded also between different species. For example, while day extensions given with R light were effective for the prevention of dormancy in *Weigela florida*, in *Picea abies* the same treatment was rather ineffective and dormancy was considerably prevented only when FR light was added during the day extension (Vince-Prue, 1984).

Reversibility of short day induced dormancy

In various species the induction of dormancy under short days (SD) has a quantitative character and the depth of dormancy attained depends on the number of photoperiods that are shorter than a critical threshold (Borthwick, 1956; Kawase, 1961; Heide, 1974; Downs

and Crabbé and Barnola, 1996). After visible growth has ceased, dormancy becomes increasingly more difficult to break, until full dormancy is attained some time later (Downs and Borthwick, 1956; Kawase, 1961; Welling *et al.*, 1997). The number of short days necessary to induce dormancy varies among species. A study by Downs and Borthwick testing the response to 8 h days in a wide range of North American tree species (1956), found it ranged from 10 days in *Liriodendron tulipifera* to 20 weeks in *Ulmus americana*. During this induction period, the return to long photoperiods and warm temperatures can cause the resumption of growth (Downs and Borthwick, 1956; Kawase, 1961; Welling, 1997). This has been shown for several species, including *Betula pubescens* (Kawase, 1961; Welling, 1997) *Picea abies* (Heide, 1974), *Catalpa bignoniodes* (Downs and Borthwick, 1956) and *Gleditsia triacanthos* (Crabbé and Barnola, 1996).

2.2.2 Dormancy release and quiescence

Photoperiod has been found to interact with temperature in the resumption of growth. Some species like *Fagus sylvatica* require LD for budburst after chilling has been fulfilled (Heide, 1993b). In other species like *Betula pubescens* and *Betula pendula*, LD appear to substitute partially for the effect of low temperatures on dormancy breaking and shortens thermal time to budburst (Myking and Heide, 1995). This effect is particularly evident in partially chilled plants (Murray *et al.*, 1989; Heide, 1993a; Myking and Heide, 1995). The time to budburst is decreased by LD conditions also in *Populus* and *Alnus* (Thomas and Vince-Prue, 1997), *Pinus sylvestris* (Jensen and Gatherum, 1965) *Picea abies* (Nienstaedt, 1967) *Liquidambar styraciflua* (Farmer, 1968). On the other hand, in *Carpinus betulus* photoperiod seems to have no effect on the breaking of dormancy and the timing of budburst (Heide, 1993b).

2.2.3 Perception of photoperiod

The site of perception of the photoperiodic stimulus seems to be the youngest fully expanded or partly expanded leaves during the induction of dormancy (Kawase, 1961) and the buds during the breaking of dormancy (Wareing, 1954). It is likely that the resumption of growth does not involve a transmissible substance (like in flowering) and depends on local perception and response of individual buds (Crabbé and Barnola, 1996; van der Shoot, 1996).

Fagus sylvatica is influenced by a light dominant mechanism for the prevention of dormancy (the day must be long rather than the night short), and by a night dominant mechanism for the breaking of dormancy (the night must be short rather than the day long) (Thomas and Vince Prue, 1997). The direction of the change of the photoperiod and the age of the plant has also been shown to be important (Partanen *et al.*, 1998; Partanen *et al.*, 2005). *Picea abies* resumed growth after a longer time when exposed to gradually shortening photoperiods from 12 h than when exposed to gradually lengthening photoperiods from 40 minutes to 8 h. However, the delay in growth resumption decreased if the plants were exposed to increasing chilling duration (Partanen *et al.*, 1998). In a study examining the effects of natural chilling and photoperiod in twigs from both old and young *Picea abies*, it was shown that while the material coming from young trees resumed growth if exposed to high forcing temperature and long photoperiod in the early stages of autumn dormancy, the material coming from old trees did not show high levels of budburst at least until the vernal equinox, regardless of the forcing photoperiod (Partanen *et al.*, 2005).

Phytocrome has been known for a long time as the key regulator of light responses in plants. Quantitative trait locus analysis of progeny between poplar ecotypes with differing critical photoperiods for dormancy induction identified two major QLTs, one of which is mapped to a phytocrome-encoding gene (Frewen *et al.*, 2000). Transgenic hybrid aspens overexpressing oat Phytocrome A (PhyA) gene were not able to arrest their growth under short days and did not respond with variations in their ABA and dehydrin proteins levels to changes in daylength (Welling, 2002, 2003). According to Welling (2002, 2003), the overproduction of oat PhyA resulted in the impairment at the very beginning of a signalling cascade in dormancy development, leading to numerous physiological alterations among which there was also a failure to develop cold acclimation. These findings suggest that PhyA plays a role in perceiving the short daylength signal that initiates growth cessation and development in woody plants (Welling, 2002, 2003). PhyA has also been linked with day timekeeping in “light dominant” southern populations of *Picea abies*, relying on the perception of far-red light during the daytime for the regulation of dormancy onset (Mølmann *et al.*, 2005).

2.3 Light intensity

Light intensity has received relatively minor attention in studies investigating dormancy release, if compared with other environmental factors such as temperature and photoperiod. A study on *Picea abies* testing the effect of two levels of photon flux density on dormancy release, showed that low photon flux density increased the variance of the frequency distribution of the chilling requirement (Höyhty and Hänninen, 1991). Earlier, Chandler *et al.* (1937, as cited in Höyhty and Hänninen, 1991) reported that high photon flux density during chilling delayed budburst, probably by indirectly warming the bud and thus decreasing the effectiveness of the chilling temperatures (Chandler *et al.*, 1937 as cited in Höyhty and Hänninen, 1991). The warming effect that absorbed radiation energy has on buds has been verified by Repo *et al.* (1991, as cited in Partanen *et al.*, 2001), who found that levels of light intensity even below $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ increased the bud temperature by 2°C compared to air temperature. Such a warming effect has been reported as a possible cause of overestimation of the effects of photoperiod in advancing budburst timing (Repo *et al.*, 1991 as cited in Partanen *et al.*, 2001). Thus, the only effects on budburst timing that present evidence attributes to light intensity are indirect ones, through a change in temperature.

2.4 Nutrient availability

Few studies have focused on the effect of nutrient availability and elevated CO_2 on phenology, but they generally agree on the fact the phenophases most affected by nutrient levels are growth cessation and leaf senescence, and while improved nutrient availability delays them, increased atmospheric $[\text{CO}_2]$ may cause these phenophases to occur earlier (Sigurdsson, 2001; Roberntz, 1999; Mugasha and Pluth, 1999).

For example, *Populus trichocarpa* clones exposed to elevated atmospheric CO_2 concentrations, at natural and high nutrient availability did not show any change in their timing of budbreak or the start of shoot extension in spring (Sigurdsson, 2001). However, their time of annual growth cessation was affected by both factors: while leaf senescence was delayed by improved nutrient availability, elevated $[\text{CO}_2]$ caused senescence earlier than expected (Sigurdsson, 2001). An experimental study by Roberntz (1999) that tested the effects of elevated CO_2 and nitrogen supply on the growth of *Picea abies* branches confirmed that elevated atmospheric $[\text{CO}_2]$ and nitrogen availability had no effect on budbreak. On the other hand, Mugasha and Pluth (1999) confirmed the senescence-

delaying effect of elevated nutrient levels in a field experiment on *Larix laricina* exposed to N-fertilization in an undrained peatland.

The only study suggesting a possible effect of nutrients on budbreak timing is a correlation analysis between various environmental factors and the phenophases observed in different plant species at several sites in Norway (Wielgolaski, 2001). In this study, the amount of ions (P, K, Mg and Ca) was found to be negatively correlated with the developmental rate during spring phenophases of most species, thus possibly indicating that a high nutrient level delayed growth resumption.

2.5 Water availability

Water availability is a very important factor for plant growth, and is thus also expected to affect the timing of phenophases. However, the few experimental studies and observations that have been conducted so far do not highlight a direct effect of water on the onset of phenophases, but rather an indirect one, through the role that water plays in the general balance of the plant's metabolism. For example, while the growth of some tropical plants is known to be triggered by increased moisture availability (Reich and Borchert, 1984) some xerophytes are known to be inhibited by waterlogging. A study conducted by Khan *et al.*, (1996), on *Pseudotsuga menziesii* exposed to 6 different levels of water availability, highlighted that bud development was faster under moderate (23 to 59%) soil water contents.

However, as different species are adapted to different environmental conditions and have different moisture requirements, possibly the same moisture regime will affect the growth of different species in a different way. This was shown even for different provenances of the same species, by a study testing the effect of different moisture regimes on growth of *Fagus sylvatica* (Nielsen and Jørgensen, 2003). Whereas the north European provenances grew very slowly for high soil water contents (80% of field capacity), the same moisture regime optimised the growth of south European provenances. On the other hand, despite the effect that genotype had on the optimal water requirements for growth, the timing of spring and autumn phenophases in relation to moisture regime was not affected by the different provenances, and growth cessation was uniformly earlier in low soil moisture, (up to 25 days earlier in the treatment with soil moisture at 38% of field capacity). Thus, the

same moisture regime that resulted in optimal bud development for *Pseudotsuga menziesi* induced water stress in *Fagus sylvatica*.

Betula pubescens is another species known to be particularly well adapted to high soil moisture (Atkinson, 1992). A study by Wielgolaski (2001) confirmed that the timing of *Betula pubescens* budbreak at various Norwegian sites was in fact negatively correlated with increased bulk density of the soil and increased precipitation, both factors pointing to an increased moisture availability. Other species that showed an earlier budbreak in relation to increased moisture availability were bird cherry (*Prunus padus*) and rowan (*Sorbus aucuparia*) (Wielgolaski, 2001).

3. Theories on dormancy breaking

The mechanisms of bud dormancy are still unknown. Many theories have been formulated over the years. In the 1960s, theories that stressed the metabolic restraint (O_2 reduction) on buds during dormancy (Vegis, 1964) were replaced by a general tendency to believe that dormancy initiation and release were controlled by the balance between inhibiting and stimulating substances. However, decades of studies that monitored endogenous levels of hormones within whole buds, leaves, stems, cambium and roots in controlled conditions were not able to provide clear-cut evidence to support this hypothesis (Arora *et al.*, 2003). Recent reviewers now admit that such a linear view is too simplistic and that precise correlations between hormone levels and dormancy stages are rare (Crabbé and Barnola, 1996). Indeed, many researchers investigating the physiology of dormancy now believe that dormancy is a complex, dynamic phenomenon, involving many parallel and concomitant pathways (van der Shoot, 1996; Arora *et al.*, 2003, Ross, 1996; Horvath *et al.*, 2003; Lang *et al.*, 1987). The awareness of the multiplicity of the controls and mechanisms regulating dormancy has resulted in different approaches being adopted in recent studies on dormancy (Arora *et al.* 2003).

One approach considered the possible role of calcium and calmodulin as messengers in seed dormancy release (Ross, 1996). Ross (1996) observed that chilling exposure was accompanied in hazelnuts by the rise in calcium and calmodulin dependent enzymes, such as nicotinamide adenine dinucleotide (NAD) kinases. He hypothesized that increase in

these enzymes could be linked with the also observed increase in the rate $(\text{NADP} + \text{NADPH})/(\text{NAD} + \text{NADH})$ which would then enable the activation of the pentose phosphate pathway, at the basis of the biosynthetic reactions preceding germination.

Another approach concentrated on the change in the water status of the buds. Faust *et al.* (1991) showed that during dormancy water is found in a bound state and becomes freer at the end of dormancy. The researchers hinted at the possibility that the state of cellular water could control dormancy release. Welling *et al.* (1997) reported that SD decrease bud water content and stimulates the production of dehydrin-like proteins, which are usually associated with cell dehydration and protect the cell against dehydration stress. However, further studies on peach by Erez *et al.* (1998) suggested that bound water is probably more related with the level of cold resistance than the level of dormancy.

A recent line of investigation involves the study of the changes in plasmodesmatal connections at the apical meristem during dormancy in trees (Rinne *et al.*, 2001; Rinne and van der Schoot, 1998). Reports by Rinne (2001) and by Rinne and van der Schoot (1998) showed that in *Betula pubescens* the plasmodesmatal connection at the apical meristem are shut down by 1,3- β -D-glucan in response to SD dormancy induction. Rinne *et al.* (2001) hypothesized that chilling results in the production of gibberellins (GA) that in turn activate the production of glucanases restoring plasmodesmatal connections. This theory is in line with previous observations that GA induces dormancy release in buds and promotes glucanase genes transcription in tobacco seeds (Arora *et al.* 2003).

4. Ecology and physiology of *Betula pubescens*, *Fagus sylvatica*, *Salix aurita*, *Salix x smithiana* and *Tilia cordata*

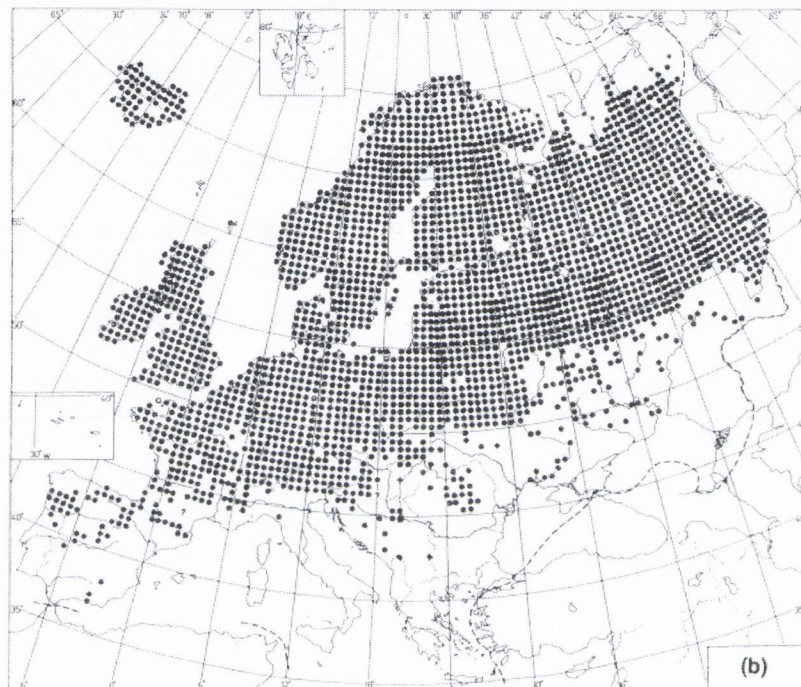
4.1 *Betula pubescens*

Betula pubescens Ehrh. (pubescent birch) is a very common tree species found throughout northern Europe and Russia (Figure 1) (Atkinson, 1992).

B. pubescens grows in a wide range of soil conditions and is very tolerant of heavy, moist and nutrient poor soil, where other species would not survive. These characteristics, as well

as its intolerance of shade make it a colonizer of bare areas and fellings. Shade intolerance is also the reason why *B. pubescens* fails to establish itself effectively even in the lowest vegetation (Kinnaird, 1974). The poor ability of young birch seedlings to penetrate the canopy is linked to the limited height they are capable of reaching while relying only on the food reserves of the seed: 2 cm compared with the 8 cm of *Pinus sylvestris* (Miles and Kinnaird, 1979).

Figure 1. The distribution of *Betula pubescens* in Europe. Reproduced from Jalas and Suominen (1976)



4.1.1 Environmental control of growth

Light

In shade, birch leaves almost cease their growth, while in open light their growth rate increases considerably (Taylor and Davies, 1985). Håbjørg (1972) studied the effects of photoperiod on *B. pubescens* and reported that apical growth increased with increasing photoperiod while growth cessation and dormancy were triggered by Short Day (SD). The light-sensitive extensibility of the cell walls of birch appears to be an important factor determining the response of this species to light (Taylor and Davies, 1988). Experimental studies demonstrated that cell-wall extensibility in *B. pendula* increases rapidly after an

increase in photosynthetically active radiation (PAR). These mechanisms could also affect *B. pubescens*.

B. pubescens can take advantage of high light irradiances due to its high photosynthetic efficiency (Davies and Pigott, 1984). However, a continuous supply of water must be provided for it to be maintained at optimal levels, as this species is very sensitive to water stress due to its shallow root system (Davies and Pigott, 1984).

Temperature

Håbjørg (1971) reports that the optimal constant temperature for height growth in *B. pubescens* from Scandinavian and Danish provenance is 18°C. Higher temperatures, such as 21 and 24 °C resulted in a decrease in height growth and a slow down in the rate of increase in dry weight. The same study indicated that fluctuating day/night temperature enhanced shoot growth when compared to constant temperature, particularly in birches from high latitudes. Maximal shoot growth in populations from 70°N and 63°N was obtained at a day/night temperature of 18/13°C and at constant 18°C for a population from 56°N (Håbjørg, 1972). The study of tree rings in Scandinavian birches growing at the treeline has suggested that the minimum mean June-September temperature for growth in *B. pubescens* was 7.0-7.8°C (Kullman, 1993).

Nutrition

The optimum proportions of mineral nutrients needed for maximum growth in *B. pubescens* were: 100N: 65K: 13P: 7Ca: 8.5 Mg (Ingestad, 1970, 1971). Fertilization with N, P and K has been shown to improve the establishment of young seedlings naturally colonizing a cleared peat area (Kaunisto, 1981). Seedlings of birch that were grown under conditions of nitrogen deficiency continued to grow, even if slowly, for at least 6 weeks, although lateral buds did not form shoots as in the control group and the leaves showed chlorophyll deficiency (Horgan and Wareing, 1980).

4.1.2 Environmental triggers of dormancy and growth resumption

Depending on the climate of the area where *B. pubescens* is growing, budbreak and leaf unfolding occur between early March and May. The female flowers emerge in spring,

before the leaves, and are usually receptive one day before the male flowers on the same tree shed pollen (Sarvas, 1952, as cited in Atkinson, 1992). Seeds are ripe between July and August and are shed from July onwards, with a peak in the months of September and October (Miles and Kinnaird, 1979). The male catkins are developed between July and September although they do not release pollen until the following year within one or two days of anthesis (Sarvas, 1952, as cited in Atkinson, 1992). Vegetative growth ceases in August and the majority of leaves are shed between September and October, with trees usually being completely bare by December. Phenological data from *B. pubescens* clones of german provenance and growing at 14 german stations indicates that there are a constant number of days between leaf colouring and leaf fall, which is about 17 days (Kramer, 1995).

Many studies have been conducted on the environmental triggers of phenological phases in *B. pubescens*, focusing on the control of dormancy and growth resumption in this species (Downs and Borthwick, 1956; Kawase, 1961; Håbjørg, 1972; Sarvas, 1972, 1974; Junttila, 1980; Myking and Heide, 1995; Myking, 1997; Welling *et al.*, 1997; Rinne *et al.*, 1994, 1997; Heide, 1993a; Heide, 2003; Junttila, 1980).

B. pubescens, like many other temperate woody species, is induced into dormancy by the gradually shortening photoperiod occurring at the end of summer (Wareing, 1956; Rinne *et al.*, 1994; Welling *et al.*, 1997). Experiments conducted on *B. pubescens* showed that half an hour light interruptions in the middle of the night, during SD treatment, were sufficient to prevent dormancy (Kawase, 1961) thus suggesting that the detection of photoperiod during dormancy induction in *B. pubescens* is based on nightlength rather than daylength. The comparison of the phenology of trees of different origins has shown that the critical daylength for growth cessation in *B. pubescens* varies depending on the latitude of origin and is generally longer for birch populations from more northerly sites (Håbjørg, 1972; Heide, 2003). The existence of photoperiodic ecotypes could be explained by the adaptive advantage brought by an earlier dormancy in response to a higher critical photoperiod for northerly birches exposed to longer and harsher winters (Li *et al.*, 2003) and by the fact that before the autumn equinox days are longer at northern latitudes.

The induction of dormancy of *B. pubescens* under short days (SD) has a quantitative character: the depth of dormancy attained depends on the number of photoperiods that are

shorter than a critical threshold (Kawase, 1961). After visible growth has ceased, dormancy becomes increasingly more difficult to break, until full dormancy is attained some time later (Kawase, 1961). The length of dormancy induction under SD in *B. pubescens* was estimated to be 2-4 weeks at 10 h photoperiod by Kawase (1961). However, the lack of a general agreement on the minimum number of SD cycles for full dormancy attainment has resulted in different dormancy-inducing experimental conditions being applied in different studies on *B. pubescens*, with SD treatments lasting 3 weeks (Welling *et al.*, 1997), 5 weeks (Heide, 2003; Myking and Heide, 1995) or 7 weeks (Rinne *et al.*, 1994). Even though photoperiod plays a major role in dormancy induction (Arora *et al.*, 2003), temperature also affects the rate of growth cessation and might enhance the effect of SD. Low constant temperatures (Downs and Borthwick, 1956; Junttila, 1980), and low night temperature associated with high day temperatures (Håbjørg, 1972) have been found to advance dormancy onset in *B. pubescens*. Recent biochemical studies on dormancy induction in this species have indicated that short photoperiod induces bud dehydration and the production of dehydrins, proteins that protect the cell against dehydration damage (Welling *et al.*, 1997).

Chilling is required by *B. pubescens* for dormancy release and growth resumption, even though many studies suggest an early dormancy release and a low chilling requirement in this species (Heide, 1993a; Sarvas, 1974; Kawase 1961; Myking and Heide, 1995). Heide (1993a) showed that *B. pubescens* is already completely released from dormancy in December. Despite the focus placed by experimental studies on chilling temperatures between 0 and 10°C (Sarvas, 1974) or 0 and 12-15°C (Myking and Heide, 1995), dormancy in this species has also been found to be broken effectively by the exposure to short periods of freezing temperature, the lower the more effective (Rinne *et al.*, 1997).

The thermal time requirements in birch depend on the amount of chilling already accumulated, decreasing as chilling progresses (Myking and Heide, 1995; Heide, 1993a). The effect of temperature however does not seem to be affected by diurnal oscillations (Myking, 1997) so that thermal time requirements for budburst in this species can be adequately approximated using the 24 h arithmetic mean. Sarvas (1972) provided conclusive experimental evidence for a sigmoidal relationship between temperature and the rate of development of flower buds of several tree species, including *B. pubescens*, which is also likely to be the case also in vegetative buds over the same temperature range.

During dormancy release and quiescence, photoperiod also affects the amount of time to budburst in *B. pubescens*. In many studies, long days have been found to enhance budburst and to shorten the time to budburst whereas short days appear to inhibit budburst (Myking and Heide, 1995; Heide 1993a; Kawase, 1961). Myking and Heide (1995) noted that the dormancy releasing effect of LD decreased with chilling duration, indicating that after full dormancy release, time to budburst in birch depended solely on temperature. The growth inhibiting effect that SDs have on partially chilled buds has probably a stabilizing effect on budburst during warm spells in the middle of the winter, and avoids growth resumption when frost risk is still high (Myking and Heide, 1995).

4.2 *Fagus sylvatica*

Fagus sylvatica L. (European beech) is a very common tree species growing central Europe and in mountainous areas in the south of Europe (Figure 2). Its distribution is under clear climatic control, being limited by its buds resistance to winter cold at its eastern limits, by late frosts at its western and northern limits and by drought at its southern limits (Peters, 1997). *F. sylvatica* can be generally found on well drained soils, and tends to form dense and shady forests where it is the main tree species dominating the canopy. *F. sylvatica* is a late succession, shade tolerant tree species, strongly competitive with respect to most other tree species in well drained soils and oceanic climates with plenty of precipitation (Peters, 1997). On the other hand, waterlogging appears to have a negative effect on its growth. Dreyer (1994) demonstrated that the growth of beech stopped completely when the soil was waterlogged.

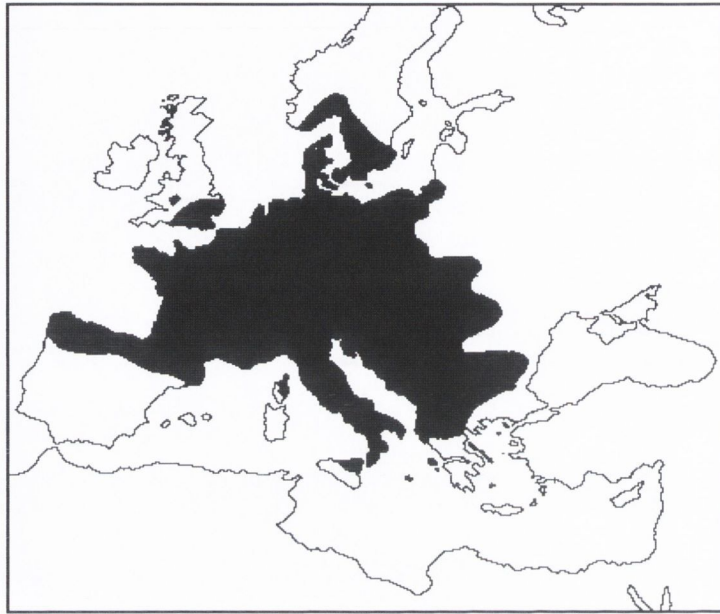
4. 2.1 Environmental control of growth

Light

F. sylvatica is tolerant of shade and can survive at very low levels of light irradiance for several years (Peters, 1997). Harbinson and Woodward (1984) showed that the photosynthetic rate of shade leaves of *F. sylvatica* were saturated at irradiances of only about 1% of the maximum incident on the canopy on a sunny day. *F. sylvatica* seedlings survive under reduced light conditions with reduced growth rates and adapted leaf morphology by reducing the mesophyll thickness. When light levels increase, this species quickly increases its growth rate and changes its leaf morphology (Harbinson and

Woodward, 1984). The dense shade beeches cast eliminates less shade tolerant competitors (Peters, 1997).

Figure 2. The distribution of *Fagus sylvatica* in Europe. Source: www.pennine.demon.co.uk/Arboretum/Fasy.htm (accessed July 2006).



Temperature

Beech grows well in temperate climates (Peters, 1997). During leaf unfolding its leaves are very vulnerable to late frosts, which can cause seed production to fail and seedlings to die (Becker, 1981). During the summer, it requires fairly warm mean temperatures to grow. If these are too low, beech has insufficient surplus production and declines (Nomoto, 1964). Huntley *et al.* (1989), through the analysis of palynological records showed that in Europe and America beech pollen percentages were related to mean January and July temperature and had optima at -1 to -4°C in January and 18°C in July.

Nutrition

F. sylvatica occurs over a wide range of mesic soils, with a pH ranging from 3.5 to over 7. In central Europe beech dominates the major and central part of the moisture and nutrient range in forests (Ellemberg, 1988). Soil nutrients improve beech growth rates in the shade (Peters, 1997). However, the positive action of improved nutrient availability is visible only when moisture conditions are not limiting: in France, Teissier du Cros *et al.* (1981) showed

that the growth of beeches was determined by water availability more than nutrient availability of the site.

4.2.2 Environmental triggers of dormancy and growth resumption

Depending on the climate of the area where *F. sylvatica* is growing, budbreak and leaf unfolding occur between the end of April and May. Leaf colouring and leaf fall occur in September and October (Brügger and Vassella, 2003).

Under favourable moisture conditions, the length of the growing season of *F. sylvatica* increases with an increase in temperature (Peters, 1997). Kramer (1995) showed that higher mean temperatures corresponded to earlier beginning of the growing season but did not affect leaf fall. On the other hand, moisture availability appeared to affect growth cessation but had no effect on flushing. Nielsen and Jorgensen (2003) tested the phenology of beeches of different provenances under different soil water treatments and concluded that while low water regimes advanced the timing of growth cessation by up to 25 days, the timing of flushing was unaffected by this factor.

After the development of full dormancy, a period of chilling temperatures is required by beech for dormancy release (Falusi and Calamassi, 1990; Heide, 1993b). However, until late October, beech is in a predormant state during which growth resumption is possible if it is exposed to long photoperiods (Heide, 1993b).

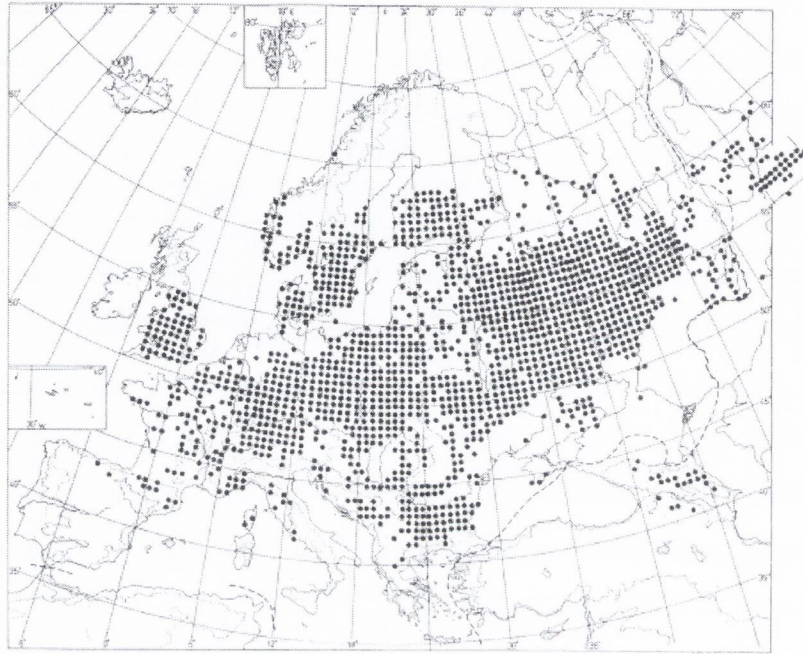
A study conducted by Heide (1993b) suggests that beech might require long days in addition to chilling for normal budburst. Fully chilled *Fagus sylvatica* twigs brought indoors and placed in growing cabinets at 21°C at an 8 h photoperiod showed incomplete budburst and took three times longer to flush than twigs in a 24 h photoperiod.

4.3 *Tilia cordata*

Tilia cordata Mill. (lime) is widely distributed throughout the deciduous forests of the temperate lowlands of Europe and Russia (Figure 3). The eastern limit of its distribution extends into Siberia (Pigott, 1991). In the southern part of its range, in the Mediterranean region, it typically grows in mountainous regions and near rivers that do not dry during the

summer, as it prefers moist and heavy soils and is fairly intolerant to periods of extended drought (Pigott, 1991).

Figure 3. The distribution of *Tilia cordata* in Europe. Reproduced from Pigott (1991).



T. cordata is a typical dominant species of climax forests: its seedlings are tolerant of shade and can continue growing under a closed canopy, it can reach a height of 30 m (more than most other competitors) and it is long lived (Horn, 1971). However, there is evidence that this species is outcompeted by *F. sylvatica* on well drained soils in regions of high rainfall (more than 800 mm), where *T. cordata* establishes successfully only in waterlogged patches (Pigott, 1991). This species is also very sensitive to human disturbances and clearing: in England human activity has been partly responsible for the gradual decline of this species, which was very abundant in the past (3000 b.c.), as inferred from palynological records (Pigott and Huntley, 1981). The vulnerability of this species to exploitation is due to its frequent failure to regenerate from seed, so that once it is destroyed it does not readily re-establish (Pigott, 1991).

4.3.1 Environmental control of growth

Light

T. cordata is a shade-tolerant species. In a comparative field study of the photosynthetic capacity of *Quercus robur*, *Fraxinus excelsior* and *T. cordata*, *T. cordata* showed typical features of low light acclimation, with high photosynthetic rates at the top of the canopy and efficient light capture per unit of area under the canopy (Kazda *et al.*, 2000). *T. cordata* and *Betula pendula* grown in pots and exposed to shaded and non-shaded treatments showed typical responses of a late-succession dominant species and a colonizing species of open spaces (Davies and Pigott, 1982). Their growth rate was similar in the shaded treatment but in the non-shaded one *B. pubescens* had a significantly higher growth rate and after one month had a larger biomass and height (Davies and Pigott, 1982). However, field observations confirmed that, in contrast to *T. cordata*, birch soon declined and died off when overtopped (Pigott, 1991).

Temperature

T. cordata is very resistant to winter cold and can remain undamaged in the extreme winter temperatures of the Russian plains. It also escapes late winter frosts due to its relatively late timing of budburst and its early leaf fall (Pigott, 1991). However, in order for its fruits to be fertile, it requires a fairly warm summer: in Britain, its absence from Scotland has been related to a failure to regenerate through seed due to the low summer temperatures (Pigott and Huntley, 1981). The northern limit of its distribution at present correlates with the July isotherm for the mean daily maximum temperature of 19-20°C (Pigott, 1991).

Nutrition

Despite the fact that *T. cordata* grows on a wide variety of soils, it has been shown that fertilization increases its height and weight. This response was shown mainly to increased levels of K and N, while P did not result in an increase in biomass (Pigott, 1991).

4.3.2 Environmental triggers of dormancy and growth resumption

Depending on the climate of the area where *T. cordata* is growing, budbreak and leaf unfolding occur between the end of April and May (Brügger and Vassella, 2003). Flowering occurs between June and July and fruits ripen between August and September.

Leaf colouring and leaf fall of some of the leaves start as early as August (Brügger and Vassella, 2003), these phenophases are completed later in the autumn.

According to Pigott (1991), a period of 6-8 weeks with temperatures below 5°C was necessary for *T. cordata* to break dormancy. Wilson *et al.* (2002) reported that *T. cordata* seedlings receiving only 200 chilling hours (hours at temperatures < 7.6°C) showed erratic budburst and eventually declined and died. However, a progressive increase in the hours of chilling from 200 to 1200 lead to a gradual decrease in the number of hours at a temperature > 22.2°C required for budburst (Wilson *et al.*, 2002). The chilling requirement of *T. cordata* is usually fulfilled by the end of January, after which increasing temperatures advance the time of budburst (Pigott, 1991). After transfer of chilled plants into growing cabinets maintained at a 12 h photoperiod, the number of days to budburst decreased from 78 to 28 with an increase in temperature from 8 to 22°C (Pigott, 1991).

4.4 *Salix aurita* and *Salix x smithiana*

The genus *Salix* comprises approximately 400 species and more than 200 listed hybrids (Newsholme, 1992). Willows are pioneer woody plants that commonly occur on floodplains and riverbanks (Verwijst, 2001). They have an ability to tolerate repeated disturbances and after being coppiced, new shoots sprout from the stump, forming a stool (Sennerby-Forsse *et al.*, 1992).

S. aurita L. is a locally common shrub of acid heaths, woods and moorlands in Scotland, Ireland and Wales. It is common on barren soils and lower slopes in these regions, while absent at altitudes above 800 m (Meikle, 1984). In continental Europe it is widely distributed in central and northern regions, eastwards to the limits of European Russia (Meikle, 1984).

S. x smithiana Willd. is a very common *Salix* hybrid deriving from a cross between *S. cinerea* x *S. viminalis* and widespread in continental Europe and in Britain and Ireland (Meikle, 1984). Like most *Salix* species it grows well in most soils, including wet, ill-drained or intermittently flooded soils, but prefers a damp, heavy soil in a sunny position (Huxley, 1992). It rarely thrives on chalk and is shade intolerant (Huxley, 1992).

Willows are notably shade intolerant species and do not generally grow under a closed canopy. Junttila (1980) showed that *S. pentandra* responded to low levels of light irradiance by stopping apical growth, but that this response depended on the latitude of origin of the clone, with northern clones exhibiting a faster response.

4.4.1 Environmental triggers of dormancy and growth resumption

In Great Britain and Ireland budbreak and leaf unfolding occur in March-April for *S. x smithiana* and in April and early May for *S. aurita*, with catkins appearing in advance of the leaves for both species (Meikle, 1984). Leaves are shed between October and November.

Growth cessation and dormancy are developed by *Salix* in response to short photoperiods. However, low temperature appears to enhance dormancy induction (Lennartsson, 2003). Lennartsson (2003) reports that *Salix* flushed even in short photoperiods if previously exposed to a dormancy induction temperature as high as 15°C, suggesting that low temperature is necessary to induce dormancy. Once dormancy is attained, the state of chilling influences the amount of thermal time required for budburst. Murray *et al.* (1989) measured the thermal times to budburst in a set of tree species growing in Britain including *S. viminalis*, and found that this species does not have a large heat requirement in comparison with late flushing species such as *F. sylvatica* (Murray *et al.* 1989). Its chilling requirement also appeared to be fulfilled relatively early (Murray *et al.* 1989). The threshold temperatures for the accumulation of heat units in *S. viminalis* were investigated by Lennartsson (2003), who found that they differed very little among clones and were all around 1.0-1.2°C.

METHODS

1. The plant material

The plant material utilized in this study was taken from the trees growing at the International Phenological Gardens (IPGs). The IPG network was established in 1959 to collect phenological data from sites across Europe (Chmielewski and Rötzer, 2001). In all gardens genetically identical trees and shrubs are planted in order to make large-scale comparisons among the timing of different phenophases of plants. The original idea behind this network was to make large-scale and standardized phenological observations. For the purpose of obtaining easily comparable data, all gardens are situated in similar surroundings (mainly flat areas with meadows and some trees), have detailed phenological observation-guides and are professionally looked after by the staff of the institutes, botanical gardens *etc* where they are located. At the moment, 69 IPGs are present in 19 European countries (Figure 4). The cuttings for the propagation were taken at the IPG located in the John Fitzgerald Kennedy arboretum (IPG N°14, Co. Wexford) unless otherwise indicated.

2. Species selection

The species selected for our study were *B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana* and *T. cordata*. The selection of these species was made on the basis of their presence in the Irish IPGs, where we took the cuttings for the propagation (Figure 5), and on their ease of propagation.

S. aurita and *S. x smithiana* were chosen because they are relatively easy to propagate and vigorous growers, *B. pubescens*, *F. sylvatica* and *T. cordata* were chosen because they are common to all Irish IPGs and were, for this reason, already selected as biological indicators of climate change in a previous study funded by the Irish Environmental Protection Agency (EPA) (Sweeney *et al.*, 2000). Not all species are present in all studies. The selection of the species used in each experiment was made on the basis of the availability of the plant material and on the information already available on the species phenology. For example,

after testing the interaction between chilling duration and forcing photoperiod in experiment 3.a, the two species that revealed most responsiveness to photoperiod (*B. pubescens* and *F. sylvatica*) were selected to conduct experiment 3.b, in which a wider range of forcing photoperiods was tested after varying chilling duration. However, only the results for *B. pubescens* are reported, due to the fast dessication of the *F. sylvatica* twigs during the experiment. Similarly, experiment 1.b, testing the effect of nutrient availability on budburst timing, did not involve *S. aurita* whose clones were not available at the time of the experiment, nor *F. sylvatica* that had been grafted on non-IPG rootstock coming from different genotypes and was thus not homogeneous as to its nutrient-absorbing capacities.

In the later phases of the study we decided to concentrate on the phenological responses of only one species, *B. pubescens*, which was also the subject of the modelling study. *B. pubescens* was of particular interest because it has already been the subject of numerous phenological studies (Sarvas, 1972, 1974; Kawase, 1961; Håbjørg, 1972; Heide, 2003; Myking and Heide, 1995; Welling *et al.*, 1997; Rinne *et al.*, 1994) and because it is widespread in northern Europe (Atkinson, 1992), where climate is warming at a fast rate (IPCC, 2001).

Figure 4. Map of the locations of the IPGs across Europe. Source: http://www.agrar.hu-berlin.de/pflanzenbau/agrarmet/ipg_net.html (accessed July 2006).

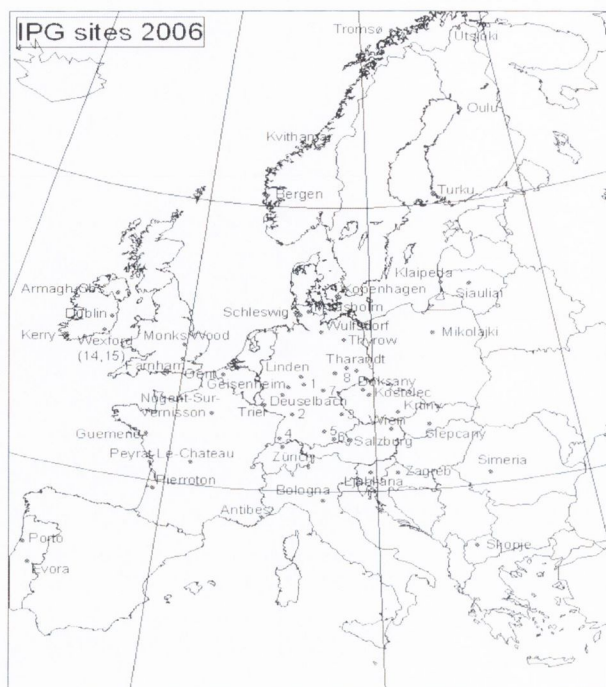


Figure 5. *B. pubescens* IPG clone at the John Fitzgerald Arboretum (IPG N°14, Co. Wexford).



3. Vegetative propagation

Plants can regenerate either sexually (through seeds or spores) or asexually. The latter form of regeneration is defined asexual or vegetative propagation and is a process where a new plant is produced from plant parts such as leaves, stems, buds, or roots. This form of replication produces plants or clones that are genetically identical to the mother plant, from where the plant part was taken (Landis *et al.*, 1999). In horticulture, vegetative propagation is achieved through various techniques to obtain identical plants with useful or amenable characters (cultivars) or to reproduce species that are difficult to propagate by seeds. The propagation techniques used in this study are propagation by stem cuttings and propagation by grafting.

3.1.1 Vegetative propagation by cuttings

Vegetative propagation by stem cuttings involves the use of detached vegetative plant parts which, upon being exposed to conditions favourable for regeneration, will develop into a complete plant, similar in all characteristics to the parent plant (Hartmann and Kester, 1983). During vegetative propagation, the cambium cells of the cutting will form new meristematic growing points and differentiate into adventitious roots that by developing will produce independent plants (Hartmann *et al.*, 1997).

Cuttings can be made from a variety of vegetative parts of a plant, such as stems, rhizomes, tubers, corms, bulbs, leaves or roots (Hartmann and Kester, 1983). According to the type and maturity of the tissue (Hartmann *et al.*, 1997), stem cuttings can be classified as hardwood, semi-hardwood and softwood cuttings. Hardwood cuttings consist of mature woody tissue of last season's growth, and typically are collected during the dormant period. Semi-hardwood cuttings are collected from the semi-lignified tissue of actively growing plants and require specialized propagation environments, such as a greenhouse with mist or a rooting chamber. Softwood cuttings are collected from the soft succulent new shoots during the growing season, cannot be stored and must be carefully handled. As softwood cuttings are very sensitive to desiccation, a mist bed or rooting chamber and treatments with hormones are usually necessary (Landis *et al.*, 1999).

The establishment of the cutting depends on a variety of factors, including the part of the shoot from where the cutting was taken, the size of the cutting, the health and age of the

parent plant, the use of rooting hormones, the rooting medium, the conditions (temperature and moisture) of the propagation environment and the season in which the cutting was taken (Hartmann and Kester, 1983; Wilson, 1993).

3.1.2 Vegetative propagation by grafting

Grafting is a very precise technique that involves physically binding two plant parts together so that they will establish a vascular connection and grow into a single individual. The upper portion of the graft that will develop into the new shoot system (**the scion**), is bonded to the **rootstock**, which is an established plant of the same or a closely related species (Landis *et al.*, 1999). The healing of the tissue between the two structures creates a meristematic area and a callus, that subsequently differentiates into a vascular cambium. This will in turn produce the vascular system of the new individual (Fabbri *et al.*, 2004) that will enable the scion to obtain water and minerals from the rootstock. The events that lead to a successful graft are the adhesion of the rootstock to the scion, the formation of a “callus bridge” at the graft interface and the differentiation of the callus into vascular tissue (Fabbri *et al.*, 2004).

3.2 Propagation of *B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana* and *T. cordata*.

3.2.1. Propagation of *B. pubescens*.

Summer 2003. On the 23rd June 2003, in the late morning (between 11 am and 12 am), 100 softwood shoots 20-30 cm long were cut from the bottom third of the crown of the parent plant, wrapped in wet newspapers and immediately placed in plastic bags, in order to prevent water loss during the return trip to the Teagasc Horticultural Center in Kinsealy (Dublin), where propagation took place. Upon arrival, the cuttings were transferred into the cold room (6°C), where they were left for a night, and on the following morning they were divided into a total of 200 cuttings 8-10 cm long and with a diameter of approximately 2 to 4 mm. The cuttings, whose basal part had been cut at an angle to increase the absorbing surface, were taken either from the base (basal cuttings), or the apex of the shoot (apical cuttings). Working on the humid propagation bench, bottom leaves were stripped off, leaving only the top two-three leaves. The cuttings were then washed in a fungicide (Captan powder, 48.9% N-trichloromethylthion ~ cyclohexene -1,2- dicarboximide)

solution and dipped in rooting hormone powder (Doff hormone powder, 0.4% NAA). Excess rooting powder was tapped off before planting. In order to avoid excessive brushing of the powder before planting, a trench was made in the rooting medium with a stick. The cuttings, were then planted directly into the moist rooting medium (1/3 perlite and 2/3 peat) to a depth of 2-3 cm, in propagating containers 40 x 25 x 10 cm (width, breath and depth), each holding 40 cuttings before being transferred into the mist propagation units, where they received fine water mist several times each hour until they rooted (Figures 6 and 7). During this period, the dead leaves were periodically removed from the containers to avoid fungus formation.

On the 29th September, (14 weeks after the start of the propagation), the containers were removed from the direct action of the mister and transferred to a less humid part of the mist unit, in order to gradually acclimate to a drier regime 4 days before transplanting into pots.

On the 4th October 2003 the 98 cuttings that had rooted (49% propagation success) were finally transplanted into 11 plastic pots filled with John Innes N^o3 compost and transferred into a thermostatically-controlled glasshouse, where the temperature never fell below 12°C to avoid chilling effects. The cuttings remained in these conditions until the beginning of the first experiments on the 11th November 2003.

Summer 2004. On the 30th June 2004 between 2 and 3 p.m., 200 softwood shoots were taken from the parent plant following the procedure adopted the previous year (described above). Similar to the procedure adopted in 2003, the shoots were stored for one night in the cold room (set at 4°C), and divided into 300 cuttings which were planted in rooting medium the following day. The cuttings remained in the mist unit for 14 weeks and dead leaves were removed periodically.

Figure 6. Propagation of B. pubescens (bottom) and T. cordata (top).

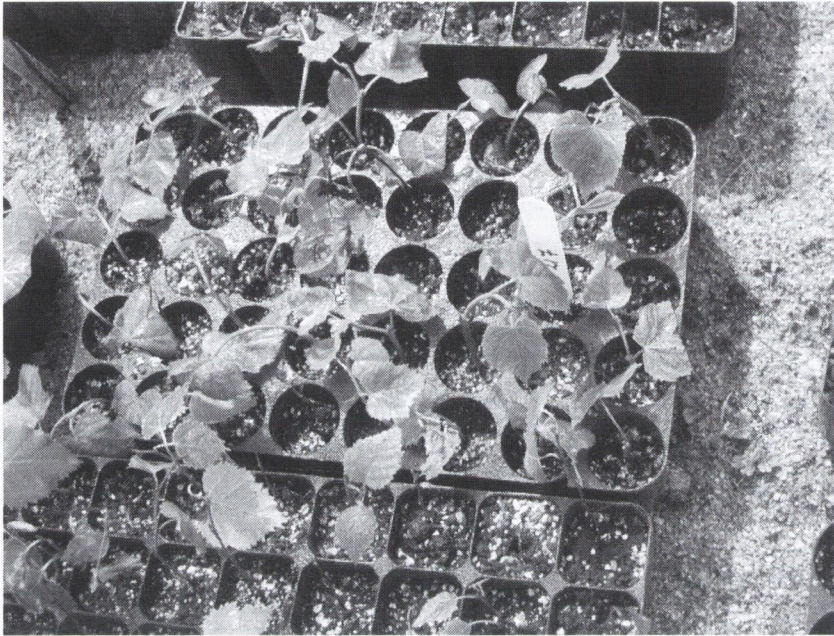


Figure 7. B. pubescens rooting. The photograph was been taken on the 11th August 2003.



On the 11th October 2003 (14 and a half weeks after the start of the propagation), after a one-week gradual acclimation to a drier regime (as described for 2003), the 180 cuttings that had rooted (60% propagation success) were finally transplanted into 1 l plastic pots filled with John Innes N^o 3 compost and transferred into a shaded part of a non-heated glasshouse until 2nd November 2004.

In November, when the newly established cuttings were transferred to the glasshouse bench, they had set buds and were showing signs of senescence and leaf abscission. The cuttings were left in the glasshouse until February 2005, and were exposed during this period to natural chilling, as the glasshouse was non-heated and shaded for most of the day by a nearby building. The humidity of the glasshouse made watering unnecessary during this period.

3.2.2. Propagation of *F. sylvatica*

At the beginning of March 2003, 100 two-year old seedlings of *F. sylvatica* were purchased from a state owned Coillte nursery (Co. Wicklow, Ireland) and kept in a cold room at 4°C until the time of grafting when they were used as rootstocks. On the 4th March 2003, in the early afternoon, 100 hardwood shoots 20-40 cm long were cut from the bottom third of the crown of the parent plant in the Dublin IPG (IPG N^o16, National Botanic Gardens), placed in plastic bags and carried back to the Teagasc Horticultural Center, Kinsealy (Dublin), where the propagation took place. Upon arrival, the plastic bags containing the shoots were transferred into a cold room (4°C), where they were left for one week. The grafting took place during the two weeks from 11th to 25th March 2003 according to the “cleft graft” technique, described by Hartmann *et al.* (1997). The hardwood shoots were cut into a total of 100 sections (the scions), 10-20 cm long, with a diameter of approximately 4 to 7 mm. The rootstocks were neatly cut back to 10-15 cm from the roots, perpendicular to their length, leaving only a short portion of stem that was about 1-2 cm in diameter. Afterwards, a thin vertical cut about 2-4 cm in depth was made with a razor sharp knife through the centre the previous cut. The basal part of the scions was then cut neatly, to form an inverse V shaped wedge, which was inserted in the cut, at the outer edge of the rootstock. Particular attention was given to matching the cambial tissues of the scion and the rootstock and ensuring adhesion between the two parts. The rootstock and the scion were then tied tightly together with elastic rubber bands and immersed into hot wax (50°C) from

the top of the scion to the base of the stem of rootstock, in order to seal the cut and prevent fungus infections and avoid transpiration and desiccation of the scion. The grafts were planted into 2 l plastic pots filled with John Innes N^o. 3 compost and transferred into a non-heated glasshouse where they were watered regularly.

At the beginning of June, 40 grafts appeared viable and had flushed (40% propagation success). The successful grafts were selected and moved to a non heated and automatically irrigated (once a day to field capacity) glasshouse until the 4th October, when they were transferred into a thermostatically controlled glasshouse, where the temperature remained above 12°C to avoid chilling accumulation. The grafts remained under these conditions until the beginning of the first experiments on the 11th November 2003.

3.2.3. Propagation of *S. aurita* and *S. x smithiana*

S. x smithiana. On the 4th March 2003, in the early afternoon, 100 hardwood shoots 20-40 cm long were cut from the parent plant in the Valentia IPG (IPG N^o13, Valentia Observatory, Co. Kerry), at about 1.5 m from the ground (the willows had a bushy habit), placed in plastic bags and transported back to the Teagasc Horticultural Center in Kinsealy (Dublin), where propagation took place. Upon arrival, the plastic bags containing the shoots were transferred into the cold room (4°C), where they remained for one week. On the 11th March, the hardwood shoots were divided into a total of 100 apical and basal cuttings 10-15 cm long and with a diameter of approximately 5 to 10 mm. The cuttings were then partially immersed in water to approximately half their height, in glass beakers of variable sizes (from half a litre to two litres capacity). Each beaker held between 10 and 20 cuttings. Particular attention was given to ensuring that their polarity was maintained (with the original upper side out of the water) in order to favour the rooting process. The basal part of the cutting was cut at an angle to increase the water-absorbing area. After two months, only approximately 50% of the cuttings had rooted, but all of them were still viable (showing green bark below the cortex), so that they were all nonetheless transplanted into 1 l pots filled with John Innes N^o. 3 compost and transferred to a non-heated glasshouse, where they were automatically irrigated once a day to field capacity. The cuttings that had not previously rooted in water did so upon being transplanted, so that by the end of September 91 cuttings (91% propagation success) were successfully established and growing vigorously (Figure 8)

Figure 8. Rooted cuttings of *S. aurita* (left) and *S. x smithiana* (right) growing vigorously (September 2003).



On the 4th October (approximately 7 months after the start of the propagation) the established cuttings were transferred to a thermostatically-controlled glasshouse, where the temperature remained above 12°C to avoid chilling effects after dormancy onset. The cuttings remained in these conditions until the beginning of the first experiments on the 11th November 2003.

S. aurita. The propagation of *S. aurita* followed exactly the same course of action, apart from the fact that the rooting success was not as high, and only 60 cuttings (60% propagation success) developed roots of the initial 100 that were treated for propagation.

3.2.4. Propagation of *T. cordata*

On the 23rd June 2003, in the late morning (between 11 am and 12 pm.), 40 softwood shoots 40-50 cm long were cut from the suckers growing at the base of the parent plant, wrapped in wet newspapers and immediately placed in plastic bags, in order to prevent water loss during the return trip to the Teagasc Horticultural Center Kinsealy (Dublin), where propagation took place. Upon arrival, the cuttings were transferred into a cold room (6°C), where they remained overnight, and on the following morning they were divided into a total of 100 cuttings 8-10 cm long with a diameter of approximately 4 to 10 mm. The

cuttings were all taken from the base of the shoot and each of them contained one node with one leaf. They were then treated according to the propagation procedure followed for *B. pubescens*, apart from the fact that, due to the larger size of the leaves, it was necessary to cut them back leaving only a small leaf portion (approximately 5 x 5 cm) to avoid excessive transpiration.

On the 4th October 2003 (14 weeks after the start of the propagation) the 60 cuttings that had rooted (60% propagation success) were finally transplanted into 1 l plastic pots filled with John Innes N^o 3 compost and transferred into a thermostatically controlled glasshouse, where the temperature remained above 12°C to avoid chilling accumulation. The cuttings remained under these conditions until the beginning of the first experiments on the 11th November 2003.

4. Experimental designs

Between 2003 and 2005 eight experiments were conducted in fully or partially controlled environmental conditions on the propagated clones of *B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana* and *T. cordata*.

The environmental factors tested included temperature, photoperiod, light intensity and nutrient availability. The experiments were designed to investigate the main effects and interactions of these factors during different phases of winter rest. In order to approach the problem of the lack of observable markers of dormancy, we decided to divide our experiments in three phases whose timing and duration was arbitrarily decided, and subsequently infer, based on budburst response, how different conditions and their timing affected actual bud ontogenesis. These three phases were referred to as: **dormancy induction**, during which the buds received a series or more of less dormancy inductive conditions, **chilling (or chilling fulfilment)**, during which the buds received a series of more or less dormancy releasing conditions, and **forcing**, during which the buds received a series of more or less budburst inductive conditions. A summarised description of the dormancy induction, chilling and forcing treatments applied in the performed experiments is presented in Table 1.

The experimental designs used in this study include one way designs, factorial designs and fractional factorial designs.

One way designs: experimental designs with only a single differentiating factor or predictor, although this factor can include several levels. The one way designs utilized during the course of this study include experiments 1.a, testing one factor (light intensity) with 2 levels, 1.b, testing one factor (nutrient availability) with 3 levels, 2.b, testing one factor (forcing temperature) with 6 levels.

Factorial designs: experimental designs incorporating two or more factors that are crossed with each other. The term crossed indicates that all combinations of the factors are included in the design and that all levels (groups) of each factor occur in combination with all levels of the other factor (Quinn and Keough, 2002). Factorial designs enable the measurement of (1) the main effects of each factor independent of other factors, (2) the interaction between factors, or how the effects of each factor depend on the level of other factors (Quinn and Keough, 2002). The factorial designs utilized during the course of this study include experiments 2.a, testing the effect of 4 levels of chilling temperature crossed with 4 levels of chilling duration; 3.a, testing the effect of 5 levels of chilling duration x 2 levels of forcing photoperiod; and 3.b, testing the effect of 4 levels of forcing photoperiod x 4 levels of chilling duration.

Fractional factorial designs: experimental designs that do not include all factor combinations. They are used when a large number of factors are manipulated and only a limited number of replicates is available. They are conducted using a subset of cells that allows for the estimation of main effects and simple interactions (Quinn and Keough, 2002). The fractional factorial designs utilized during the course of this study included experiments 3.c and 4.a. Experiment 3.c tested effect of 2 levels of dormancy induction duration x 2 levels of chilling presence/absence x 2 levels of chilling photoperiod. Obviously the two levels of chilling photoperiod could not be applied to the unchilled treatments. Experiment 4.a tested the effect of 2 levels of dormancy induction photoperiod (naturally decreasing-8h) x 2 levels of chilling photoperiod (SD-LD) x 2 levels of chilling temperature (high-low). The combination LD+high temperature during the chilling treatment was missing, as it was assumed it was equivalent to a forcing treatment on unchilled plants, whose effects had already been tested in other experiments (3.a, 3.b and

3.c). The five forcing treatments tested presence/absence of warm and cold spells (2 levels), duration of the cold/warm spells cycle (2 levels), timing of application of the cold/warm spells (2 levels) and temperature combined with photoperiod.

Table 1. Dormancy induction, forcing and chilling treatments applied in each of the experiments performed during the study. "Ambient" refers to non-controlled temperature (received outdoors or in the glasshouse) which was recorded daily or obtained from a nearby weather station as daily max and min. >12 °C refers to the temperature recorded inside a thermostatically controlled glasshouse. The cells in bold characters refer to the factors that were varied within each experiment. B.pub: B. pubescens, F.sylv: F. sylvatica, S.au: S. aurita, S.sm: S. x smithiana.

Experiment	Species	Experimental material	Dormancy Induction Treatment			Chilling Treatment			Forcing Treatment		Additional factors tested
			Temperature (°C)	Photoperiod (hours)	Duration (days)	Temperature (°C)	Photoperiod (hours)	Duration (days)	Temperature (°C)	Photoperiod (hours)	
1a	B.pub, F.sylv, S.sm, T.cor	budsticks	Ambient	Natural	NA	Ambient	Natural	NA	22	16	High and Low Light Intensity
1b	B.pub, S.sm, T.cor	potted trees	Ambient	Natural	NA	Ambient	Natural	NA	13±1	Natural	Low K, Low N
2a	B.pub, S.sm, T.cor	budsticks	>12	Natural	until 20 Nov	2, 4, 6, 10	0	30, 55, 95, 122	22	16	NA
2b	B.pub, F.sylv, S.sm, T.cor	budsticks	Ambient	Natural	NA	Ambient	Natural	NA	0, 6, 12, 18, 24, 32	16	NA
3a	B.pub, F.sylv, S.au, S.sm, T.cor	potted trees	>12	Natural	until 10 Nov	3	0	0 (no chilling), 11, 29, 55, 105	18	8, 16	NA
3b	B. pub	budsticks	>12	Natural	NA	Ambient	Natural	0(no chilling), 30, 55, 95	22	10,12,14,16	NA
3c	B. pub	potted trees	10, 18	8	30, 60 (start on 19th July)	10	0, 8	0 (no chilling), 50	20	16	NA
4a	B. pub	potted trees	18 constant 18 average	Natural, 8	60 (start on 19th July)	10, 18	8, 16	50	Constant 20, cycles 10-20, 15	16,12, 9 increasing to 16	NA

The tests were conducted either on sets of potted clones or on sets of budsticks taken from the branches of the clones and cut into segments 3-5 cm long, each containing one bud. These one-node cuttings were protected with parafilm at their distal end to prevent water loss and desiccation, and placed inside peat-filled trays before transfer into experimental conditions. The experimental material was watered regularly and the compost kept moist all the time.

All the treatments taking place in the growth cabinets (Sanyo Fitotron 600L and Fisons Fitotron 600H) received a photon flux density of $170 \pm 10 \mu\text{mol m}^{-2}$ with a red/far red ratio varying between 7 and 9, unless otherwise indicated.

4.1 Effect of light intensity and nutrients

4.1.1 Experiment 1.a - light intensity

On the 28th of February 2004, dormant twigs were removed from adult IPG clones of *B. pubescens*, *F. sylvatica*, *T. cordata* and *S. x smithiana* growing outdoors in IPG 14, Wexford (Ireland). Twenty twigs were taken from one tree of each species. The twigs were stored in the dark at 3°C for 16 days, until the start of the experiment. By this time the buds' chilling requirement was assumed to be met and thermal time to budburst to have already started to accumulate. Each twig was cut into a one-node cutting, containing a lateral bud. For each species, the cuttings were divided into two groups of 10 cuttings each, which were placed in two trays filled with peat compost. The two trays were transferred to a growth cabinet maintained at 24°C, with a 16-h photoperiod. Inside the cabinet, one of the trays was shaded with a white cotton fabric screen, which decreased the light intensity without altering the red/far red ratio. The other was openly exposed to the light. The photon flux densities in the two light intensity treatments were $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (high intensity) and $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ (low intensity). In both cases the red/far red ratio was 7 ± 1 . Ten one-node cuttings were tested in each treatment.

4.1.2 Experiment 1.b - nutrient deficiency

On the 10th June 2004 one-year old cloned plants of *B. pubescens*, *S. x smithiana* and *T. cordata* were repotted in John Innes N^o2 compost, to which slow-release nutrients had been added in different amounts to provide a low N treatment and a low K treatment. The low N compost contained 0.168 g/l N, 0.543 g/l P and 1.5 g/l K, while the low K compost contained 0.507 g/l N, 0.543 g/l P and 0.6 g/l K. In the control group, fertilisation was maintained at the optimum by regular applications of Phostrogen soluble plant food, NPK 14:10:27. Plant growth in both the low N and low K treatments was stunted and showed signs of nutrient deficiency until they became dormant. The plants were kept outdoors during the summer and on the 1st September 2004 they were moved to a temperature controlled glasshouse where temperature was maintained above 12°C. On the 21st November, the plants were transferred outside to receive natural chilling until the 13th of March, when, after 123 days of natural chilling, they were moved back to the heated glasshouse (at 13±1°C), where artificial lighting extended the photoperiod to 24 h. The light had a red/far red ratio of 3.8 with a photon flux density of 170±10 µmol m⁻² s⁻¹. Each of the 3 treatments, i.e., low N, low K and control, was applied to sets of 4 plants for each species.

4.2. Manipulation of a single experimental phase

4.2.1 Experiment 2.a – Effect of chilling temperature and duration

On the 21th November 2004, 16 one-year cloned old plants of *B. pubescens*, *S. x smithiana* and *T. cordata*, that had been propagated in 2003 and kept in a temperature controlled glasshouse (temperature never decreasing below 12°C), were moved to 4 incubators at chilling temperatures of: 2, 4, 6 and 10 °C, and in the dark. Four plants per species were incubated at each temperature.

After 30, 55, 95 and 122 days, one-node cuttings were randomly taken from the tree branches, placed in compost-filled trays and transferred into growth cabinets at 22°C with a photoperiod of 16 h. Each of the 16 treatments, i.e. 4 chilling durations x 4 chilling temperatures, was applied to sets of 8 cuttings per species.

4.2.2 Experiment 2.b – Effect of forcing temperature

This experiment investigated the effect of forcing temperature on fully chilled budsticks. On the 28th of February 2004, dormant twigs were removed from adult IPG clones of *B. pubescens*, *F. sylvatica*, *T. cordata* and *S. x smithiana* growing outdoors in IPG 14, Wexford (Ireland). Sixty twigs were taken from one tree of each species. The twigs were stored in the dark at 3°C for 16 days, until the start of the experiment. By this time the buds' chilling requirement was assumed to be met and thermal time to budburst to have already started to accumulate. Each twig was cut into a one-node cutting, containing a lateral bud. The 60 one-node cuttings were then transferred into growth cabinets and maintained at a 16-hour photoperiod. The growth cabinets were set at different temperatures of 3, 6, 12, 18, 24 and 32°C and a set of 10 one-node cuttings placed in each.

4.3 Manipulation of two experimental phases

4.3.1 Experiment 3.a – Effect of chilling duration and forcing photoperiod (8 and 16 h)

We investigated the main effects and interactions between chilling duration and photoperiod in recently propagated *B. pubescens*, *F. sylvatica*, *T. cordata* and *S. aurita* and *S. x smithiana* plants. At the start of the experiment on the 11th of November 2003, *F. sylvatica* and the two *Salix* species were already well established, as they had been propagated from winter cuttings and by grafting several months earlier, whereas *B. pubescens* and *T. cordata* had recently rooted (July-August) and possessed only 2-8 dormant buds. The plants were kept in a temperature controlled glasshouse (temperature never decreasing below 12°C), until the start of the experiment. Forty plants for each species were transferred to a cold room, permanently dark, at a constant temperature of 3°C, where they received chilling for varying lengths of time: 0, 11, 30, 55 and 105 days. Batches of 8 plants per species were identified randomly and removed from the cold room at the end of each period to be transferred into growth cabinets. These were set at 18°C with a photoperiod of either 16 h (long day treatments, LD) or 8 h (short day treatments, SD). Each of the 10 treatments, i.e. 5 chilling durations x 2 forcing photoperiods, was tested on 4 replicates for each species.

4.3.2 Experiment 3.b – Effect of chilling duration and different forcing photoperiods (10, 12, 14, 16 h)

The effects of a range of forcing photoperiods were tested on *B. pubescens* budsticks removed from trees at various stages of chilling accumulation. On the 21th November 2004, 10 one-year old IPG clones of *B. pubescens* that had been propagated in 2003 and kept in a temperature controlled glasshouse (temperature never decreasing below 12°C), were moved outdoors to receive natural chilling. After chilling durations of 0, 30, 55 and 95 days, 9 budsticks were randomly removed from the tree branches and cut into 36 one-node cuttings, placed in compost-filled trays and transferred to growth cabinets for the forcing treatments at different photoperiods of 10, 12, 14 and 16 h. In all treatments the forcing temperature was maintained at 22°C. Each of the 16 treatments (4 chilling durations x 4 forcing photoperiod) was applied to sets of 9 cuttings.

4.3.3. Experiment 3.c - Effect of dormancy induction temperature and duration and chilling photoperiod and duration.

On the 19th July 2005, 48 cloned *B. pubescens* trees were placed in two growth cabinets where they received dormancy induction at a constant temperature of 10 °C (24 plants) or 18 °C (24 plants). Half of each group remained in the growth cabinet for 30 days (short dormancy induction) and the other half for 60 days (long dormancy induction). During this time photoperiod was maintained at 8 h. Subsequently each group of plants was divided into three groups of 4 plants each and received, (1) a 50-day long chilling treatment at 10 °C, either at a 8 or (2) 0 h photoperiod (3) 50 days at 20°C and a 16 h photoperiod (unchilled treatment). After these treatments all groups were transferred into forcing condition at 20°C with a 16 h photoperiod.

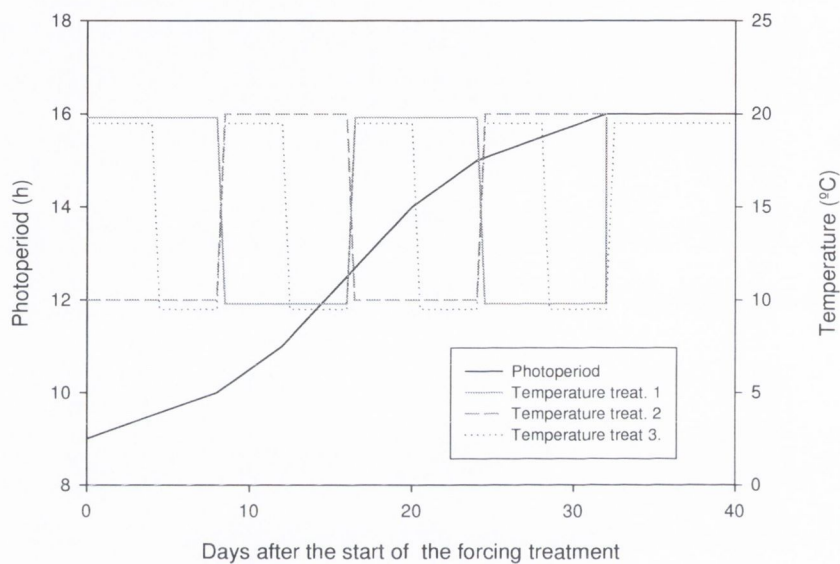
4.4 Manipulation of three experimental phases

4.4.1 Experiment 4.a - Effect of dormancy induction, chilling and forcing temperatures and photoperiods

One-hundred-twenty one year old cloned *B. pubescens* trees were tested in a fractional factorial experiment involving the manipulation of photoperiod and temperature during three different experimental phases: dormancy induction, dormancy and quiescence. The

aim of the experiment was to gain information on the effect of different combinations of environmental factors during these three phases. We were particularly interested in knowing whether warm/cold spells and their timing during spring affect the time to budburst, so that the forcing treatments were designed to have gradually lengthening photoperiod and warm and cold spells of different lengths applied at different times during this period (see Figure 9).

Figure 9. Schematic representation of the temperature regimes of the first three forcing treatments in experiment 4.a, all exposed to gradually lengthening photoperiod.



On the 19th July 2005, 60 plants were transferred into growth cabinets, where they were kept for 60 days in dormancy-inductive conditions (8 h photoperiod at 18°C). The other 60 plants were left in the glasshouse, where they received natural daylength and an average daily temperature of 17.7 °C. During this period, daylength decreased naturally from 15.7 h to 12.2 h, and daily minimum temperature never fell below 14°C. On the 17th September, after 60 days of dormancy induction, each set of 60 plants was divided into three groups, receiving the following chilling treatments during 50 days: (1) 8 h photoperiod at 10°C, (2) 8 h photoperiod at 18°C, (3) 16 h photoperiod at 10°C. Subsequently each set of 20 plants was divided into 5 groups of 4 plants each and placed in 5 different forcing treatments. Three of the treatments were exposed, for 4 days at a time, to gradually increasing photoperiods from 9 up to 16 h, over a period of 32 days. Over the 32 day period of lengthening photoperiod these three treatments also received a series of cold and warm spells as follows: (1) alternation of 8-days long warm (20°C) and cold (10°C) spells, (2)

alternation of 8-days long cold (10°C) and warm (20°C) spells, in phase opposition to the first treatment, in order to correspond with different daylengths, (3) alternation of 4-days long warm (20°C) and cold (10°C) spells. The fourth and the fifth forcing treatments received constant temperature and photoperiod of (4) 15°C at 12 h photoperiod and (5) 20°C at 16 h photoperiod.

5. Statistical analysis

5.1 Measurements

The phenophase that we selected for our study was budburst, which we refer to as the moment when the green tips of the young leaves were visible inside the opening bud. The phenological observations were carried out every day or every second day during the forcing treatments, and noted on special worksheets, where a diagram of the tree or cutting had been drawn, complete with its dormant buds, prior to the start of budburst. This method allowed recording budburst timing and enabled the calculation of the percentage of budburst after the end of the forcing treatment.

The response of budburst timing and percentage of budburst was subsequently analysed in relation to the environmental factors analysed in each experiment. When whole potted trees were used in the experiments, we analysed their mean time to budburst, which was obtained by averaging the number of days to budburst of each bud (arithmetic mean). When budsticks were used, the number of days to budburst of each budstick was considered. The count of the number of days to budburst was started from the moment of transfer into the forcing treatment. Budburst percentage in potted trees was defined as the percentage of buds flushing against the total number of buds on the trees. For one-node budsticks budburst percentage was either 0 (non-flushing) or 100% (flushing). When budburst percentage was supposedly decreased by accidental causes (for example, bud desiccation in one-node budsticks) or not recorded (as in experiment 1.b), only the data for budburst timing were used.

The minimum chilling requirement is here defined as the minimum chilling duration triggering at least 50% budburst during the forcing treatment.

5.1.1. Budburst rate and forcing rate

Budburst rate is referred to as 1/days to budburst. This transform is proportional to growth rate during quiescence and was used as a measure of the response of bud growth to forcing temperature in the graphical representation of the results of experiment 2.b.

Forcing rate was used to facilitate the comparison of the budburst rates of different species in experiment 2.b. Budburst rates were normalized to 1 at 32°C thus defining forcing rate as (days to budburst at 32°C)/(days to budburst).

5.2. Analysis of Variance

The data were subjected to one-way and factorial Analysis of Variance (ANOVA) to check for significant differences among the means of the days to budburst and budburst percentages measured in the different treatments.

The ANOVA partitions the total variation into different sources (associated with the different effects in the design), enabling the comparison of the variance due to the between-groups variability with that due to the within-group variability. Within groups variability represents variability caused by random effects within each sample, while between groups variability represents variability due to the differences between the means of the populations from which the samples were drawn (Fowler *et al.* 1998).

Under the null hypothesis that there are no mean differences between groups or treatments in the population, the variance estimated from the within-group (treatment) variability should be about the same as the variance estimated from between-group variability (Underwood, 1997).

One way ANOVA is used for the analysis of experimental designs with only a single differentiating factor or predictor, although that factor will comprise several levels or groups (Quinn and Keough, 2002). It was used for the analysis of the results of experiments 1.a (effect of light intensity), 1.b (effect of nutrient availability) and 2.b (effect of forcing temperature).

Factorial ANOVA is used for the analysis of experimental designs incorporating two or more factors that are crossed with each other. Factorial ANOVA involves partitioning of the total sums of squares of the samples into the various components that make up the total variability (Fowler *et al.* 1998): while within sample variability, represents, like in one way ANOVA, the variability caused by random effects within each sample, between groups variability is divided into more components, representing variation between samples due to both the main factors and the interaction of the different factors (Fowler *et al.* 1998). Factorial ANOVAs were used for the analysis of experiments 2.a., 3.a, 3.b, 3.c and 4.a.

5.2.1 Unbalanced Designs

Unbalanced designs were present in many experiments, decreasing the robustness of the ANOVA and complicating the determination of expected values of mean squares (Quinn and Keough, 2002).

Two different cases were identified:

1. Experimental designs with unequal sample sizes (all cells in the design had at least one observation);
2. Experimental designs with missing cells.

5.2.1.1 Experimental designs with unequal sample sizes

In spite of the fact that the experiments had been designed with equal number of observations per cell, many experiments ended up with unequal observation numbers for budburst timing. This was expected, and causally related to the manipulated environmental factors whose different levels differently affected the state of dormancy. In fact, while some treatments caused partial dormancy release and only a small portion of the buds flushed (leading to empty or semi-empty design cells), other treatments triggered complete budburst (and lead to full design cells). Experimental design with unequal sample size were analysed with Type III sum of squares as indicated by Quinn and Keough (2002). This method is based on unweighted marginal means and therefore is not influenced by the sample size in each cell. Analysing the same effects on budburst percentage did not require the same attentions; in fact absence of flushing could be conveniently indicated as 0%, leading to equal sample sizes.

5.2.1.2 Experimental designs with missing cells

Cells were missing from the fractional factorial experimental designs, and, for budburst timing, from all the cells in which none of the buds flushed (corresponding to cells with 0% budburst).

Fractional factorial designs

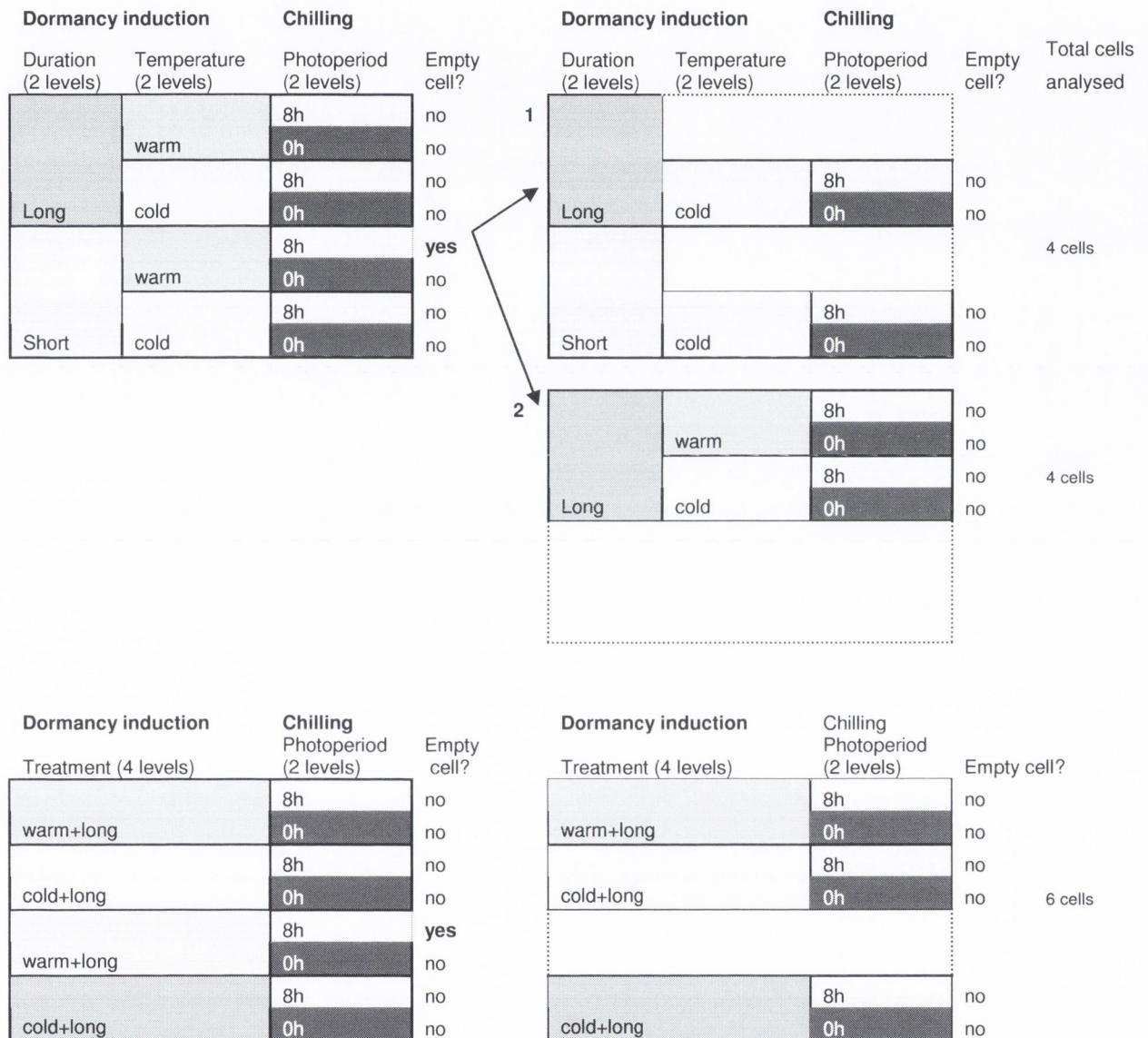
Experiment 3.c and 4.a tested the effects of a high number of factors (4 factors in experiments 3.c and 7 factors in experiment 4.a) on a limited number of replicates, which prevented the combination of all factor levels in the experimental designs.

Cells with 0% budburst

When the experimental treatments were insufficient to release dormancy, buds did not flush and no budburst timing observation could be recorded in the design cell corresponding to that particular treatment. This condition occurred in experiment 3.a, 3.c and 4.a.

These unbalanced designs were analysed by selecting subsets of the data with observations in all cells in order to test "balanced" hypotheses for lower-order effects (Quinn and Keough, 2002) and by grouping factors together in order to avoid the calculation of interaction effects among factors with incomplete levels. For example, in experiment 3.c, in order to calculate the effect of chilling photoperiod on timing of budburst it was firstly necessary to select the cells of the design with observations from treatments receiving a chilling treatment (presence of chilling), then, as data were missing from one of the cells of the design (plants exposed to short dormancy induction duration at high temperature, and chilling at 10°C and 8h photoperiod did not flush) a further selection was necessary. However, as we were dealing already with a reduced matrix, in order not to lose too much information on the effects of chilling photoperiod on timing of budburst, we chose to group the 2 factors defining the dormancy treatments (2 dormancy induction duration levels*2 dormancy induction temperature levels) into 4 levels of the same, generic "dormancy treatment" factor. This allowed us to calculate the effects and interactions of chilling photoperiod (2 levels) and dormancy treatment on a larger subset of cells than if the 2 dormancy induction factors had been kept separate (see Figure 10).

Figure 10. Schematic representation of the experimental design for budburst timing in the chilled treatments of experiment 3.c (left) and possible selections of full cell subsets for the analysis of the effects of chilling photoperiod. The figure shows how combining the 2 factors defining dormancy induction treatment (duration and temperature) allows us to test the main effect of chilling photoperiod on a larger number of cells and replicates. When the two dormancy induction factors are kept ungrouped (top) there are two possible design subsets with all cells full (1 and 2). Each of these includes only 4 cells. By grouping the two dormancy induction factors into one generic factor (bottom), it is possible to test the main effect of chilling photoperiod on 6 cells.



5.2.2 Control of the ANOVA assumptions

The preliminary assumption for hypothesis testing based on ANOVA, require that:

1. the observations are taken from normally distributed populations (normality)

2. the variances of the error terms (and of the observations in the populations being sampled) should be approximately equal in each group (variance homogeneity)
3. The error terms and the observations should be independent (independence)

The *normality* of the data was controlled with Quantile versus Quantile (Q-Q) plots (not shown). The used function plots theoretical quantiles (points taken at regular intervals from the cumulative distribution of a variable with a standard normal distribution) versus the observed quantiles (points taken at regular intervals from the cumulative distribution of the observed sample). If the distribution of the observed sample is normal, the plot will form a straight line (Dalgaard, 2002).

Fligner-Killeen's and Levene's tests were performed to check for the *homogeneity of variances* prior to the analysis (not shown). Levene's test is based on absolute deviations of each observation from its respective group mean. Where the variances are different, the absolute deviations differ, on average, among samples (Underwood, 1997). Fligner and Killeen's test is a k-sample simple linear rank which uses the ranks of the absolute values of the centered samples (Conover *et al.*, 1981). The version implemented in this study uses median centering in each of the t samples.

Independence of the samples was ensured by randomization at various stages of the experimental design, as suggested by Quinn and Keough (2002). The choice of the plants and the plant parts (cuttings) taking part in the experiments was done randomly as well as their positioning inside the growth cabinets and the glasshouses where the experiments took place. The plants were then identified through labels specifying the treatments they had received.

5.3 Post-hoc tests

Post-hoc comparison techniques are used to get information on the contribution of particular means to a statistically significant effect from the ANOVA. While performing a series of simple t-tests to compare all possible pairs of means would actually overestimate the statistical significance of mean differences, post-hoc tests specifically take into account the fact that more than two samples were taken.

For comparing the effects of different treatments after performing one-way ANOVAs, we utilised the Bonferroni procedure. This procedure involves tests of multiple *a-priori* hypotheses while controlling the experimenter-wise error rate; specifically, it can be shown that the type 1 error rate ($\alpha(\text{exp})$) for a set of comparisons will not exceed the sum of the error levels ($\alpha(\text{ind})$) for a set of m tests of significance; or $\alpha(\text{exp}) < m \cdot \alpha(\text{ind})$. The Bonferroni procedure uses this inequality to adjust the significance levels for the individual post-hoc comparisons (Quinn and Keough, 2002).

5.4 Description of the results

The data were described by quoting the significant main effects and interactions between the environmental factors tested in the experiments and their relative p-value. The means quoted in the results sections refer to the unweighted means computed by the ANOVA for the detection of effects. Unweighted means are computed by averaging the means across the levels and combinations of levels of the factors not used in the marginal means table (or plot), and then dividing by the number of means in the average. Thus, each mean that is averaged to compute a marginal mean is implicitly assigned the same weight, regardless of the number of observations on which the respective mean is based.

5.4.1 Graphical representation of budburst responses

The responses of budburst timing and percentage to changes in environmental factors were graphically represented through scatter plots, line-and-scatter plots and bar plots. In experiment 2.b, logistic (sigmoidal) three parameter curves of equation $f = a/(1+\exp(-(x-x_0)/b))$ were fitted to the data of budburst timing, in order to represent the sigmoidal growth rate during budburst development (Landsberg, 1974; Sarvas, 1974; Hänninen, 1990). In the line-and-scatter plots of experiments 3.a and 3.b, including some treatments that did not flush during the course of the forcing treatments, non-flushing groups were nonetheless graphically represented as flushing after the duration of the forcing treatment (90 days for experiment 3.a, 80 days for experiment 3.b). This was done with the purpose of giving an idea of the pattern of decreasing time to budburst at increasing chilling durations (experiment 3.a) and increasing photoperiods (experiment 3.b).

The effect plots, which were used to show significant effects, were computed by averaging the means across the levels and combinations of levels of the factors not used in the marginal means table (or plot), and then dividing by the number of means in the average.

RESULTS

1. Control of the ANOVA assumptions

1.1 Time to budburst

The preliminary control of the assumptions revealed that while the distribution of data was in most cases approximately normal, the variances were not always homogeneous among samples. In fact, in experiments testing the degree of dormancy release at various stages of chilling (2.a, 3.a, 3.b, 3.c) and experiments including treatments that released dormancy only incompletely (4.a), data from dormant plants with irregular budburst were analysed together with data from non-dormant plants, whose response was more synchronous, and thus with lower variance. Unfortunately, mathematical transformations such as square rooting did not homogenise the variance among groups. However, visual inspections of the plots of the mean times to budburst of the treatments against their variances showed that no correlation existed between them across the cells of the design for most experiments. This enabled us to carry out the ANOVAs, which are deemed to be very robust to the violation of the assumption of homogeneity of variance, unless the means are correlated with variances across cells of the design (Lindman, 1974). The only experiment that showed a correlation between the observation means and variances is experiment 2.a, whose significant results should be considered with caution.

1.2 Percentage budburst

Percentage budburst was binomially distributed as often is the case when data are percentages or portions (Underwood, 1997). This implied that variances were larger where means were near 50% than where means were large or small, and lead to heterogeneity of variance in experiment 3.a, 3.c and 4.a. The appropriate transform for these situations is the arc-sin of the square root of the proportion (Underwood, 1997), which was in this case successful at stabilizing the variances.

The results from the analyses conducted on the transformed data are reported in the ANOVA tables only when they differed from the original results with regards to the

significance of main effects or interactions. This occurred only for experiment 4.a, the only case in which the ANOVA results reported are those corresponding to the arc-sin of the square root of the proportion.

2. General effects of temperature, photoperiod, light intensity and nutrients

This section deals with the overall effects of the environmental factors under investigation and briefly compares the main responses of the different species to each factor. The main effects of temperature, photoperiod, light intensity and nutrients are summarized in Tables 2 (main effects of temperature), 3 (main effects of photoperiod) and 4 (main effects of light intensity and nutrient availability). The results of the ANOVA testing for both the overall effects of environmental factors and the effects of species in experiments 1.a, 1.b, 2.a, 2.b, 3.a (the experiments including more than one species) are presented in Table 5. Despite the fact that the ANOVAs performed were factorial, the interactions among factors are not reported in the tables as a detailed study for each species has been presented in the next result section. These analyses as well as graphs and complete ANOVA tables reporting both main and interaction effects of environmental factors on single species can be found in result section 3.

Table 2. P-values for the main effects of temperature during the different experimental phases on on days to budburst (TB) and percentage budburst (B %) of *B. pubescens*, *F. sylvatica*, *S. aurita*, *S.x smithiana* and *T. cordata*. The experiments are described in Table 1.

Effect of temperature													
Experiment Response		2a		2b		3a		3b		3c		4a	
		TB		TB		TB	B%	TB	B%	TB	B%	TB	B%
Dormancy induction		<i>B. pubescens</i>								0.040	<0.001		
Chilling	Temperature	<i>B. pubescens</i>		0.079								<0.001	<0.001
		<i>S. x smithiana</i>		0.003									
		<i>T. cordata</i>		0.584									
	Duration	<i>B. pubescens</i>		<0.001		<0.001	<0.001	<0.001	<0.001				
		<i>F. sylvatica</i>				0.001	<0.001						
		<i>S. aurita</i>				<0.001	<0.001						
		<i>S. x smithiana</i>		<0.001		<0.001	<0.001						
<i>T. cordata</i>		<0.001		<0.001	<0.001								
Forcing		<i>B. pubescens</i>			<0.001								
		<i>F. sylvatica</i>			<0.001								
		<i>S. x smithiana</i>			<0.001								
		<i>T. cordata</i>			<0.001								

Table 3. P-values for the main effects of photoperiod during the different experimental phases on days to budburst (TB) and percentage budburst (B%) of *B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana* and *T. cordata*. The experiments are described in Table 1.

		Effect of photoperiod							
		Experiment		3a		3b		3c	
		Experimental phase	Response	TB	B%	TB	B%	TB	B%
		Dormancy induction	<i>B. pubescens</i>						
		Chilling	<i>B. pubescens</i>					0.125	0.169
		Forcing	<i>B. pubescens</i>	<0.001	0.008	0.051	<0.001		
			<i>F. sylvatica</i>	<0.001	0.001				
			<i>S. aurita</i>	0.501	0.029				
			<i>S. x smithiana</i>	0.001	0.032				
			<i>T. cordata</i>	0.018	0.187				

Table 4. P-values for the main effects of light intensity during forcing and nutrient deficiency (long-term) on days to budburst(TB) on *B. pubescens*, *F. sylvatica*, *S.x smithiana* and *T. cordata*. The experiments are described in Table 1.

Effect of light intensity and nutrient availability			
Experiment		1a	1b
Response		TB	TB
Light intensity	<i>B. pubescens</i>		0.071
	<i>F. sylvatica</i>		<0.001
	<i>S. x smithiana</i>		0.347
	<i>T. cordata</i>		0.008
Nutrient availability	<i>B. pubescens</i>	<0.001	
	<i>S. x smithiana</i>	<0.001	
	<i>T. cordata</i>	<0.001	

Table 5. General analyses of variance testing for overall main effects and species effects on percentage budburst and time to budburst in experiments investigating more than one species. (a) experiment 2.a, (b) experiment 3.a, (c) experiment 2b, (d) experiment 1a, (e) experiment 1b.

The ANOVAs performed were factorial, but the interactions among factors are not reported in the tables as a detailed study for each species has been presented in result section 3. Due to missing blocks, the analysis of experiment 2.b was performed on a reduced matrix, excluding the cells corresponding to the treatments receiving a forcing temperature of -3°C , in which none of the species flushed. For the same reason, a general ANOVA could not be conducted on the budburst timing data from experiment 3.a. The experiments are described in Table 1.

(a) Exp 2a: time to budburst					
	df	SS	MS	F	p
Chilling temperature	3	39.8	13.3	3.35	0.02
Chilling Duration	3	2745.7	915.2	231.46	<0.001
Species	2	1428.9	714.4	180.68	<0.001

(b) Exp 3a: percentage budburst					
	df	SS	MS	F	p
Chilling duration	4	151532.1	37883	76.31	<0.001
Photoperiod	1	30269.3	30269.3	60.98	<0.001
Species	4	18813.8	4703.4	9.47	<0.001

(c) Exp 2b: time to budburst					
	df	SS	MS	F	p
Forcing temperature	4	2777.0	694.0	264.50	<0.001
Species	3	3760.0	1253.0	477.60	<0.001

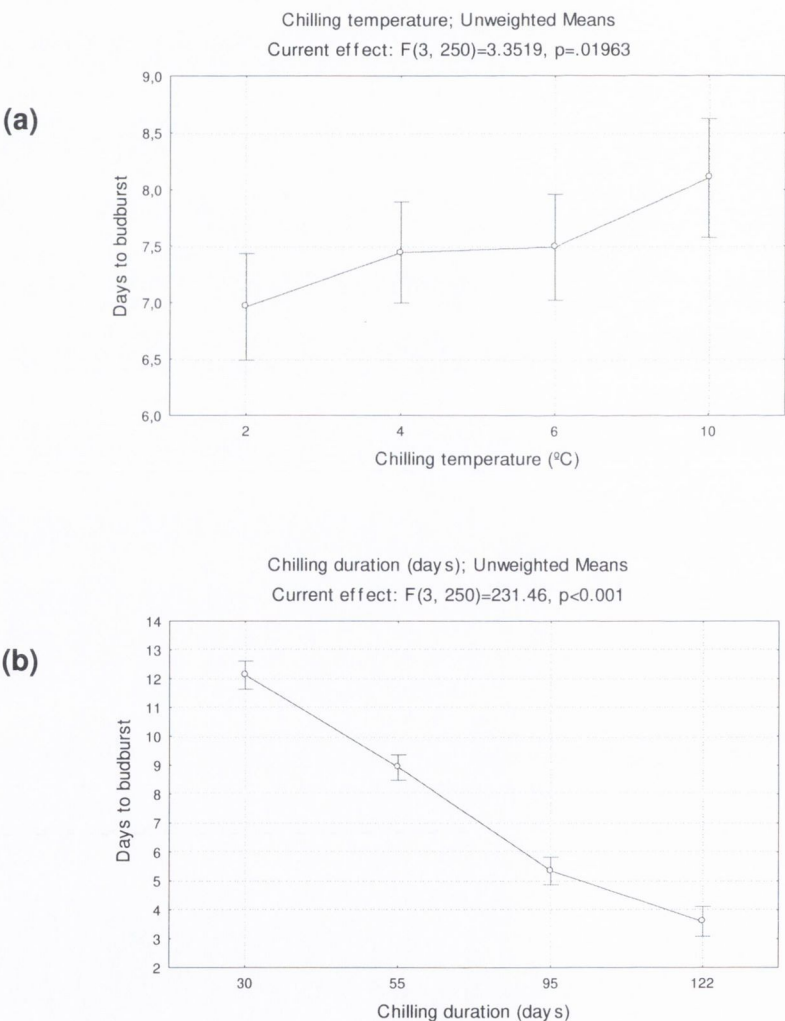
(d) Exp 1a: time to budburst					
	df	SS	MS	F	p
Light intensity	1	29.9	29.9	16.25	<0.001
Species	3	487.7	162.6	88.21	<0.001

(e) Exp 1b: time to budburst					
	df	SS	MS	F	p
Nutrient deficiency	2	47.3	23.7	6.59	0.006
Species	2	417	208.5	58.04	<0.001

2.1 Temperature

Temperature during dormancy induction significantly affected the timing ($P= 0.040$) and percentage of budburst ($P<0.001$) of *B. pubescens*, the only species that was tested for this effect (Table 2). High temperature during dormancy induction increased percentage budburst and delayed the onset of budburst (for a more detailed description of this effect, see result section 3).

Figure 11. General effect of (a) chilling temperature and (b) chilling duration on time to budburst in one-node budsticks of *B. pubescens*, *S. x smithiana* and *T. cordata*, combined dataset. Data are from experiment 2.a (effect of chilling temperature and duration). Vertical bars denote 0.95 confidence intervals.



General effects

Low chilling temperature and long chilling duration generally advanced budburst and increased percentage budburst (Figures 11.a, 11.b and 12.a). The general ANOVAs revealed that chilling duration and temperature had significant effects on both responses in experiments 2.a and 3.a (P -values<0.05) (Table 5.a and b).

However, as different species differed as to their timing and percentage of budburst in relation to chilling temperature and duration (P -values<0.001) (Tables 5.a and b), individual analyses for each species were performed.

Main effects and differences among species

Chilling temperature significantly affected the timing of budburst of *B. pubescens* (P <0.001) in experiment 4.a (testing the effect of 10 and 18°C during chilling, in combination with different dormancy induction, chilling and forcing temperatures and photoperiods), but not in experiment 2.a (testing the effect of 2, 4, 6 and 10°C applied for 4 different chilling durations) (Table 2). Chilling temperature also significantly affected the timing of budburst of *S. x smithiana* (P = 0.003), but not that of *T. cordata* (P = 0.584), (experiment 2.a) and the percentage budburst in *B. pubescens* in experiment 4.a (P <0.001) (Table 2, but for a detailed description of these effects see result section 3).

Chilling duration was another important factor for the percentage and timing of budburst in the analysis of the data pertaining to single species, significantly affecting both responses in all the species under investigation. In fact, the ANOVAs of the data from experiments 3.a, 2.a and 3.b indicate that chilling duration had significant effects on both percentage budburst and timing of budburst in all species under study: *B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana* and *T. cordata* (P -values<0.005) (Table 2). In general, the effects of chilling duration were most evident at the initial stages of chilling, with the decrease in time to budburst being sharpest as chilling duration increased from 11-30 to 55 days (depending on the species). This pattern was also present for percentage budburst, with the sharpest increase between 11 and 55 days of chilling for most species (the general effect can be seen in Figure 12.a).

Finally, in experiment 3.c, chilling absence/presence significantly affected the percentage budburst of *B. pubescens* ($P<0.001$), but not its timing of budburst (Table 2).

Temperature played an important role during forcing: in experiment 2.b, fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. x smithiana*, and *T. cordata* that were exposed to a series of forcing temperatures, advanced their time to budburst at higher temperatures (Figures 13 and 14). Despite this effect being highly significant ($P<0.001$) in a general ANOVA there was also a significant difference among the 4 species tested ($P<0.001$) (Table 5.c), which made it necessary to analyze each species individually. The effect of forcing temperature on the timing of budburst was significant for all species (Table 2).

The rates of budburst (1/ days to budburst) of the 4 species all showed a sigmoidal increase when plotted against raising forcing temperature, highlighting the presence of an optimum temperature for bud development (for more details see result section 3). The normalization of the timing of budburst from experiment 2.a into “rates of forcing” (days to budburst at 32°C/days to budburst) enabled a same-scale comparison of the species-specific patterns of increasing growth rates at increasing forcing temperature, which was not possible by comparing the rates of budburst (on different scales). The rates of forcing of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*, all ranging from 0 at -3°C, to 1 at 32°C, were plotted and fitted with sigmoidal curves of equation $y=1/(1+e^{b(x-c)})$, where b expresses the slope of the curve and c the position of the inflexion point in the curve, or the temperature at which the curve ascends more rapidly (Figure 15, for their parameters and R^2 see Table 6). The comparison between the shapes of the curves showed *F. sylvatica* and *T. cordata* as having a very similar response to temperature, in that their rate of forcing increased rapidly at low temperatures but slowly to the optimum, contrasting with the responses of *B. pubescens* and *S. x smithiana*, whose rates of forcing rose slowly at low temperatures and more rapidly as temperature neared 32°C. The similarity of the responses of *F. sylvatica* and *T. cordata* was confirmed by the similarity of the fitted parameters (Table 6), and the initially more rapid increase in their rate of forcing was shown by the lower value fitted to the c parameter.

2.2 Photoperiod

The effects of the length of the photoperiod during dormancy induction and chilling were tested only in *B. pubescens*. This factor significantly affected the time to budburst ($P=0.003$) and the percentage budburst ($P<0.001$) of *B. pubescens* in experiment 4.a (Table 3) (see also result section 3). On the other hand, the manipulation of photoperiod during chilling in *B. pubescens* did not affect time to budburst nor percentage budburst in experiment 3.c (comparison between 0 h and 8 h chilling photoperiods) and experiment 4.a (comparison between 8 h and 16 chilling photoperiods) ($P\text{-values}>0.005$) (Table 3).

Figure 12. General effect of (a) chilling duration and (b) forcing photoperiod on percentage budburst of *B. pubescens*, *F. sylvatica*, *S. x smithiana*, *S. aurita* and *T. cordata*, combined dataset. Data are from experiment 3.a (effect of chilling duration and forcing photoperiod). Vertical bars denote 0.95 confidence intervals.

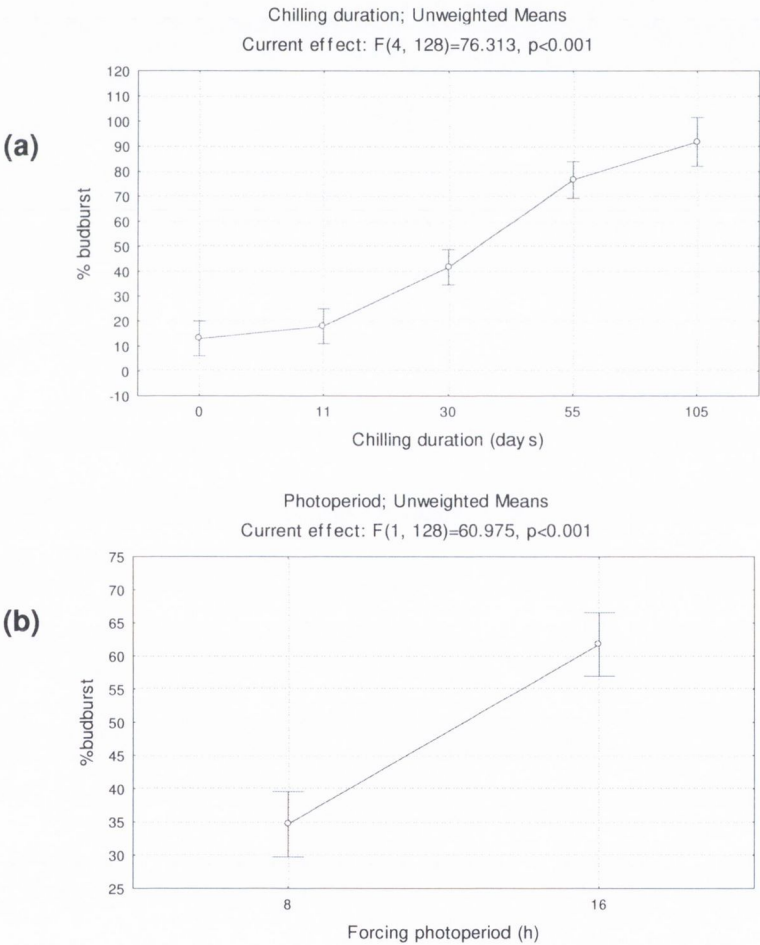


Figure 13. General effect of forcing temperature on timing of budburst in fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*, combined dataset. Data are from experiment 2.b (effect of forcing temperature). Vertical bars denote 0.95 confidence intervals.

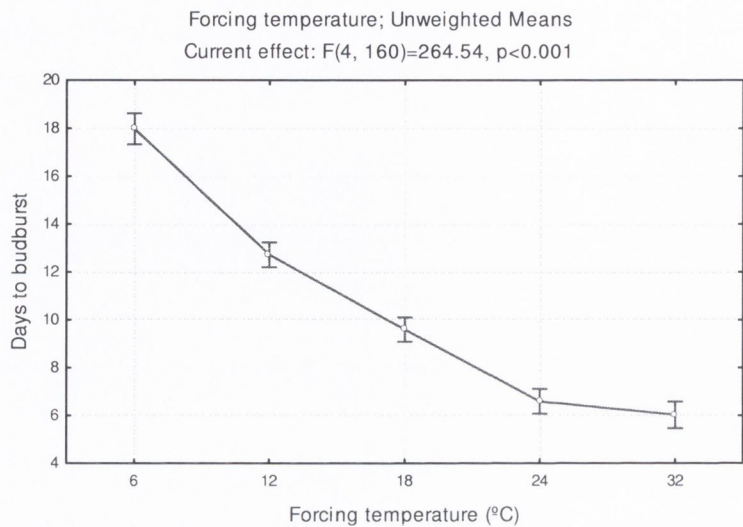


Figure 14. Photograph showing the more advanced phenological stage of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata* exposed to higher forcing temperatures (experiment 2.b).

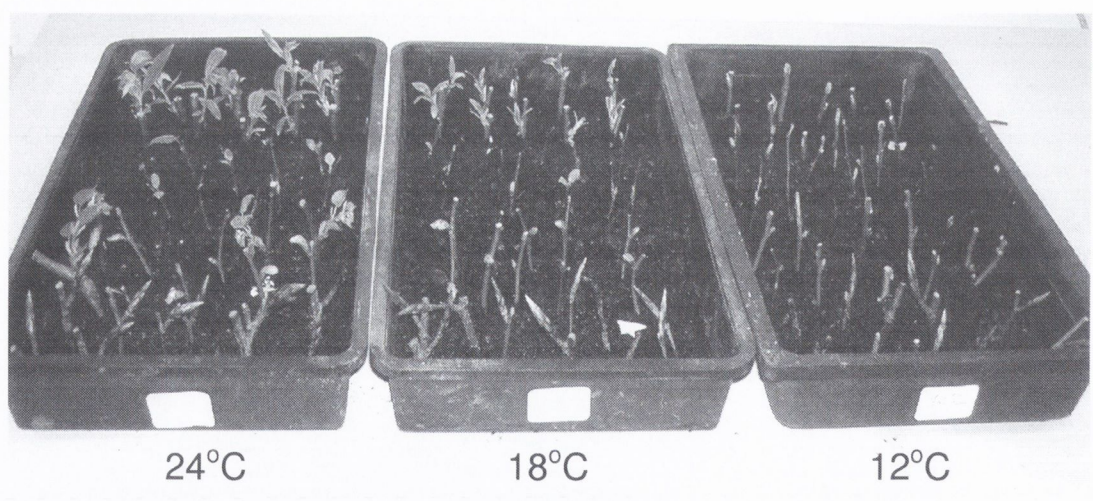


Figure 15. Relationship between forcing temperature and rate of forcing, (days to budburst at 32 °C / days to budburst) in fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*. The data are from experiment 2.b (effect of forcing temperature) and have been fitted with sigmoidal curves, whose coefficients and R^2 are given in Table 6.

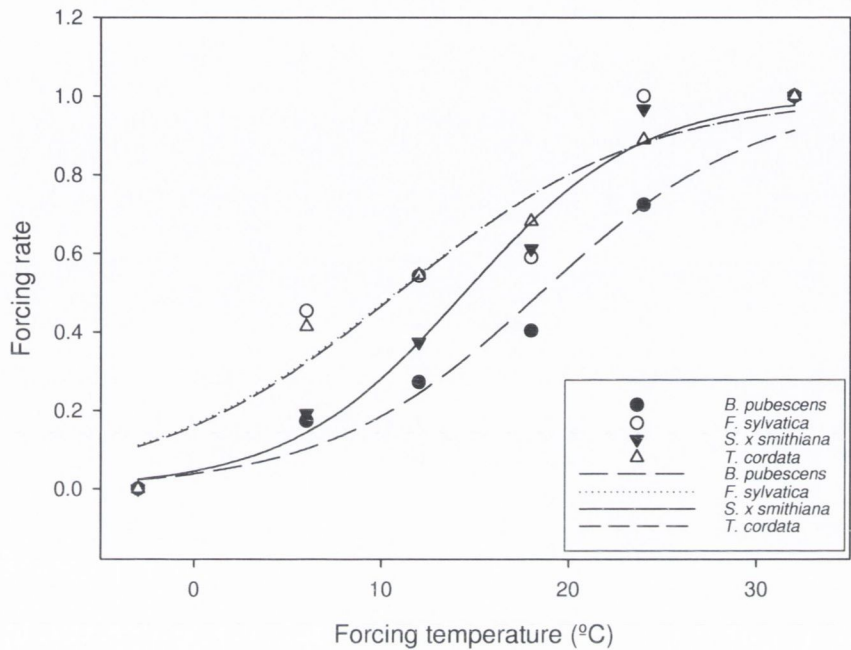


Table 6. Coefficients and R^2 of the sigmoidal curves $y = 1/(1+e^{b(x-c)})$ fitted to the rates of forcing of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata* (see Figure 15).

	<i>B. pubescens</i>	<i>F. sylvatica</i>	<i>S. x smithiana</i>	<i>T. cordata</i>
R^2	0.97	0.96	0.98	0.90
b	-0.174	-0.152	-0.211	-0.151
c	18.5	10.87	14.43	10.76

Main effects and differences among species

The significant species effect found in experiment 3.a suggested that we perform individual analyses for each species, which confirmed that forcing photoperiod significantly affected the timing of budburst in *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata* (P -values < 0.05) but not in *S. aurita* ($P=0.501$), and percentage budburst in *B. pubescens*, *F. sylvatica*, *S. aurita* and *S. x smithiana* (P -values < 0.05) but not in *T. cordata* ($P= 0.187$) (Table 3, see result section 3 for more details on this effect).

2.3 Light intensity and nutrients

High light intensity during forcing generally advanced the timing of budburst compared with low light intensity (Figure 16). A general ANOVA confirmed the significance of this effect in the 4 species investigated (*B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*) but also indicated that there was a significant difference among species (Table 5.d). The individual species analysis revealed significant effects of light intensity in *F. sylvatica* ($P < 0.001$) and *T. cordata* ($P=0.008$), but not in *B. pubescens* ($P= 0.071$) and *S. x smithiana* ($P=0.347$), (Table 4).

Nutrient deficiency generally delayed the timing of budburst compared with the control treatment (Figure 17). A general ANOVA confirmed the significance of the overall effect of nutrient availability on the timing of budburst in the 3 species investigated (*B. pubescens*, *S. x smithiana*, and *T. cordata*) ($P=0.006$) but also indicated that there was a significant difference among species as to time to budburst ($P < 0.001$) (Table 5.e). The individual species analysis revealed that the 3 species were all significantly affected by nutrient deficiency (P -values < 0.001) (Table 4).

Figure 16. General effect of forcing light intensity on timing of budburst in fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*, combined dataset. Data are from experiment 1.a (effect of light intensity). Vertical bars denote 0.95 confidence intervals.

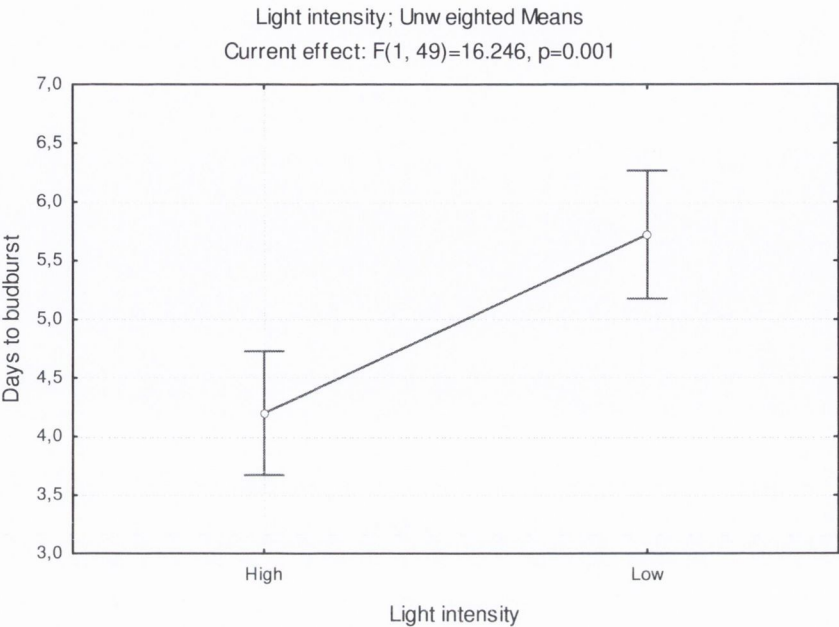
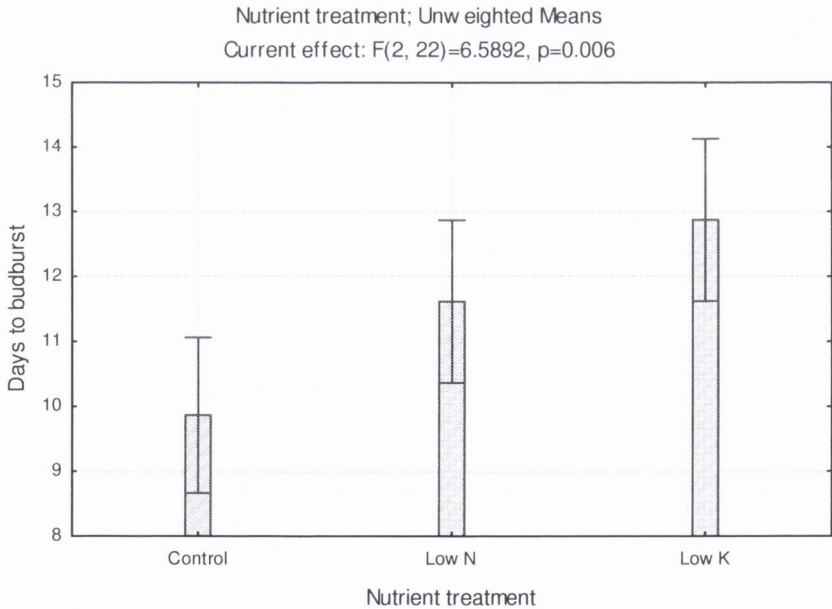


Figure 17. General effect nutrient deficiency on timing of budburst in *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*, combined dataset. Data are from experiment 1.a (effect of nutrient deficiency). Vertical bars denote 0.95 confidence intervals.



3. Effects and interactions of temperature, photoperiod, light intensity and nutrients on species

This section deals with the effects of the environmental factors under investigation for each of the species considered in the experiments. The main effects of temperature, photoperiod, light intensity, nutrient deficiency and their significant interactions are described in detail.

3.1 Temperature

3.1.1 Dormancy induction

3.1.1.1 *B. pubescens*

Low temperature (10°C) during dormancy induction increased the percentage of budburst and advanced the onset of budburst in chilled plants. These effects can be seen in Figure 18, which shows the variation in percentage budburst and time to budburst in chilled *B. pubescens* at the two dormancy induction temperatures used (10 and 18°C).

The ANOVA results (Table 7.b) confirmed the significance of the effect of dormancy induction temperature on both the timing of budburst and percentage budburst ($P = 0.04$ and $P < 0.001$, respectively). The main effects of dormancy induction temperature on time to budburst and percentage budburst are shown in Figure 19. The increase in dormancy induction temperature increased the number of days to budburst from 21 to 26 and decreased percentage budburst from 61% to 37% (Figure 19.a and b).

The direction of the effect of temperature during dormancy induction appeared to depend on the state of chilling: while high temperature advanced budburst from 32 to 22 days in unchilled plants, it delayed it from 21 to 31 (Figure 20.a) in chilled plants (interaction between dormancy induction temperature and presence/absence of chilling treatment on the timing of budburst, $P = 0.041$ Table 7.a). Correspondingly, high temperature during dormancy induction increased the percentage budburst from 8 to 30% in unchilled plants and decreased it from 61 to 37% in chilled plants (interaction between dormancy induction temperature and presence/absence of chilling treatment on percentage budburst, $P < 0.001$) (Table 7.a) (Figure 20.b).

Table 7. Analysis of variance for the time to budburst and percentage budburst in *B. pubescens*. (a) All data. (b) Subset of all chilled plants. (b.i) Effects of chilling photoperiod and dormancy induction conditions. Due to the missing data for timing of budburst in non-flushing plants receiving short dormancy induction at 18°C and chilling at 10°C and 8h photoperiod, for the analysis of the effect of chilling photoperiod it was necessary to group dormancy induction duration (DID) and temperature (DIT) into a single factor (DID+T) with 4 levels. A subset of 3 of these levels which had observations in all cells was then analysed, including 1. DID=30days and DIT=10°C; 2. DID=60days and DIT=10°C; 3. DID=60days and DIT=18°C; (b.ii) Effects of dormancy induction duration and dormancy induction temperature, subset of chilled plants. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration).

(a) All data											
Time to budburst						Percentage budburst					
	df	SS	MS	F	p		df	SS	MS	F	p
Dormancy Induction Duration (DID)	1	48.5	48.5	0.68	0.417	DID	1	2120.0	2120.0	5.55	0.024
Dormancy Induction Temperature (DIT)	1	43.4	43.4	0.61	0.442	DIT	1	4.0	4.0	0.01	0.923
Chilling presence/absence (Chill y/n)	1	64.9	64.9	0.91	0.349	Chill y/n	1	9522.0	9522.0	24.94	<0.001
DID*DIT	1	4.8	4.8	0.07	0.797	DID*DIT	1	837.0	837.0	2.19	0.147
DID*Chill y/n	1	62.8	62.8	0.88	0.357	DID*Chill y/n	1	19700.0	19700.0	51.58	<0.001
DIT*Chill y/n	1	331.4	331.4	4.64	0.041	DIT*Chill y/n	1	5552.0	5552.0	14.54	<0.001
DID*DIT*Chill y/n	1	41.3	41.3	0.58	0.454	DID*DIT*Chill y/n	1	1468.0	1468.0	3.85	0.057
(b) Chilled plants											
Time to budburst						Percentage budburst					
i)	df	SS	MS	F	p		df	SS	MS	F	p
Chilling Photoperiod (ChPht)	1	50.5	50.5	2.62	0.125						
Dormancy induction treatment (DID+T)	2	320.7	160.3	8.33	0.003	DID	1	25400.0	25400.0	115.71	<0.001
ChPht*DID+T	2	194.0	97.0	5.04	0.020	DIT	1	4299.0	4299.0	19.60	<0.001
ii)						ChPht	1	442.0	442.0	2.00	0.169
DID	1	216.9	216.9	7.89	0.011	DID*DIT	1	59.0	59.0	0.30	0.610
DIT	1	131.9	131.9	4.80	0.040	DID*ChPht	1	181.0	181.0	0.80	0.373
DID*DIT	1	17.5	17.5	0.64	0.433	DIT*ChPht	1	106.0	106.0	0.50	0.493

Figure 18. Mean percentage of budburst (bar plot) and mean days to budburst (line and scatter plot) in chilled *B. pubescens* exposed to dormancy induction durations of (a) 30 and (b) 60 days and temperatures of 10 and 18 °C. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration). 0% budburst was observed in plants receiving a 30 days dormancy induction at 18°C and a chilling photoperiod of 8h.

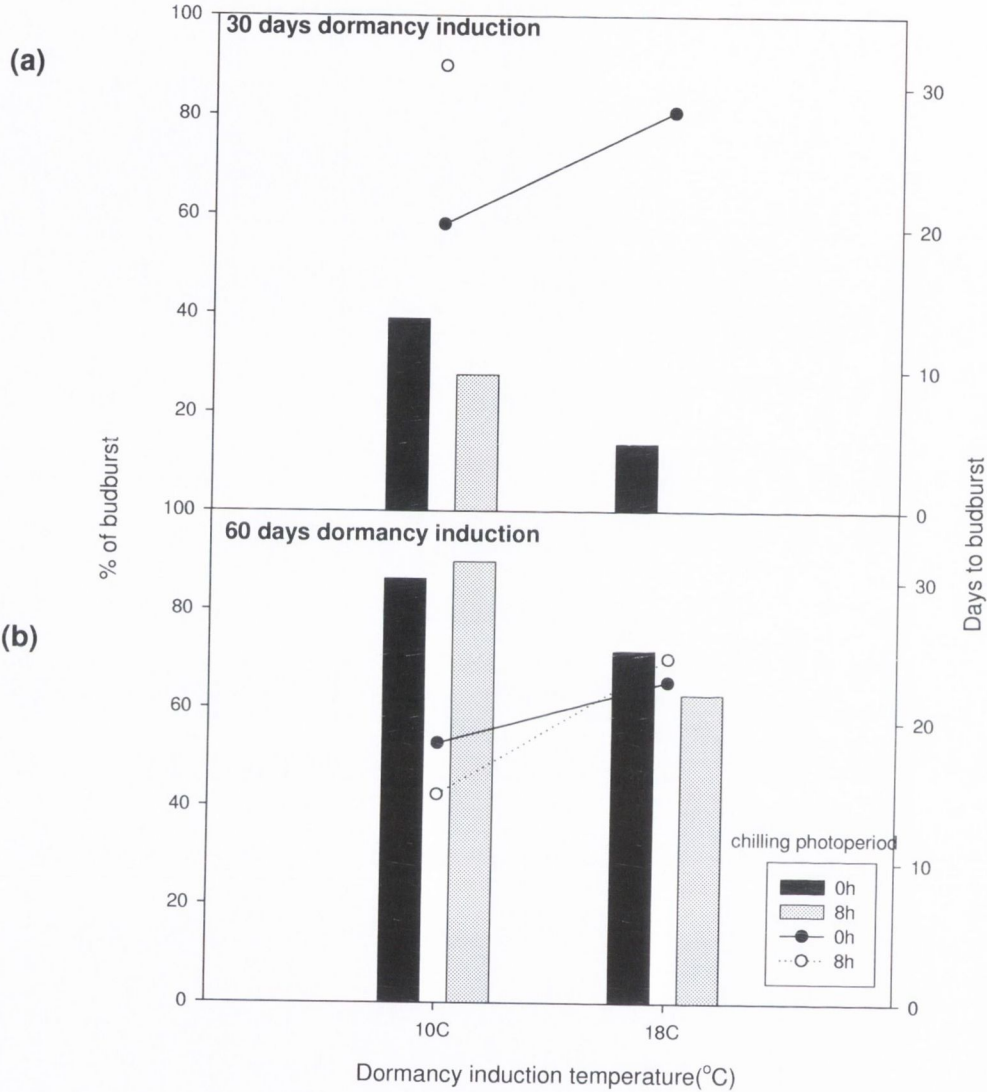


Figure 19. Effect of dormancy induction temperature on (a) time to budburst and (b) percentage budburst in chilled *B. pubescens*. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration). Vertical bars denote 0.95 confidence intervals.

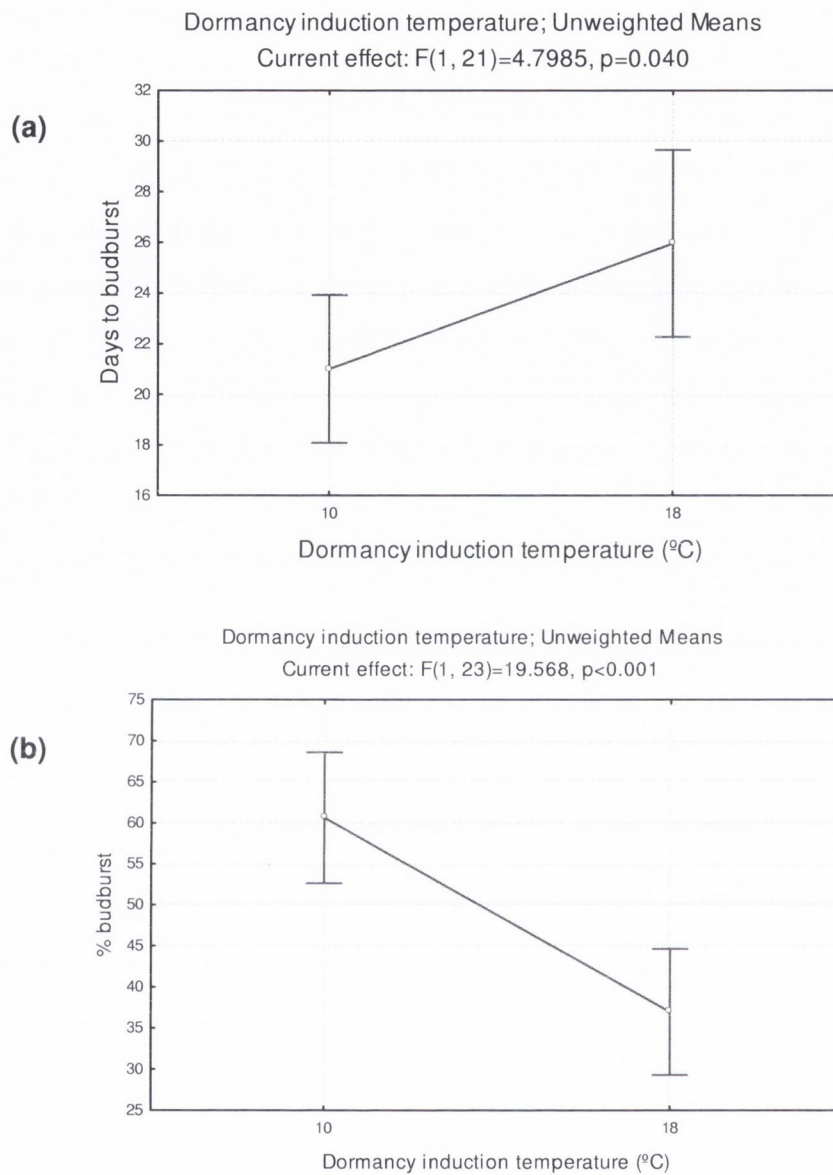
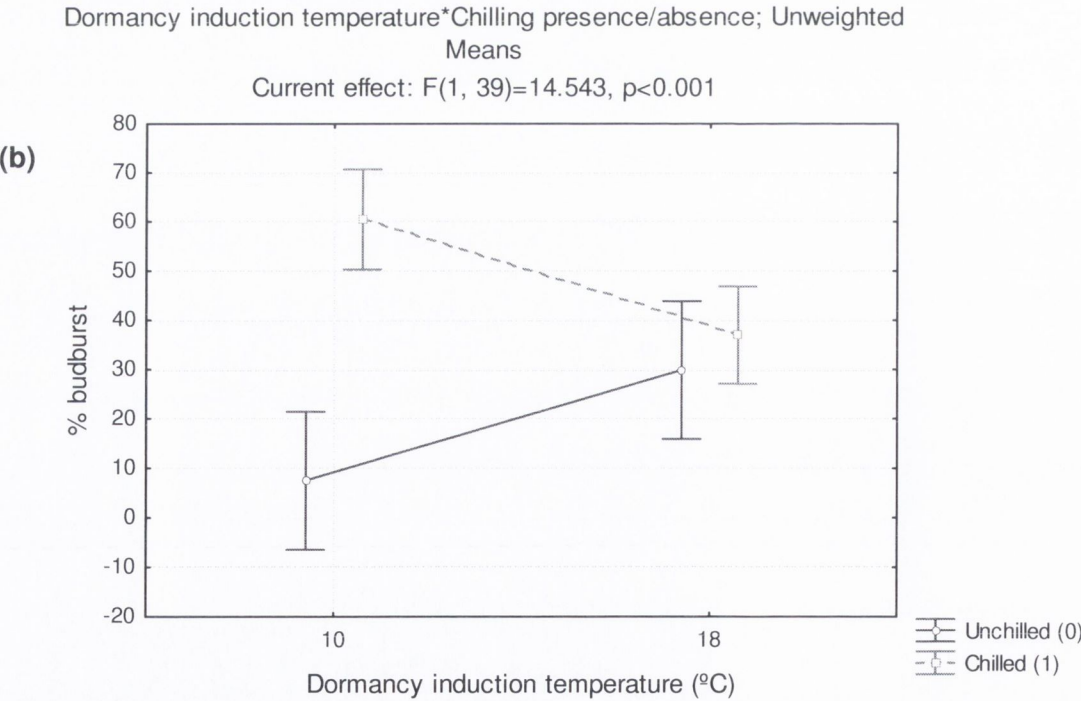
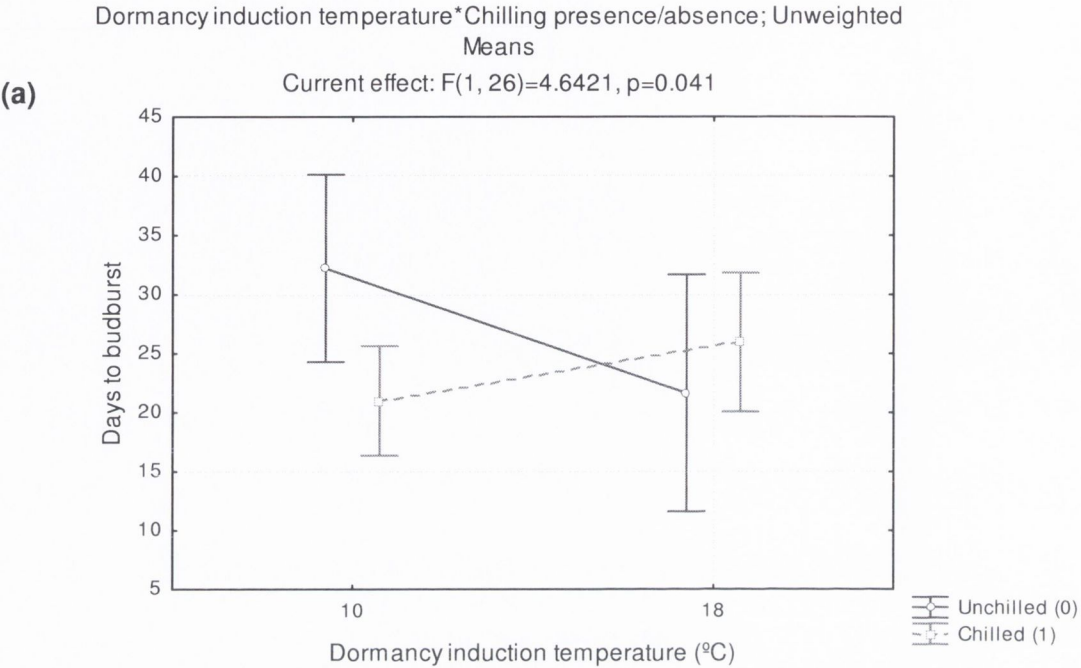


Figure 20. Effect of dormancy induction temperature on (a) time to budburst and (b) percentage budburst in chilled and unchilled *B. pubescens*. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration). Vertical bars denote 0.95 confidence intervals.



3.1.2 Chilling

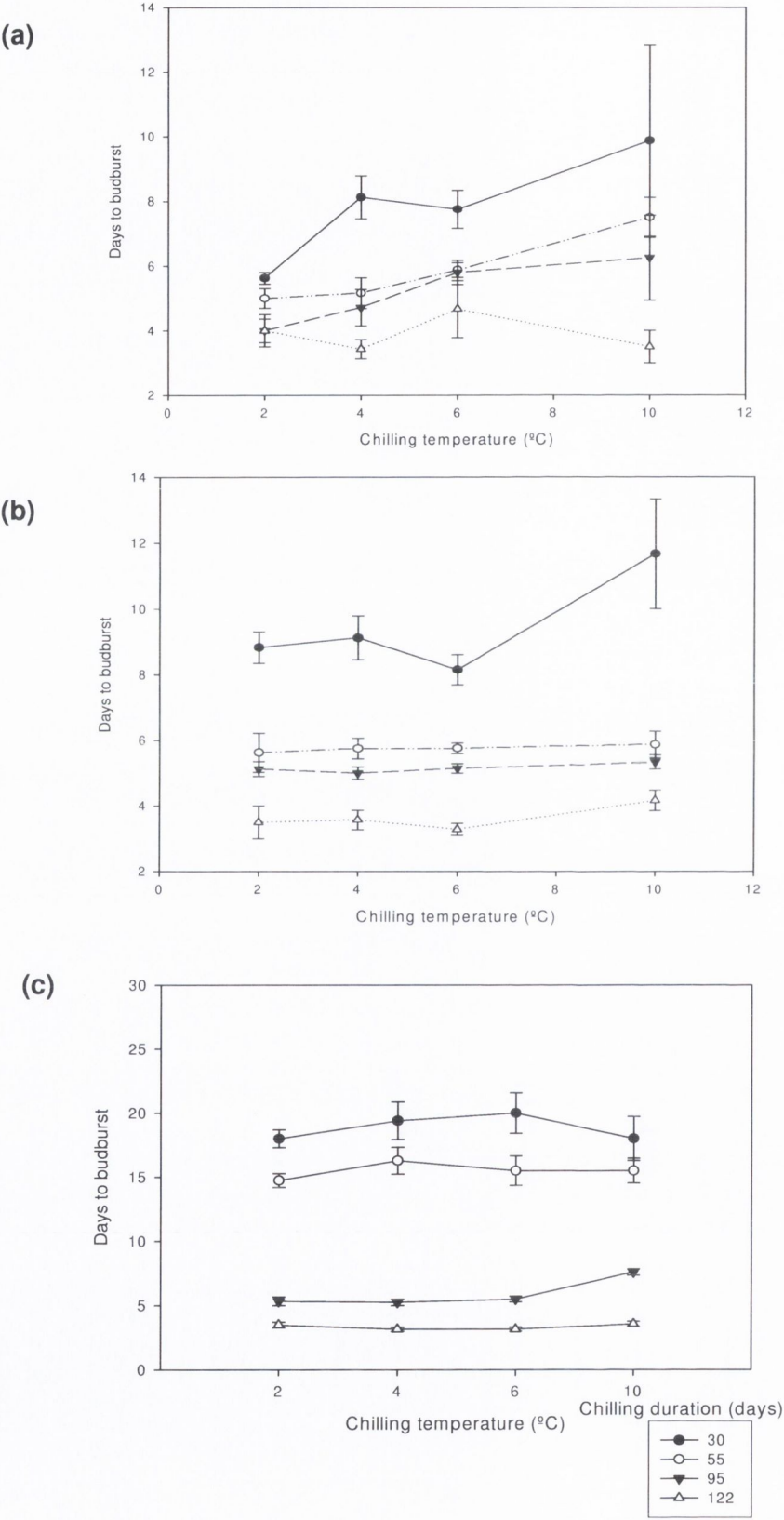
3.1.2.1 *B. pubescens*

Chilling duration had a significant effect on both the timing (Tables 8.a, 8.b, 9 and 10.a) and the percentage budburst (Tables 9 and 10.b) of *B. pubescens* (P -values < 0.05). Chilling temperature affected timing of budburst in experiment 4.a (Table 11.b) and in a subset of the results of experiment 2.a (Table 8.b). In general, lower chilling temperature and longer chilling duration advanced the timing of budburst and increased its percentage. Figure 21.a shows the interaction of chilling temperature with chilling duration on timing of budburst in experiment 2.a. Figure 22.a and 23.a show the effect of chilling duration and forcing photoperiod (SD and LD) on the timing and percentage of budburst in experiment 3.a. Figures 24.a and 24.b show the interaction between chilling duration and forcing photoperiod (different levels) on the timing and percentage of budburst in experiment 3.b.

Table 8. Analysis of variance for the effects of chilling temperature and duration on time to budburst in *B. pubescens* (a) all data (b) subset of plants receiving only 30, 55 and 95 chilling days, (c) subset of plants receiving 122 chilling days (d), *S. x smithiana* and (e) *T. cordata*. Data are from experiment 2.a (effect of chilling temperature and duration).

(a) <i>B. pubescens</i> (all data)					
	df	SS	MS	F	p
Chilling Temperature (ChT)	3	34.4	11.5	1.59	0.198
Chilling Duration (ChD)	3	225.7	75.3	10.43	<0.001
ChT*ChD	9	41.7	4.6	0.64	0.757
(b) <i>B. pubescens</i> (chilling durations of 30, 55 and 95 days)					
	df	SS	MS	F	p
ChT	3	71.2	23.7	2.89	0.041
ChD	2	152.2	76.1	9.26	<0.001
ChT*ChD	6	24.6	4.1	0.5	0.808
(c) <i>B. pubescens</i> (chilling duration of 122 days)					
	df	SS	MS	F	p
ChT	3	4.2	1.4	3.23	0.065
(d) <i>S. x smithiana</i>					
	df	SS	MS	F	p
ChT	3	17.9	6.0	4.93	0.003
ChD	3	410.1	136.7	113.22	<0.001
ChT*ChD	9	19	2.1	1.75	0.089
(e) <i>T. cordata</i>					
	df	SS	MS	F	p
ChT	3	7	2	0.7	0.584
ChD	3	3429	1143	339.1	<0.001
ChT*ChD	9	32	4	1.1	0.400

Figure 21. Relationship between chilling temperature and number of days to budburst following different chilling durations for one-node budsticks of *B. pubescens* (a), *S. x smithiana* (b) *T. cordata* (c). Data are from experiment 2.a (effect of chilling temperature and duration). Vertical bars denote standard errors of the mean.



Chilling temperature

In experiment 2.a, chilling temperatures of 2 and 4°C decreased the mean time to budburst for *B. pubescens* when compared to 10°C. In fact, the average number of days to budburst across all chilling durations for 2 and 4°C were, respectively, 4.9 and 5.4 as opposed to 6.4 days at 10°C. Similarly, chilling duration progressively decreased the mean time to budburst from 8.1 days (average number of days to budburst across all chilling temperatures for 30 days of chilling) to 3.9 days (average number of days to budburst across all chilling temperatures for 122 days of chilling). However, the effect of chilling temperature on timing of budburst was significant only when considering the first three chilling durations (30, 55 and 95 days), ($P=0.041$) and excluding the longest chilling duration (122 days) from the analysis (see Tables 8.a and b). Accordingly, the analysis of the subset of one-node budsticks that received 122 days of chilling did not show any effects of chilling temperature on timing of budburst (Table 8.c). These effects can be seen in Figure 21.a, showing the number of days to budburst in relation to chilling temperature, grouped by different chilling durations. In the graph the delaying effects of high chilling temperature are evident only for plants receiving 30, 55 and 95 days of chilling, while after 122 days of chilling the range of investigated chilling temperatures elicits similar responses. However, the significance of these effects should be considered with caution, due to the observed variance heterogeneity and its correlation with the group means.

Optimal chilling temperature

Despite the significance of the effects of chilling temperature on timing of budburst after short chilling durations, a post-hoc pairwise comparison confirmed that, in the range of chilling temperature tested (2-10°C), 2, 4 and 6°C had equal effects on the timing of budburst (results not shown). There was no consistent effect of chilling temperature on subsequent time to budburst, nor was an unequivocally optimal chilling temperature found: the most effective temperature for dormancy release depended on the duration of the chilling treatment and appeared to be 2, 2-4, 2 and 4°C after 30, 55, 95 and 122 days of chilling, respectively (Figure 21.a). In addition, the effect of chilling temperature in the tested range was smaller in magnitude than that of chilling duration. In fact, while the largest mean difference in timing of budburst attributable to chilling temperature in the range 2-10°C was 1.5 days (time to budburst at 10°C averaged across chilling durations –

time to budburst at 2°C averaged across chilling durations), increasing chilling duration (from 30 to 122 days) caused a budburst advance of 4.2 days (time to budburst after 122 days of chilling averaged across chilling temperatures –time to budburst after 30 days of chilling averaged across chilling temperatures).

Chilling duration

Chilling duration affected the timing of budburst and percentage budburst in experiment 3.a ($P<0.001$, $P<0.001$, respectively) (Table 9) and experiment 3.b ($P<0.001$, $P<0.001$, respectively) (Table 10). In experiment 3.a, the mean time to budburst decreased from 48 to 3 days in LD as chilling duration increased from 0 to 105 days, and from 35 to 5 days in SD as chilling duration increased from 29 to 105 days. Similarly the mean percentage budburst (averaged across the two forcing photoperiods) increased from 31.3% to 100% as chilling duration progressively increased from 0 to 105 days of chilling. These effects can be seen in Figures 22.a and 23.a, representing the responses of timing of budburst and percentage budburst to increasing chilling duration in long and short forcing photoperiods. The graphs show that increasing chilling duration decreases the time to budburst (Figure 22.a) and progressively increases percentage budburst (Figure 23.a) in both photoperiods.

In experiment 3.b the mean time to budburst decreased from 19 to 10 days and the mean percentage budburst increased from 21% to 97% (averaged across photoperiods) as chilling duration progressively increased from 0 to 95 days of chilling. Figures 24.a and 24.b represent the response of timing of budburst and percentage budburst to a range of forcing photoperiods after various chilling durations (0, 30, 55 and 95 chilling days) in experiment 3.b. The line plots in the two figures show that as chilling duration increased, time to budburst became progressively shorter (Figure 24.a) and percentage budburst increased (Figure 24.b).

By contrast, in experiment 3.c, (testing the presence or absence of chilling duration), the timing of budburst was not affected by the absence of chilling when compared to chilled treatments, differently from percentage budburst that was significantly affected by this factor ($P < 0.001$) (Table 7). The absence of chilling resulted in a decrease in percentage budburst from 48.7% to 18.7%, shown in Figure 25. The combined effects of dormancy induction duration, temperature and chilling presence/absence on percentage budburst and

time to budburst are shown in Figure 26, showing that the exposure to chilling reverses the effects of dormancy induction temperature on timing and percentage of budburst (see previous section on the effects of temperature on dormancy induction).

Table 9. Analysis of variance for the time to budburst and percentage budburst of *B. pubescens*, *F. sylvatica*, *S. aurita*, *S.x smithiana* and *T. cordata*. Data are from experiment 3.a (effect of chilling duration and forcing photoperiod). Percentage budburst: all species analysed for main and interaction effects of chilling duration (0, 11, 30, 55 and 105 days)*photoperiod (LD and SD). Time to budburst: due to missing blocks, caused by absence of budburst in treatments receiving short chilling durations, subsets of the data with observations in all cells were selected. **B. pubescens**: main and interaction effects of chilling duration (30, 55 and 105 days)*photoperiod (LD and SD). **F.sylvatica**: main effects of chilling duration (all levels) on LD plants, main effects of photoperiod (LD and SD) on plants exposed to 105 chilling days. **S. aurita**: main and interaction effects of chilling duration (55 and 105 days)*photoperiod (LD and SD). **S. x smithiana**: main and interaction effects of chilling duration (30, 55 and 105 days)*photoperiod (LD and SD). **T. cordata**: main and interaction effects of chilling duration (55 and 105 days)*photoperiod (LD and SD).

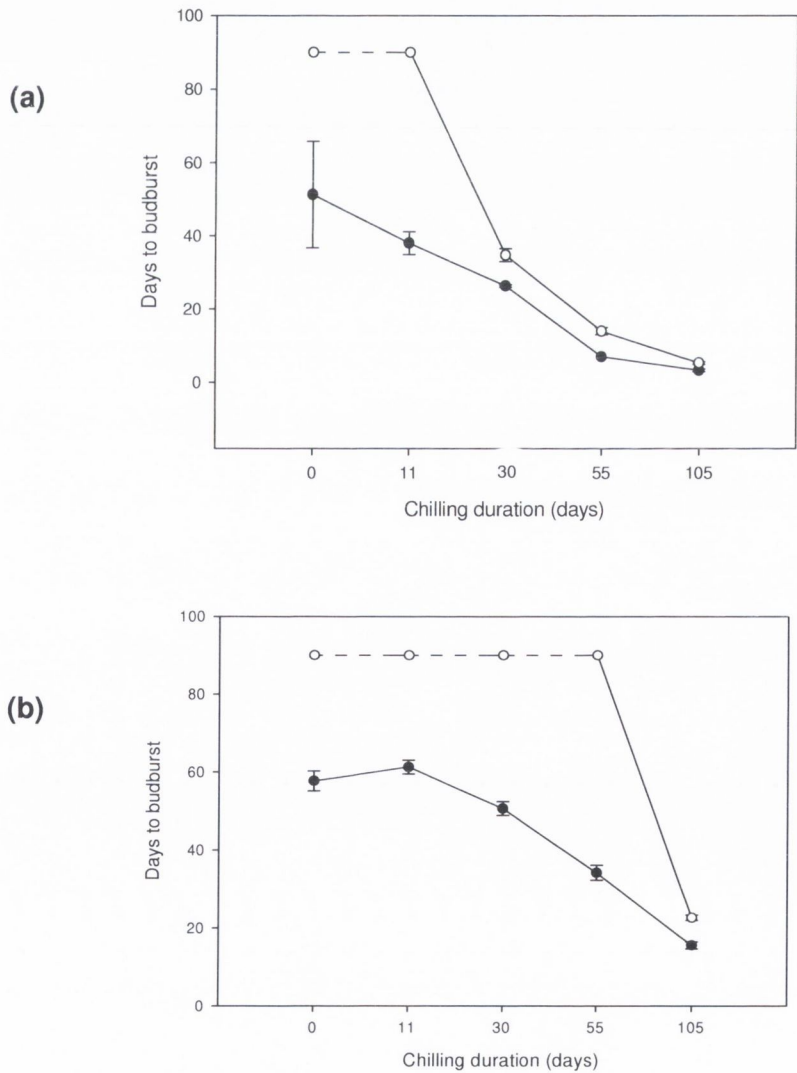
		Time to budburst					Percentage budburst				
		df	SS	MS	F	p	df	SS	MS	F	p
B. pubescens	Chilling Duration (ChD)	2	2182	1091	1323	<0.001	4	24900	6231	10.22	<0.001
	Photoperiod (Pht)	1	148	148	179	<0.001	1	14400	14400	23.55	<0.001
	ChD*Pht	2	36	18	22	<0.001	4	10900	2723	4.47	0.008
F.sylvatica	ChD	4	5743	1436	104.1	<0.001	4	15115.7	3778.9	17.92	<0.001
	Pht	1	99.41	99.41	37.72	0.001	1	39133.8	39133.7	185.55	<0.001
	ChD*Pht						4	6926.4	1731.6	8.21	<0.001
S.aurita	ChD	1	195	195	34.89	<0.001	4	45400	11300	131.1	<0.001
	Pht	1	2.7	2.7	0.49	0.501	1	460	460	5.3	0.029
	ChD*Pht	1	1.5	1.5	0.27	0.617	4	928	232	2.7	0.052
S. x smithiana	ChD	2	2773	1386	123.5	<0.001	4	2448.3	1224.2	109.05	<0.001
	Pht	1	216	216	19.3	0.001	1	161.6	161.6	14.4	0.002
	ChD*Pht	2	217	108	9.7	0.002	4	216.7	108.3	9.65	0.002
T. cordata	ChD	1	444.2	444.2	50.73	<0.001	4	56600	14200	16.1	<0.001
	Pht	1	65.2	65.2	7.45	0.018	1	1608	1608	1.83	0.187
	ChD*Pht	1	32.2	32.2	3.68	0.079	4	2199	550	0.63	0.648

Table 10. Analysis of variance for the time to budburst (a) and percentage budburst (b) of *B. pubescens*. Data are from experiment 3.b (Effect of chilling duration and forcing photoperiod (10, 12, 14, 16 h)).

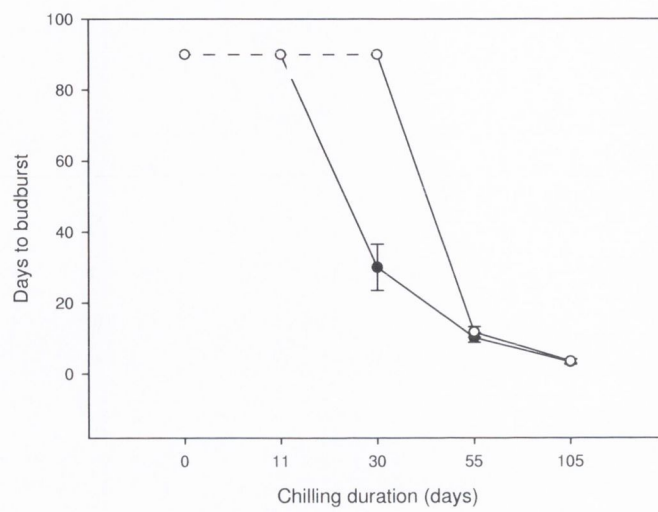
(a)Time to budburst					
	df	SS	MS	F	p
Chilling Duration (ChD)	2	2012.0	1006.0	14.16	<0.001
Photoperiod (Pht)	2	452.0	226.0	3.18	0.051
ChD*Pht	4	309.0	77.0	1.09	0.374

(b) Percentage budburst					
	df	SS	MS	F	p
Chilling Duration (ChD)	3	8.7	2.9	19.25	<0.001
Photoperiod (Pht)	3	4.9	1.6	10.91	<0.001
ChD*Pht	9	2.9	0.3	2.13	0.032

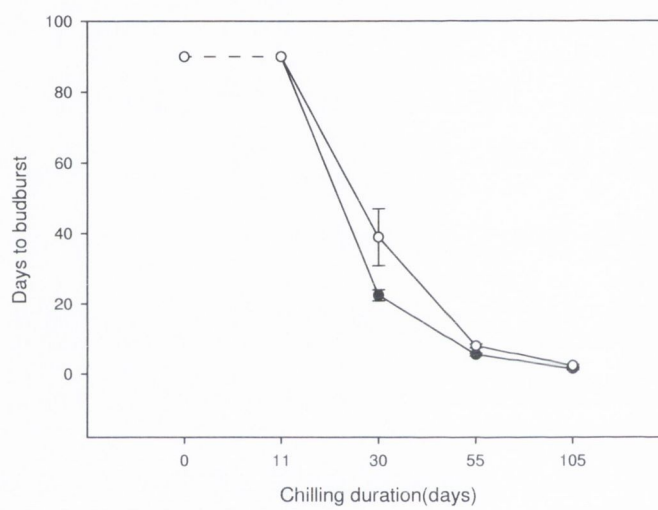
Figure 22. Relationship between chilling duration and number of days to budburst for (a) *B. pubescens*, (b) *F. sylvatica*, (c) *S. aurita*, (d) *S. x smithiana*, (e) *T. cordata*, grouped by forcing photoperiod: 16 h (filled symbols) and 8 h (open symbols). Non flushing plants have been symbolically represented as flushing after 90 days (data connected with a broken line), which was the total duration of the forcing treatment. Data are from experiment 3.a (effect of chilling duration and forcing photoperiod). Vertical bars denote standard errors of the mean.



(c)



(d)



(e)

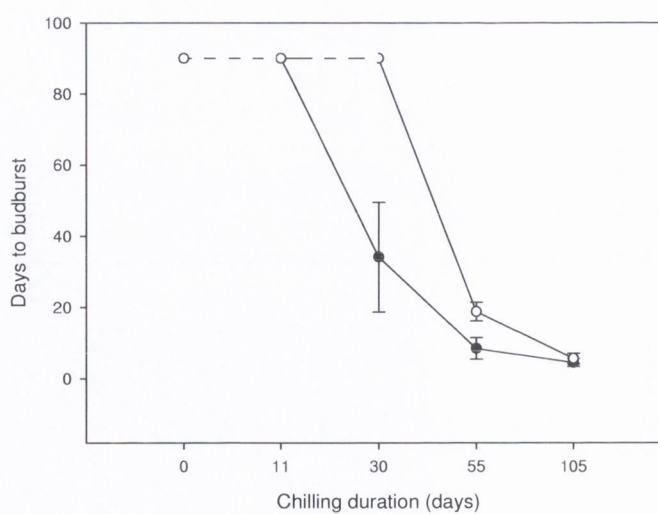
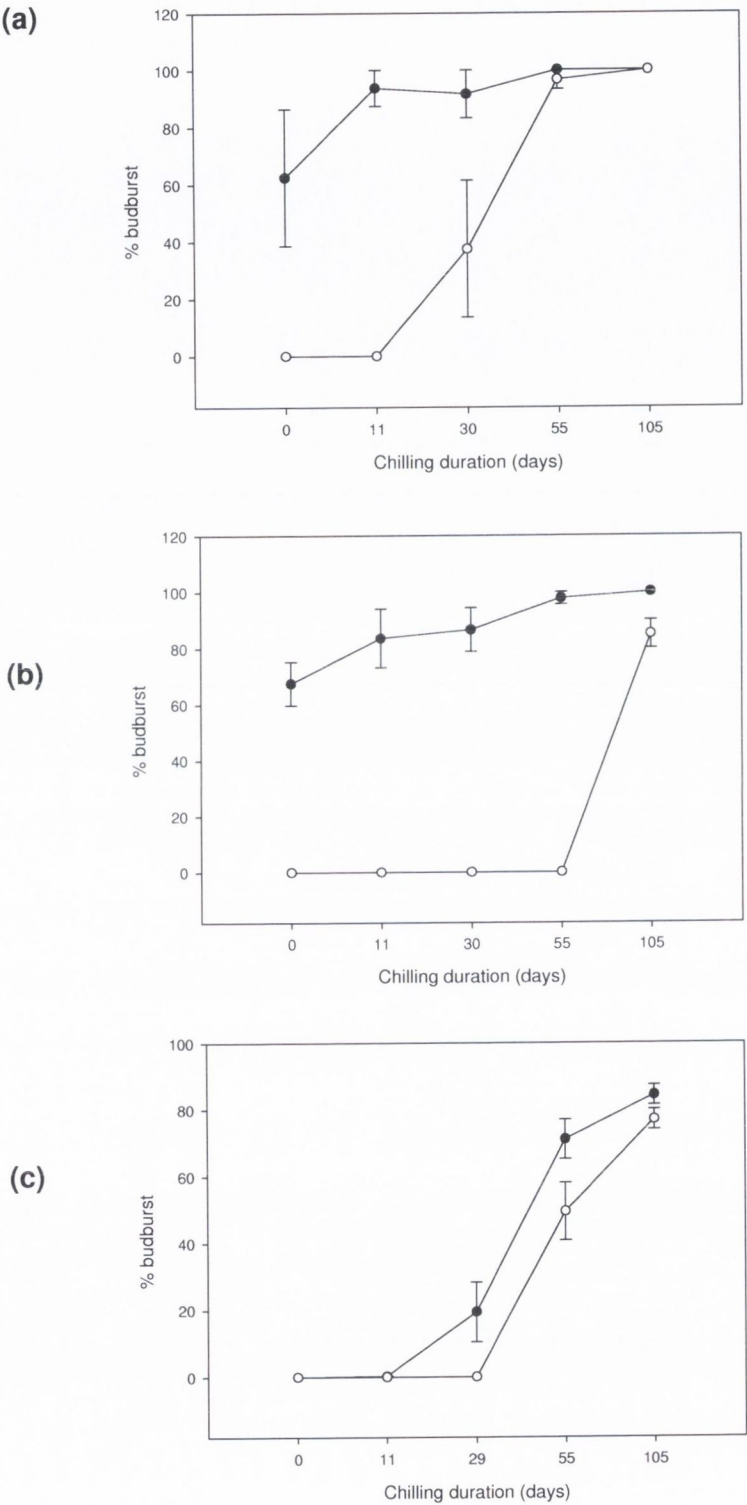
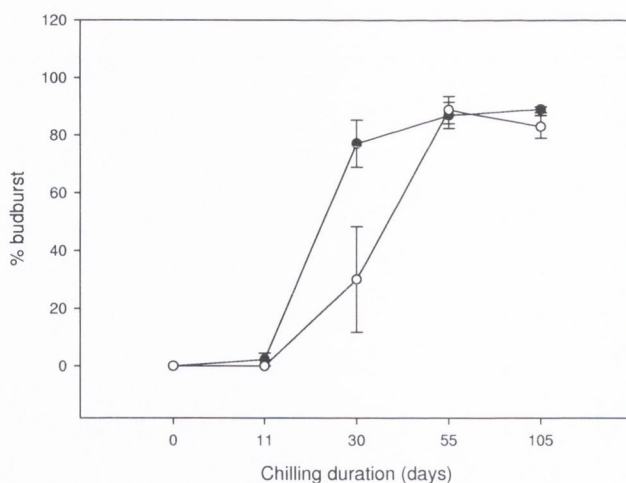


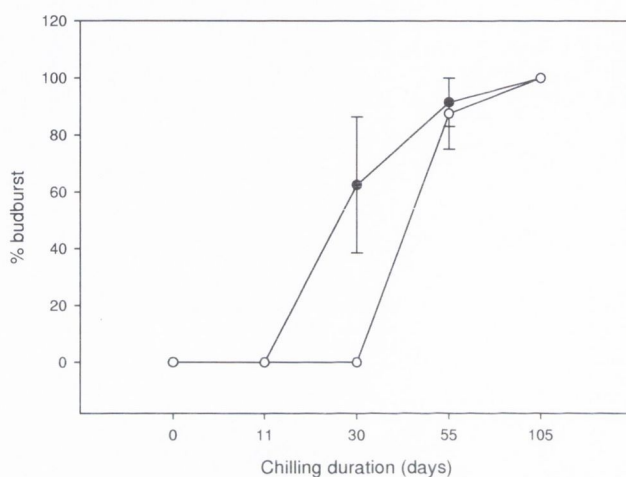
Figure 23. Relationship between chilling duration and percentage budburst for (a) *B. pubescens*, (b) *F. sylvatica*, (c) *S. aurita*, (d) *S. x smithiana*, (e) *T. cordata*, grouped by forcing photoperiod: 16 h (filled symbols) and 8 h (open symbols). Data are from experiment 3.a (effect of chilling duration and forcing photoperiod). Vertical bars denote standard errors of the mean.



(d)



(e)



Minimum chilling requirement

There were inconsistencies among the experiments also as to the minimum chilling requirement for dormancy release (minimum chilling duration leading to a percentage budburst equal or >50%) in *B. pubescens*, which varied among the experiments and depended on the forcing photoperiod. In experiment 3.c., LD-forced unchilled plants could not generally resume growth (only 2 plants out of 16 showed a percentage budburst > 50%, both having received short dormancy induction treatments) while in experiment 3.a, LD-forced unchilled plants were generally able to flush (3 out of 4 plants showed a percentage budburst of 50% or more). On the other hand, the minimum chilling requirement for SD-forced plants was between 30 and 55 days in experiment 3.a (in which chilling took place in the dark at a constant temperature of 3°C and was followed by 8 h, 18°C forcing) (Figure 22.a) and between 55 and 95 days in experiment 3.b, (in which natural chilling took place outside at a mean temperature of 6.5°C, and was followed by 10 h, 22°C forcing) (Figure 24.a).

Figure 24. Relationship between forcing photoperiod and (a) number of days to budburst and (b) percentage budburst in *B. pubescens* one-node budsticks exposed to different chilling durations. Non flushing plants have been represented as flushing after 80 days (data connected by a broken line), which was the total duration of the forcing treatment. Data are from experiment 3.b (effect of chilling duration and different forcing photoperiods (10, 12, 14, 16 h)). Vertical bars denote standard errors of the mean.

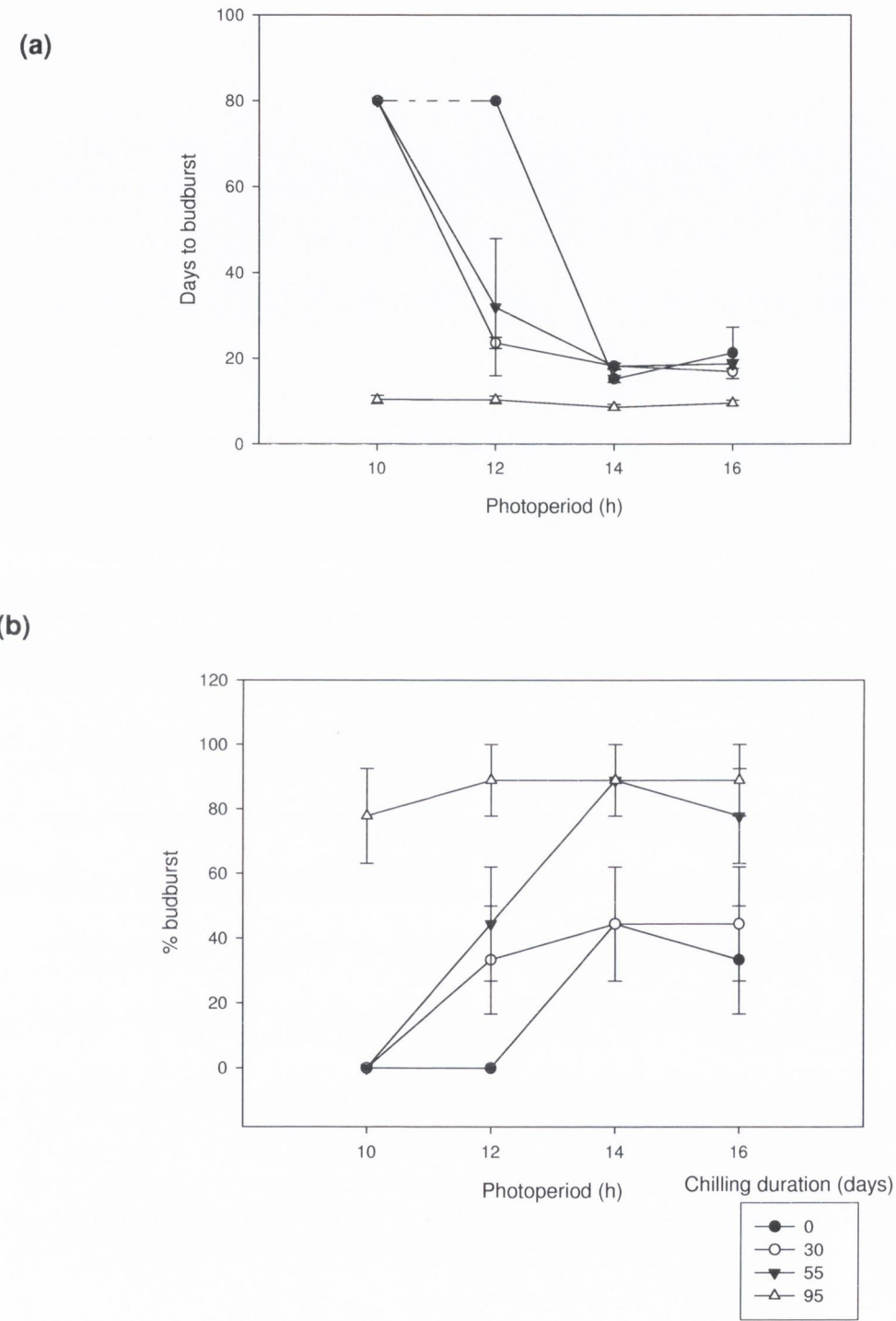
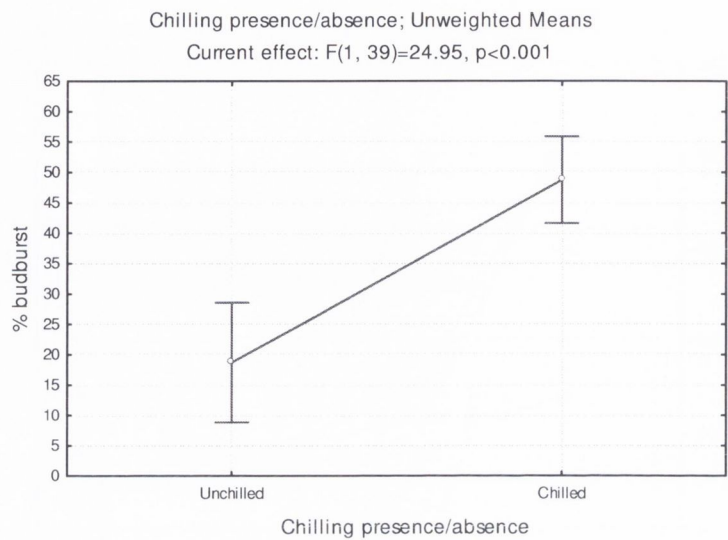


Figure 25. Effect of chilling presence/absence on percentage budburst (b) in *B. pubescens*. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration). Vertical bars denote 0.95 confidence intervals.



Interaction between chilling temperature and the photoperiod received during dormancy induction

Chilling temperature interacted significantly with the photoperiod received during dormancy induction ($P<0.001$) on the arcsin transform of percentage budburst ($\arcsin(\sqrt{\text{percentage budburst}/100})$), which is proportional to percentage budburst (Table 11). In fact, plants chilled at lower temperature showed a larger percentage budburst, but this increase was larger if the photoperiod received during dormancy induction was short (from 5.2 to 85.5 %) rather than long (from 7.8 to 52.3 %). The significant interaction of chilling temperature and dormancy induction photoperiod on the arcsin transform of budburst is shown in Figure 27.

Table 11. Analysis of variance for the time to budburst and percentage budburst of *B. pubescens*. Percentage budburst was arc-sin transformed to homogenise variance. Due to missing blocks, caused by either the experimental design or the absence of budburst in treatments receiving chilling at 18°C and forcing at a constant temperature of 15°C and photoperiod of 12 h (forcing treatment 5), subsets of the data with observations in all cells were selected. These subsets include (a) **time to budburst**: all observations except those receiving forcing treatment 5; **percentage budburst**: all data; (b) **time to budburst**: all observations but those receiving chilling at a photoperiod of 16 h and those receiving forcing treatment 5; **percentage budburst** all observations but those receiving chilling at a photoperiod of 16 h; (c) **time to budburst**: all observations but those receiving chilling at a temperature of 18 °C and those receiving forcing treatment 5; **percentage budburst** all observations but those receiving chilling at a temperature of 18°C. Data are from experiment 4.a (effect of dormancy induction, chilling and forcing temperatures and photoperiods).

	Time to budburst					arcsin($\sqrt{(\% \text{Budburst}/100)}$)				
	df	SS	MS	F	p	df	SS	MS	F	p
(a)										
Dormancy Induction Photoperiod (DIPht)	1	160	160	9.88	0.003	1	0.9	0.9	13.9	<0.001
Chilling Treatment	2	490	245	15.12	<0.001	2	16.8	8.4	129.8	<0.001
Forcing Treatment	3	2840	946.6	58.41	<0.001	4	0.4	0.1	1.4	0.228
(b)										
Chilling Temperature (ChT)	1	709000	709000	55.53	<0.001	1	14	14	229	<0.001
DIPht*ChT	1	14200	14200	1.11	0.297	1	1	1	15.7	<0.001
(c)										
Chilling Photoperiod (ChPht)	1	1247	1247	0.83	0.365	1	0	0	0.07	0.786
DIPht*ChPht	1	1103	1103	0.74	0.395	1	0	0	0.08	0.777

3.1.2.2 *F. sylvatica*

Chilling duration

Chilling duration had significant effects on both percentage budburst and timing of budburst in *F. sylvatica* (P -values < 0.001) (experiment 3.a, Table 9). Longer chilling duration enhanced percentage budburst (Figure 23.b) and advanced its timing (Figure 22.b). In LD treatments, the mean time to budburst decreased from 57.7 to 15.6 days, while in SD treatments, no budburst was observed until the plants had received at least 105 days of chilling, and even then flushing in SD was delayed (22.7 days as opposed to 15.6 days). Correspondingly, the percentage budburst (averaged across forcing photoperiods) increased from 62.5% to 99% as chilling duration increased from 0 to 105 days, even though, as said above, these mean values are not very informative, due to the strong effect of photoperiod (see section 3.2.3.2 for more details on this effect). These patterns are presented in Figure

22.b and 23.b, showing the effect of chilling duration on time to budburst and percentage budburst, respectively. The plots, grouped by photoperiod, show the effects that chilling duration exerts on timing and percentage of budburst.

Minimum chilling requirement

The minimum chilling requirement for budburst in *F. sylvatica* differed in different forcing photoperiods. While unchilled plants were able to flush in LD with percentage budbursts of 50% or more, SD forcing triggered budburst only after 105 days of chilling at 3°C (experiment 3.a) (Figure 23.b).

3.1.2.3 S. aurita

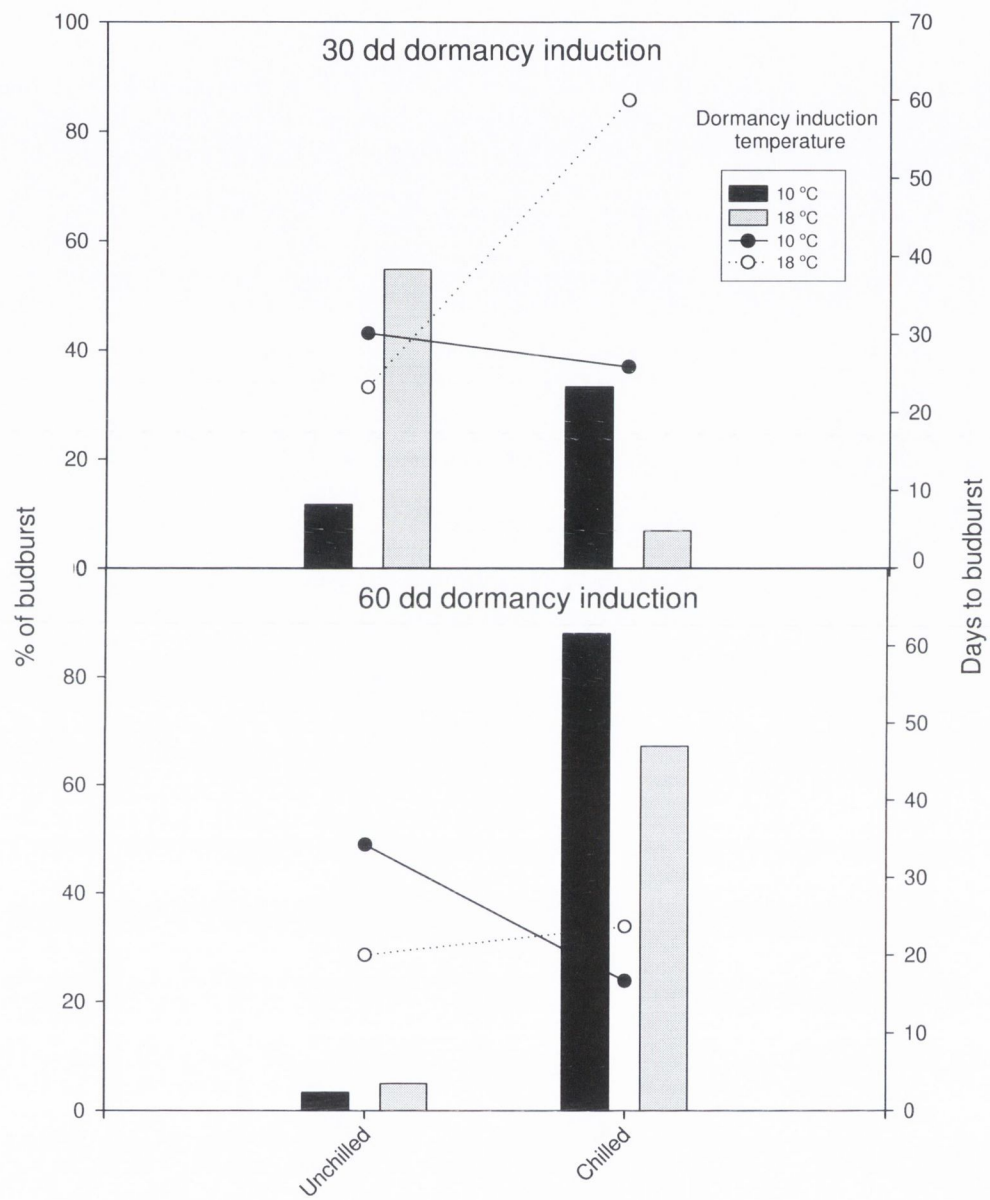
Chilling duration

Chilling duration had a significant effect on both percentage budburst and timing of budburst in *S. aurita* ($P < 0.001$, $P < 0.001$; respectively) (experiment 3.a, Table 9). Longer chilling duration enhanced percentage budburst and advanced its timing. The mean time to budburst decreased from 30 to 3.4 days in LD as chilling duration increased from 30 to 105 days, and from 11 to 3.6 days in SD as chilling duration increased from 55 to 105 days (Figure 22.c). As chilling duration progressed from 0 to 105 days, percentage budburst (averaged across forcing photoperiods) increased from 0 to 74.5% (Figure 23.c).

Minimum chilling requirement

The minimum chilling requirement at 3°C for budburst in *S. aurita*, as shown by experiment 3.a, was between 30 and 55 days for both LD and SD forcing treatments. The percentage budburst after a chilling duration of 55 days was 71% in LD and approximately 50% in SD (Figure 23.c).

Figure 26. Mean percentage of budburst (bar plot) and mean number of days to budburst (line and scatter plot) in chilled and unchilled *B. pubescens* exposed to dormancy induction durations of (a) 30 and (b) 60 days, and temperatures of 10 and 18 °C. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration).



3.1.2.4 *S. x smithiana*

Chilling temperature and duration

Chilling temperature and duration had a significant effect on both percentage budburst and timing of budburst in experiment 2.a (effects of chilling temperature and duration $P<0.01$) (Table 8.d) and 3.a (effect of chilling duration $P<0.001$) (Table 9). Low chilling temperatures and longer chilling durations increased percentage budburst and advanced its timing in all the experiments testing these effects (Figures 21.b, 22.d and 23.d).

In experiment 2.a, chilling temperatures of 2, 4 and 6°C resulted in a decrease in time to budburst from 6.8 (average of the days to budburst across the temperatures of 2, 4 and 6°C and all chilling durations) to 5.8 days (average of the days to budburst at 10°C across all chilling durations), and the mean time to budburst progressively decreased from 9.4 to 3.6 days (averaged across chilling temperatures) as chilling duration increased from 30 to 122 chilling days (Figure 21.b). However, the significance of these effects should be considered with caution, due to the observed variance heterogeneity and its correlation with the group means.

In experiment 3.a., as chilling duration increased from 30 to 105 days the time to budburst decreased from 22.4 to 1.5 days in LD and from 39 and 2.3 in SD (Figure 22.d). Percentage budburst (averaged across forcing photoperiods) increased from 0 to 86.6% as chilling duration increased from 0 to 105 days (Figure 23.d).

Minimum chilling requirement

The minimum chilling requirement at 3°C for budburst of *S. x smithiana*, as shown by experiment 3.a, was between 11 and 30 days in LD (the mean percentage budburst was 77%) and between 30 and 55 days in SD (the mean percentage budburst was 88%). (Figure 23.d).

Optimal chilling temperature

Similar to the results obtained for *B. pubescens*, it was not possible to identify an optimal chilling temperature in the range 2-10°C, but rather a threshold (between 6 and 10°C) under which chilling was more effective, but uniformly so. The number of days to budburst after

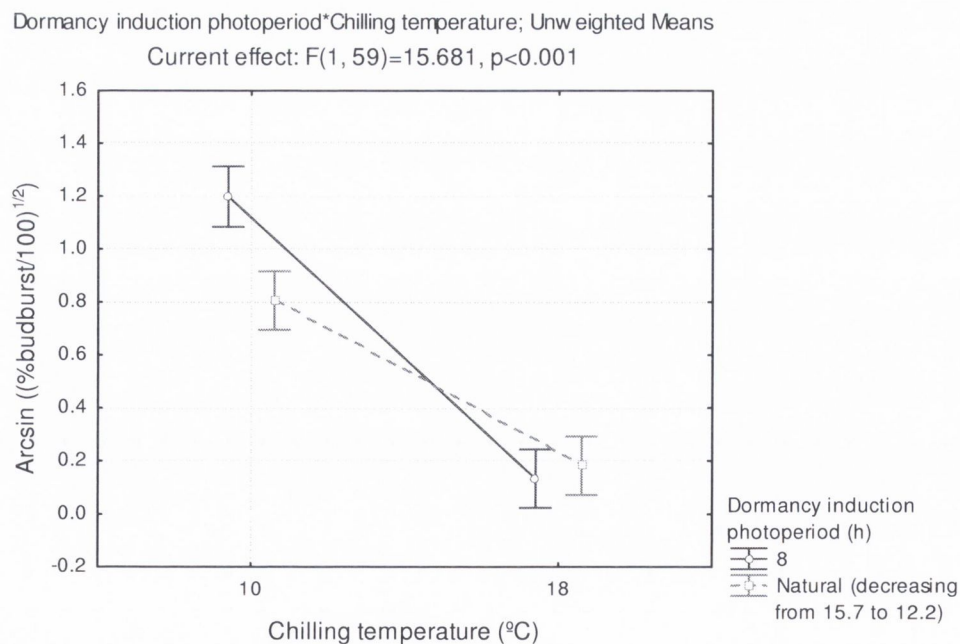
chilling at 10°C was 6.8 days as opposed to 5.6, 5.9 and 5.8 after chilling at respectively, 6, 4 and 2°C (averages for each chilling temperature across all chilling durations).

3.1.2.5 *T. cordata*

Chilling duration

Chilling duration had a significant effect on both percentage budburst and timing of budburst ($P<0.001$) (Tables 8.e and 9). Longer chilling durations increased percentage budburst and advanced its timing in both the experiments testing these effects, 2.a and 3.a (Figures 21.c, 22.e and 23.e). In experiment 2.a chilling duration progressively decreased the mean time to budburst from 19.1 to 4 days (averaged across chilling temperatures) as it increased from 30 to 122 chilling days (Figure 21.c). In experiment 3.a., the time to budburst decreased from 38 to 4.6 days in LD as chilling duration increased from 30 to 105 days, and from 38 to 6 days in SD as chilling duration increased from 55 to 105 days (Figure 22.e). Percentage budburst (averaged across forcing photoperiods) increased from 0 to 97.9% as chilling duration increased from 0 to 105 days (Figure 23.e).

Figure 27. Interaction of chilling temperature and dormancy induction photoperiod on the arcsin transform of percentage budburst in *B. pubescens*. Data are from experiment 4.a (effect of dormancy induction, chilling and forcing temperatures and photoperiods). Vertical bars denote 0.95 confidence intervals.



Chilling temperature

Chilling temperature in the investigated range did not have any significant effects on timing of budburst ($P=0.584$) (Table 8). Differently from *B. pubescens*, we could not identify a subset of chilling durations in which chilling temperature affected budburst. The uniform response of timing of budburst to chilling temperature can be seen in Figure 21.c, which shows the relationship between number of days to budburst and chilling temperature after different chilling durations.

Minimum chilling requirement

The minimum chilling requirement at 3°C for budburst of *T. cordata*, as shown by experiment 3.a, was between 11 and 30 days in LD (the mean percentage budburst was 62.5%) and between 30 and 55 days in SD (the mean percentage budburst was 87.5%) (Figure 23.e).

3.1.3 Forcing

3.1.3.1 B. pubescens

Forcing temperature significantly affected timing of budburst of *B. pubescens* ($P<0.001$) (Table 13). Higher forcing temperatures lead to earlier budburst in fully chilled *B. pubescens* one-node budsticks (Figure 28). In experiment 2.b, testing the effect of a range of forcing temperatures from -3°C to 32°C, none of the cuttings undergoing forcing at -3°C flushed, and as forcing temperature increased from 6°C to 32°C the time to budburst progressively decreased from 12.1 to 2.1 days. Correspondingly, budburst rate (1/mean time to budburst) progressively increased from 0.08 to 0.48 days⁻¹ over the same temperature interval. The relationship between budburst rate and temperature clearly showed a logistic pattern ($r^2=0.99$, $P=0.0126$), rising slowly until 12°C then more rapidly up to 32°C (Figure 29.a).

Cold and warm spells

The enhancing effect of forcing temperature on bud growth was confirmed also in experiment 4.a, where this factor combined with long photoperiod, exerted the only significant impact on budburst timing ($P<0.001$) (Table 12.c) among the different

combinations of environmental factors tested in the forcing treatments. In particular, the results from this experiment indicated that the timing of budburst depended on the average forcing temperature, rather than the presence of warm and cold spells (non significance of warm and cold spells, Table 12.d), or their duration and the timing of their application during forcing (non significance of temperature cycles duration and timing, Table 12.a and b).

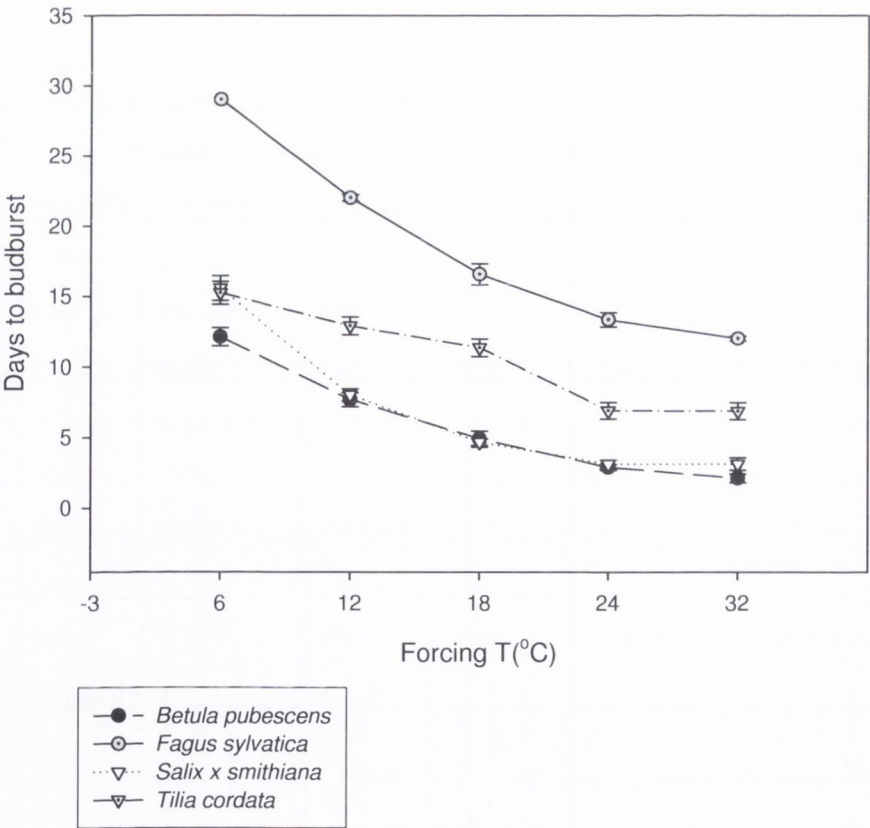
Table 12. Analysis of variance for the time to budburst of *B. pubescens*. Due to missing blocks, caused by both the fractional factorial experimental design and the absence of budburst (plants in treatments receiving chilling at 18 °C and forcing at a constant temperature of 15 °C and photoperiod of 12 h did not flush), subsets of the data with observations in all cells were analysed **(a) Effect of cycle duration** (Dormancy induction photoperiod*chilling treatment*cycle duration). Subset of plants chilled at 10°C including only forcing treatment 1 (alternation of 4 days long warm and cold spells) and 3(alternation of 8 days long warm and cold spells). **(b) Effect of cycle timing** (Dormancy induction photoperiod*chilling treatment*cycle timing). Subset of plants chilled at 10°C including only forcing treatment 1(see above) and 2 (alternation of 4 days long cold and warm spells). **(c) Effect of combined action of temperature and photoperiod** (dormancy induction photoperiod*chilling treatment*temperature+photoperiod). Subset of plants chilled at 10°C including only forcing treatments 4 (constant temperature of 20°C and photoperiod of 16h) and 5 (constant temperature of 15°C and photoperiod of 12h) **(d) Effect of presence/absence of cold and warm spells cycles** (dormancy induction photoperiod*chilling treatment* presence/absence of cold and warm spells). Subset of plants chilled at 10°C including only forcing treatments 1, 2, 3 and 5. Similar analyses were not conducted for percentage budburst as it resulted that there were no significant effects of forcing treatment on this response. Data are from experiment 4.a (Effect of dormancy induction, chilling and forcing temperatures and photoperiods).

	Time to budburst				
	df	SS	MS	F	p
(a)					
Cycle Duration (CycD)	1	1444	1444	0.14	0.714
Dormancy Induction Photoperiod (DIPht) *CycD	1	16500	16500	1.56	0.221
(b)					
Cycle Timing (CycTim)	1	1774	1774	0.14	0.708
DIPht*CycTim	1	2495	2495	0.2	0.657
(c)					
Forcing Temperature+Photoperiod (ForcTPht)	1	1707	1707	132.5	<0.001
DIPht*ForcTPht	1	6	6	0.5	0.985
(d)					
Cold/warm spell cycles presence/absence (CycY/N)	1	0.5	0.5	0.017	0.896
DIPht*CycY/N	1	175	175	5.638	0.021

Table 13. Analysis of variance for the effect of forcing temperature on the time to budburst of fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana* and *T. cordata*. Data are from experiment 2.b (effect of forcing temperature).

	df	SS	MS	F	p
<i>B.pubescens</i>	4	599.4	149.9	69.71	<0.001
<i>F.sylvatica</i>	3	586.0	195.4	80.06	<0.001
<i>S.x smithiana</i>	4	987.6	246.9	108.16	<0.001
<i>T.cordata</i>	4	478.2	119.6	32.18	<0.001

Figure 28. Relationship between forcing temperature and number of days to budburst in fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*. At -3°C, no budburst was observed in any of the species. Data are from experiment 2.b (effect of forcing temperature). Vertical bars denote standard errors of the mean.



The advance of budburst at high, constant temperature (20°C) and 16 h photoperiod compared with cold and warm spells (average temperature of 15°C) at increasing photoperiod (from 9 to 16h), and with constant temperature of 15°C and 12 h photoperiod can be seen in Figure 30, showing the interaction between dormancy induction and forcing conditions in each of the three different chilling treatments (on separate plots).

3.1.3.2 *F. sylvatica*

Forcing temperature significantly affected timing of budburst of *F. sylvatica* ($P<0.001$) (Table 13). Higher forcing temperature advanced budburst in fully chilled *F. sylvatica* budsticks (Figure 28). In experiment 2.b none of the budsticks undergoing forcing at -3°C flushed, and as forcing temperature increased from 6°C to 32°C the time to budburst progressively decreased from 29 to 12 days. Correspondingly, budburst rate (1/days to budburst) progressively increased from 0.03 to 0.08 over the same temperature interval. The relationship between budburst rate and temperature clearly showed a sigmoidal pattern ($r^2=0.96$), rising rapidly as temperature increased to 12-18°C and then slowing down above 18°C (Figure 29.b).

3.1.3.3 *S. x smithiana*

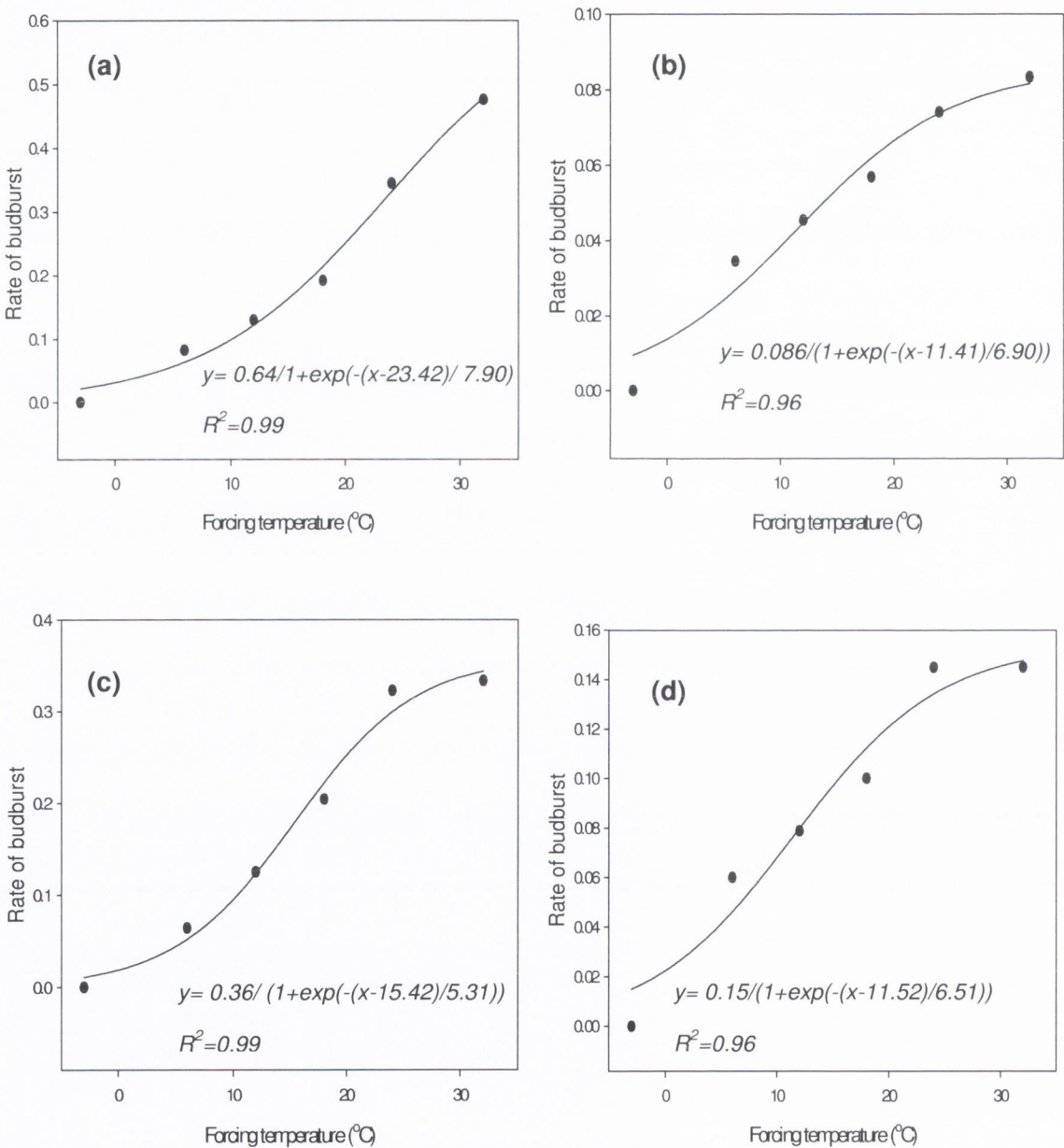
Forcing temperature significantly affected timing of budburst of *S. x smithiana* ($P<0.001$) (Table 13). Higher forcing temperature advanced budburst in fully chilled *S. x smithiana* cuttings (Figure 28). In experiment 2.b none of the budsticks undergoing forcing at -3°C flushed, and as forcing temperature increased from 6°C to 32°C the time to budburst progressively decreased from 15.6 to 3 days. Correspondingly, budburst rate progressively increased from 0.06 to 0.33 over the same temperature interval. The relationship between budburst rate and temperature clearly showed a sigmoidal pattern ($r^2=0.99$), rising slowly as temperature increased to 6°C then more rapidly up to 24-32°C (Figure 29.c).

3.1.3.4 *T. cordata*

Forcing temperature significantly affected the timing of budburst of *T. cordata* ($P<0.001$) (Table 13). Higher forcing temperature advanced budburst in fully chilled *T. cordata* cuttings (Figure 28). In experiment 2.b none of the budsticks undergoing forcing at -3°C flushed, and as forcing temperature increased from 6°C to 32°C the time to budburst

progressively decreased from 15.2 to 6.9 days. Correspondingly, budburst rate progressively increased from 0.07 to 0.15 over the same temperature interval. The relationship between budburst rate and temperature showed a sigmoidal pattern ($r^2=0.96$), rising rapidly as temperature increased to 12-18°C and then slowing down above 18°C (Figure 29.d).

Figure 29. Relationship between forcing temperature and rate of budburst (1/days to budburst) in fully chilled one-node budsticks of (a) *B. pubescens* (b) *F. sylvatica*, (c) *S. x smithiana*, (d) *T. cordata*. The data, from experiment 2.b. (effect of forcing temperature) have been fitted with sigmoidal curves.



3.2 Photoperiod

3.2.1 Dormancy induction

3.2.1.1 *B. pubescens*

Dormancy induction photoperiod

Dormancy induction photoperiod significantly affected the timing ($P = 0.003$) and the variance stabilizing transform of the percentage of budburst ($\arcsin \sqrt{(\text{percentage budburst}/100)}$) of *B. pubescens* ($P < 0.001$) (Table 11.a). This transform is proportional to percentage budburst, and thus reflects the effects that the environmental factors tested in the analysis have on percentage budburst. Long photoperiods during dormancy induction increased subsequent time to budburst from 36.6 to 39.8 days (Figure 31.a) and decreased the percentage of budburst from 50% to 32%, corresponding to a decrease in $\arcsin \sqrt{(\text{percentage budburst}/100)}$ of 0.78 to 0.60 (Figure 31.b).

The interaction between dormancy induction photoperiod and chilling temperature affected the transform of the percentage of budburst $\arcsin \sqrt{(\text{percentage budburst}/100)}$ ($P < 0.001$) (Table 11.b), but not timing of budburst. In fact, while all plants receiving high as opposed to low chilling temperature showed a decrease in percentage budburst, this decrease was more pronounced in plants exposed to an 8 h photoperiod rather than natural photoperiod (naturally decreasing from 15.7 h to 12.2 h, thus longer) during dormancy induction. This effect was reflected by $\arcsin \sqrt{(\text{percentage budburst}/100)}$, as shown in Figure 31.c. The interactions between dormancy induction photoperiod and the 5 different forcing treatments to which the *B. pubescens* plants were exposed in experiment 4.a can also be seen in Figure 30.

Dormancy induction duration

The duration of dormancy induction had significant effects on both the timing of budburst ($P = 0.011$) and percentage budburst ($P < 0.001$) in chilled *B. pubescens* (Table 7.b). The time to budburst decreased from 26.6 to 18.7 days (Figure 32.a) and the percentage budburst increased from 20.5 to 77.6% (Figure 32.b) as dormancy induction duration increased from 30 to 60 days.

Figure 30. Response to different forcing treatments by mean percentage of budburst (bar plot) and mean days to budburst (line and scatter plot) on *B. pubescens* clones exposed to dormancy induction at 8h and natural photoperiods, after three different chilling treatments, lasting 50 days: (a) 18°C at a 8h photoperiod, (b) 10°C at a 8h photoperiod, (c) 10°C at a 16h photoperiod. Forcing treatments: (1) alternation of 8-days long warm (20 °C) and cold (10°C) spells at increasing photoperiod, (2) alternation of 8-days long cold (10°C) and warm (20°C) spells at increasing photoperiod, (3) alternation of 4-days long warm (20°C) and cold (10°C) spells at increasing photoperiod, (4) constant temperature of 20°C and photoperiod of 16h, (5) constant temperature of 15°C and photoperiod of 12h. Data are from experiment 4.a (effect of dormancy induction, chilling and forcing temperatures and photoperiods).

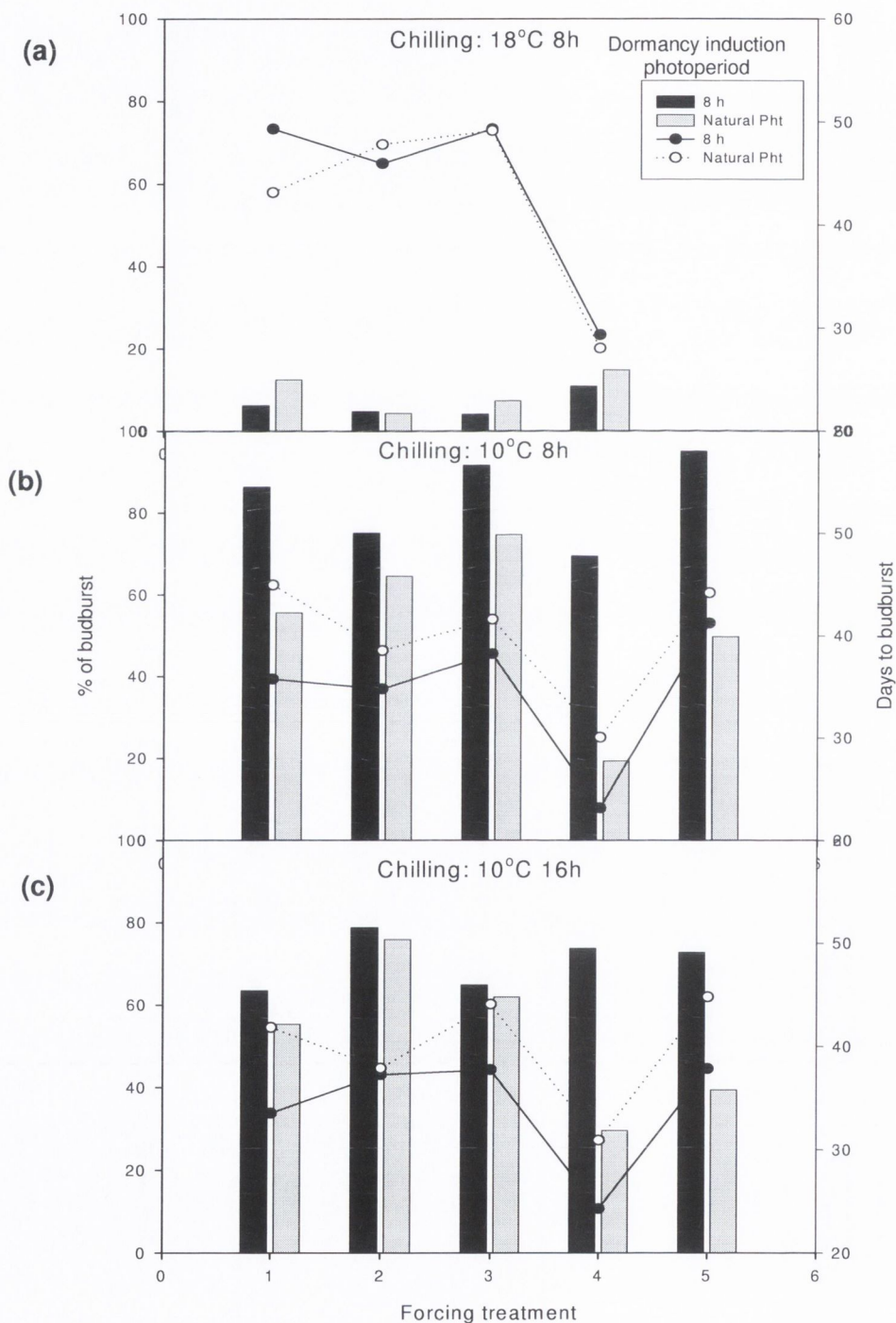
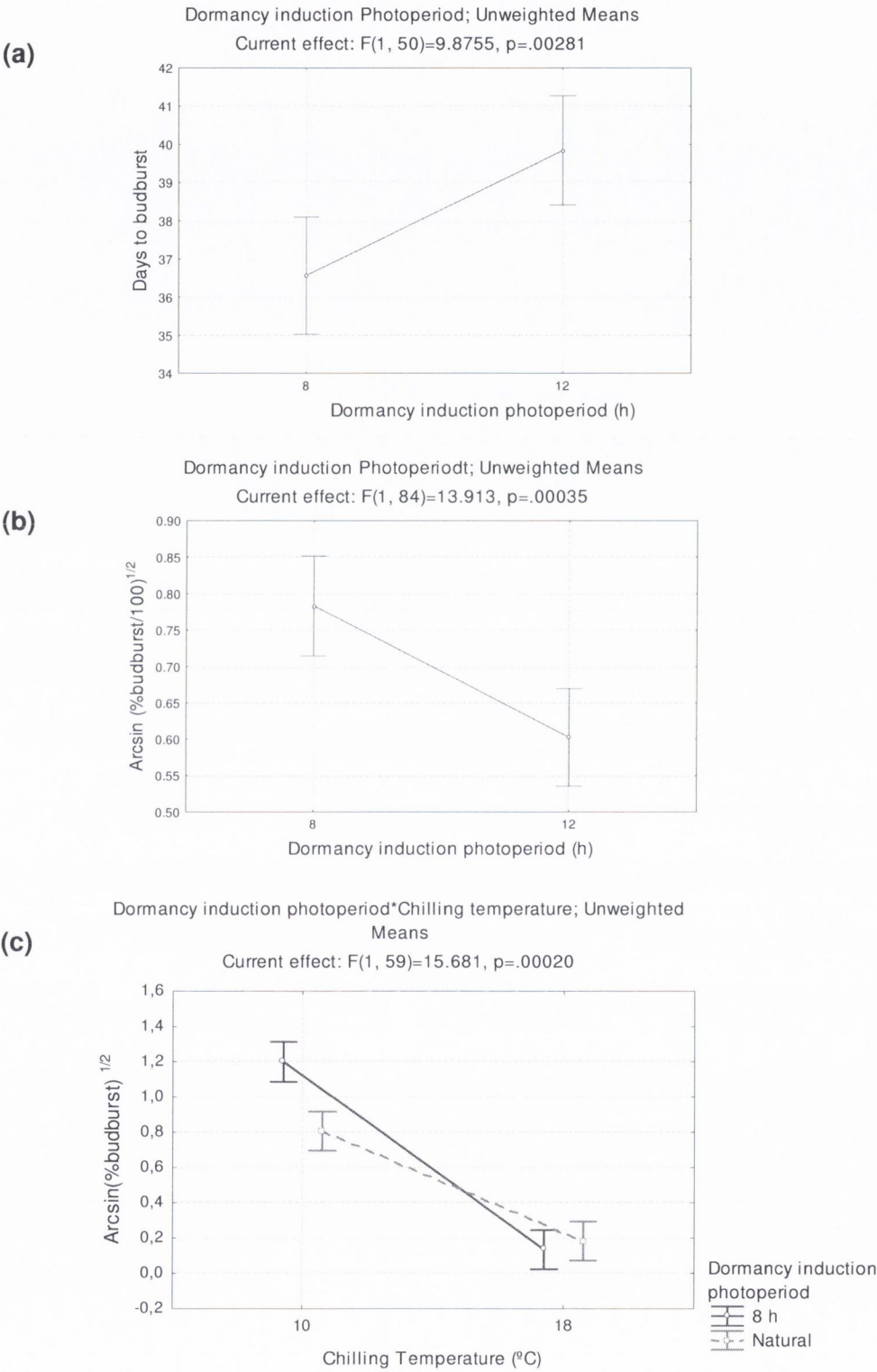


Figure 31. *B. pubescens*. (a) Effect of dormancy induction photoperiod on time to budburst (days). (b) Effect of dormancy induction photoperiod on $\arcsin\sqrt{(\text{percentage budburst}/100)}$. (c) Interaction between dormancy induction photoperiod and chilling temperature, on $\arcsin\sqrt{(\text{percentage budburst}/100)}$. Data are from experiment 4.a (effect of dormancy induction, chilling and forcing temperatures and photoperiods). Vertical bars denote 0.95 confidence intervals.



Dormancy induction duration and chilling presence/absence interacted significantly on percentage budburst: while an increase in dormancy induction duration increased percentage budburst from 20.1 to 77.4 % in chilled plants, it instead decreased it from 33.4 to 4.2% in unchilled plants. This effect can be seen in Figure 32.c and in Figure 26, showing the interactions between dormancy induction photoperiod and chilling presence/absence on two separate plots, each corresponding to a different dormancy induction duration.

3.2.2 Chilling

3.2.2.1 *B. pubescens*

Chilling photoperiod did not affect the timing of budburst in experiment 3.c, in which 0 h and 8 h chilling photoperiods were compared (Table 7.b), nor in experiment 4.a, comparing 8 h and 16 h chilling photoperiods (Table 11.c) (P -values>0.05). In experiment 3.c, total darkness decreased the mean time to budburst in plants exposed to short dormancy induction at 10°C, and long dormancy induction at 18°C (markedly in the first dormancy induction treatment, and slightly in the second) while it increased it in plants exposed to long dormancy induction at 10°C (Figure 33). This interaction between dormancy induction treatment and chilling photoperiod on timing of budburst was significant ($P= 0.020$) (Table 7.b).

3.2.3 Forcing

3.2.3.1 *B. pubescens*

Forcing photoperiod had a significant effect on percentage budburst of *B. pubescens* in both the experiments testing this factor, 3.a and 3.b (P -values<0.001) (Tables 9 and 10.b). Long days during the forcing period increased percentage budburst, especially after short chilling periods. The promoting effect of photoperiod on percentage budburst is shown in Figure 23.a, representing this response in relation to increasing chilling duration for both SD and LD forced plants and in Figure 24.b showing the response of percentage budburst to forcing photoperiod after 4 different chilling durations. However, the effect of photoperiod was not constant with chilling duration: in experiment 3.a, long photoperiods during forcing enhanced the percentage of budburst in plants that received chilling durations of 0, 11 and 30 days, whereas after 105 days of chilling the percentage of buds opening was

approximately the same regardless of photoperiod (interaction between chilling duration and forcing photoperiod, $P<0.01$) (Table 9).

Figure 32. *B. pubescens*. (a) effect of dormancy induction duration on time to budburst (days), (b) effect of dormancy induction duration on percentage budburst, (c) interaction between dormancy induction duration and presence/absence of chilling on percentage budburst. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration). Vertical bars denote 0.95 confidence intervals.

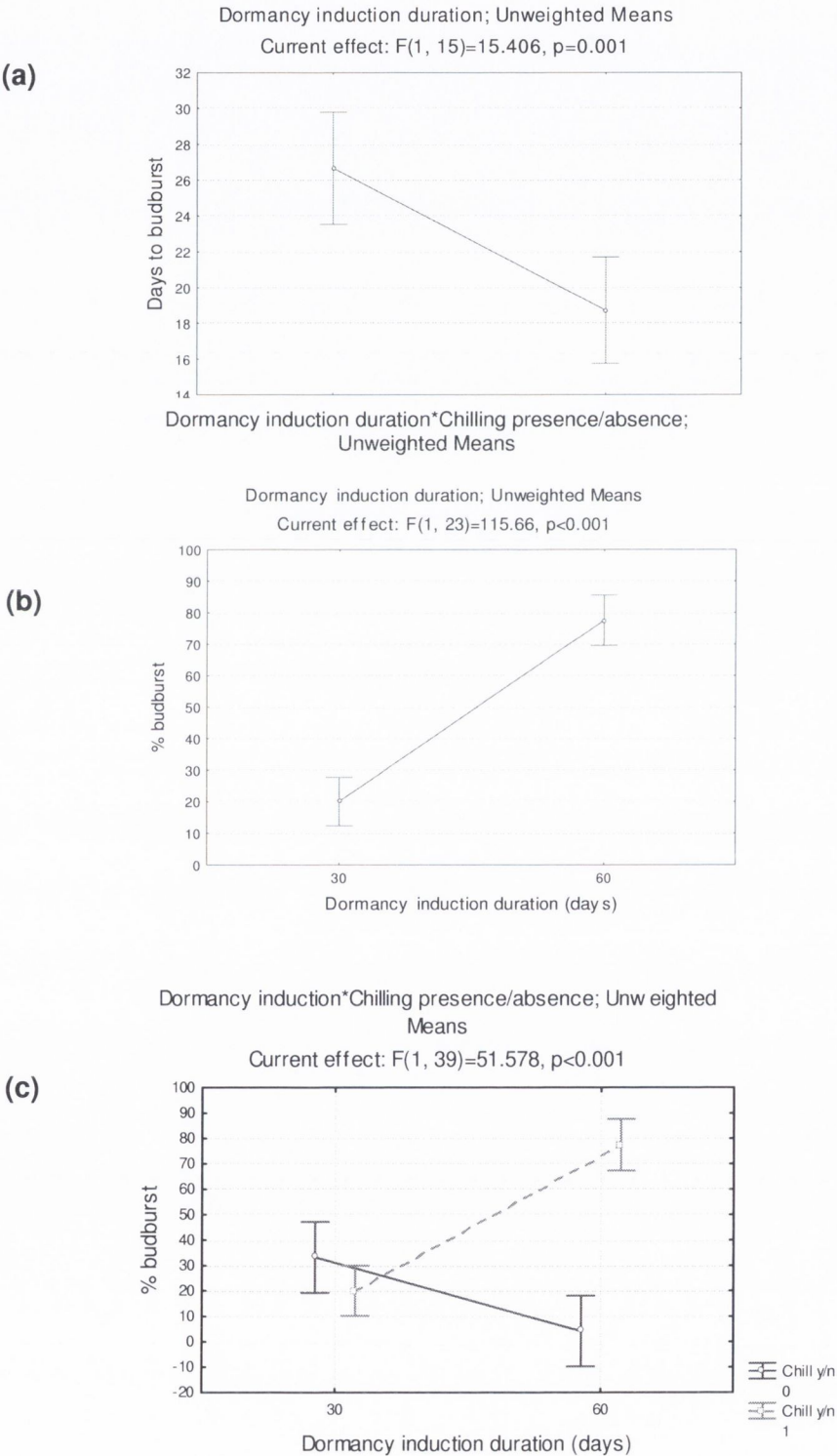
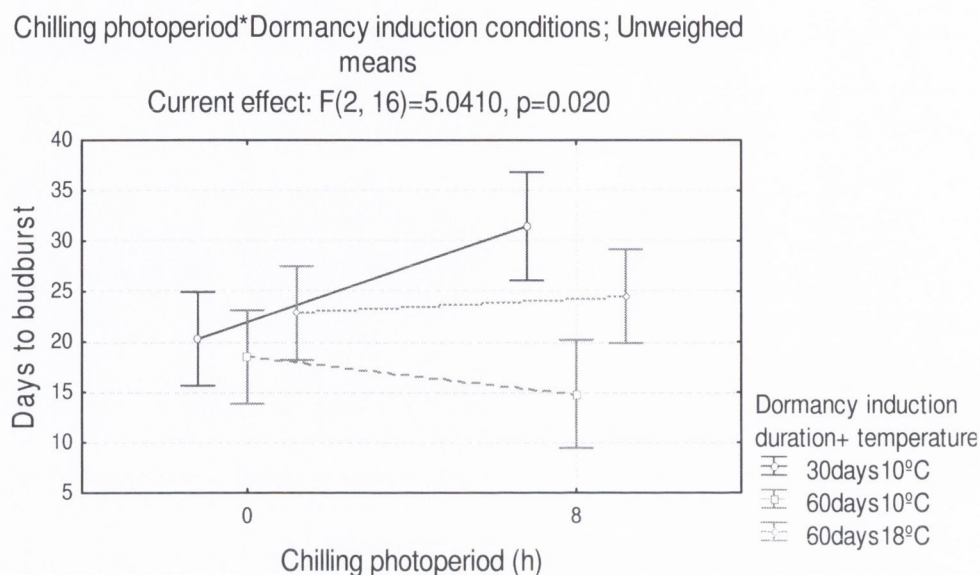


Figure 33. Interaction between dormancy induction treatment and chilling photoperiod on time to budburst in *B. pubescens*. Data are from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration). Vertical bars denote 0.95 confidence intervals.



Similarly, in experiment 3.b, the increase in percentage budburst caused by LD, was more pronounced after short chilling durations (interaction between forcing photoperiod and chilling duration, $P<0.05$) (Table 10.b).

Figure 24.b shows that the effects of photoperiod on percentage budburst at different stages of chilling. The graph shows that budburst was triggered by progressively shorter photoperiods following increasing chilling durations. This threshold photoperiod progressively decreased from 14 - 16 h in unchilled treatments to 10 h or less after 95 days of chilling at 6°C.

In the same experiment, timing to budburst was not significantly affected by forcing photoperiod and did not show large variations at photoperiods above the critical threshold for budburst occurrence ($P=0.051$) (Table 10.a) (Figure 24.a). This contrasted with the results of experiment 3.a, in which a long photoperiod advanced timing of budburst (significant effect of photoperiod on time to budburst, $P<0.001$; Table 9). In addition, in experiment 3.a, the promoting effect that LD exerted on time to budburst was more pronounced for short than long chilling durations (interaction between chilling duration and forcing photoperiod, $P<0.01$) (Table 9). This interaction is shown in Figure 22.a, in that the

gap between the plots of the timing of budburst in SD and LD fills out after increasing chilling durations.

3.2.3.2 *F. sylvatica*

Forcing photoperiod had a significant effect on both percentage budburst and timing of budburst (P -values <0.01) (Table 9). In experiment 3.a, LD forcing conditions triggered budburst in unchilled plants and plants that had received 11, 30, 55 and 105 days of chilling, while SD forcing triggered budburst only in plants receiving the longest chilling duration (105 days). These effects were confirmed by the significance of the main effect of forcing photoperiod on percentage budburst ($P<0.001$) (Table 9) and by the significance of the interaction between chilling duration and forcing photoperiod on percentage budburst (the LD-triggered increase in percentage budburst was smaller after 105 days of chilling than after shorter chilling durations) ($P<0.001$) (Table 9). These effects can be seen in Figure 23.b, showing percentage budburst in relation to chilling duration for both SD and LD forced plants. As for timing of budburst, it was not possible to test any interactions between forcing photoperiod and chilling duration, as in only one out of five chilling durations budburst occurred in both SD and LD forcing treatments. However, the ANOVA for main effects indicated that photoperiod had a significant effect on the timing of budburst ($P=0.001$) (Table 9). In fact, even after 105 days of chilling, LD forcing advanced the mean time to budburst from 22.7 to 15.6 days compared with SD forcing (Figure 23.b).

3.2.3.3 *S. aurita*

In experiment 3.a, a long forcing photoperiod decreased the mean time to budburst (Figure 22.c) and increased the percentage budburst (Figure 23.c). The ANOVA indicated that photoperiod had a significant effect on percentage budburst ($P= 0.029$) but not on time to budburst ($P=0.501$), and that there were no interactions between photoperiod and chilling duration on timing of budburst nor percentage budburst ($P>0.05$) (Table 9).

3.2.3.4 *S. x smithiana*

Forcing photoperiod had a significant effect on both percentage budburst ($P= 0.002$) and timing of budburst ($P= 0.001$) (Table 9). Long days during the forcing period decreased the mean time to budburst (Figure 22.d) and increased the percentage budburst (Figure 23.d).

In experiment 3.a., LD increased percentage budburst in plants that had previously received chilling durations of 11 and 30 days, whereas after 55 and 105 chilling days the percentage of buds opening was approximately the same regardless of photoperiod (interaction between chilling duration and forcing photoperiod, $P=0.002$) (Figure 23.d). Similarly, the LD-triggered advance in the timing of budburst was larger in plants that had received only 30 chilling days than longer chilling durations (interaction between chilling duration and forcing photoperiod, $P=0.002$). Figure 22.d shows that the gap between the timing of budburst in SD and LD narrows as chilling duration decreases from 30 to 105 days.

3.2.3.5 *T. cordata*

Forcing photoperiod had a significant effect on timing of budburst ($P=0.018$) but not on percentage budburst ($P=0.187$) (Table 9). In experiment 3.a, LD decreased the mean time to (Figure 22.e) but increased only slightly the percentage budburst (Figure 23.e). There were no interactions between photoperiod and chilling duration on timing of budburst or percentage budburst ($P>0.05$) (Table 9).

3.3 Light intensity and nutrients

3.3.1 *B. pubescens*

High light intensity slightly advanced budburst (0.95 days on average) in *B. pubescens* one-node budsticks, decreasing the time to budburst from 2.75 to 1.8 days (Figure 34). The more advanced phenological stage of *B. pubescens* receiving high light intensity is shown in Figure 35. However, this difference was not significant ($P>0.05$) (Table 14).

On the other hand, nutrient deficiency had a significant effect on the time to budburst ($P<0.001$), (Table 15). Compared with the control treatment, nutrient deficiency delayed budburst by 0.3 days on average in low K regime (from 6.5 to 6.8 days) and 1.8 days on average in low N regime (from 6.5 to 8.3 days) (Figure 36). However, post-hoc pairwise comparisons indicated that only the low N regime had a significant effect on the time to budburst (Table 16).

3.3.2 *F. sylvatica*

High light intensity markedly advanced budburst (4.13 days on average) in *F. sylvatica* one-node budsticks, decreasing the time to budburst from 11.3 to 7.2 days (Figure 34). This difference was significant ($P < 0.001$), (Table 14).

3.3.3 *S. x smithiana*

High light intensity did not affect timing of budburst in *S. x smithiana* one-node budsticks ($P > 0.05$) (Table 14), which flushed all at the same time (4 days after the transfer into forcing conditions) in high and low light conditions (Figure 34).

On the other hand, nutrient deficiency had a significant effect on the time to budburst ($P < 0.001$), (Table 15). Compared with the control treatment, nutrient deficiency delayed budburst by 1.9 days on average in low N regime (from 9.3 to 11.2 days) and 4.3 days on average in low K regime (from 9.3 to 13.6 days) (Figure 36). A Post-hoc pairwise test indicated that the control treatment was significantly different from both the low N and low K regimes and these two differed from each other (Table 16).

3.3.4 *T. cordata*

High light intensity slightly advanced budburst (1 day on average) in *T. cordata* one-node budsticks, decreasing the time to budburst from 4.8 to 3.8 days (Figure 34). This difference was significant ($P = 0.008$), (Table 14).

Nutrient also had a significant effect on the time to budburst ($P < 0.001$), (Table 15). Compared with the control treatment, nutrient deficiency delayed budburst by 1.7 days on average in low N regime (from 13.8 to 15.5 days) and 4.2 days on average in low K regime (from 13.8 to 18 days) (Figure 36). Post-hoc pairwise comparisons indicated that the control treatment was significantly different from both the low N and low K regimes and these two differed from each other (Table 16)

Table 14. Analysis of variance for the effect of light intensity on the time to budburst of fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata*. Data are from experiment 1.a. (effect of light intensity during forcing)

	df	SS	MS	F	p
<i>B.pubescens</i>	1	4.0	4.0	3.75	0.071
<i>F.sylvatica</i>	1	80.9	80.9	19.21	<0.001
<i>S.x smithiana</i>	1	0.0	0.0	1.00	0.347
<i>T.cordata</i>	1	2.5	2.5	12.50	0.008

Figure 34. Mean number of days to budburst in fully chilled one-node budsticks of *B. pubescens*, *F. sylvatica*, *S. x smithiana* and *T. cordata* exposed to high and low light intensity. Data are from experiment 1.a (effect of light intensity). Vertical bars denote standard errors of the mean.

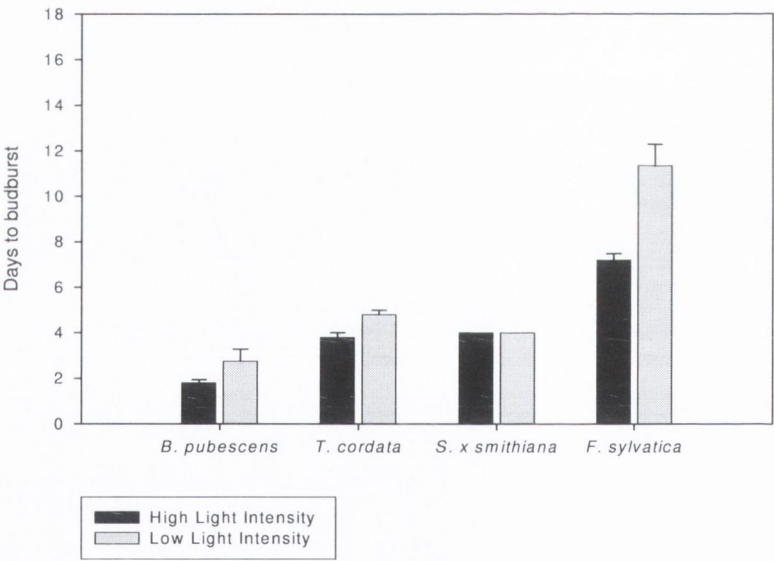


Figure 35. High light intensity slightly advanced budburst in *B. pubescens* one-node budbusticks exposed to high light intensity (above).

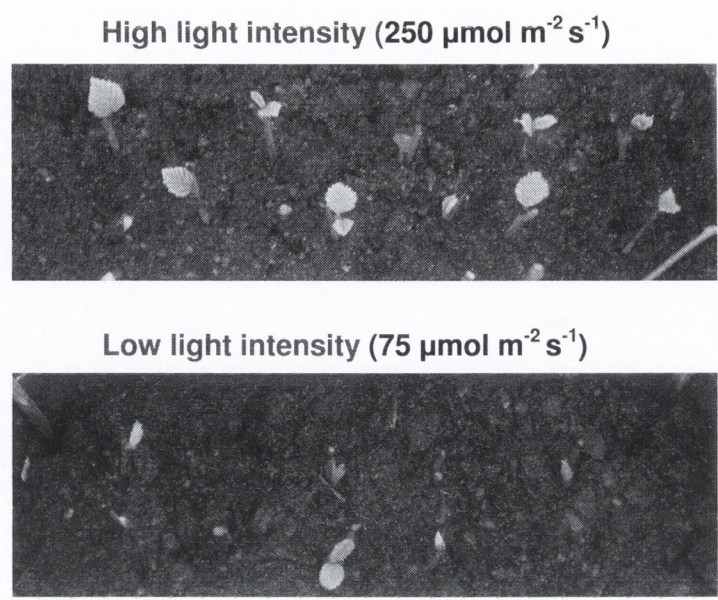


Figure 36. Mean number of days to budburst in fully chilled *B. pubescens*, *S. x smithiana* and *T. cordata* grown in low K, low N and control conditions (long term application). Data are from experiment 1.b (effect of nutrient deficiency). Vertical bars denote standard errors of the mean.

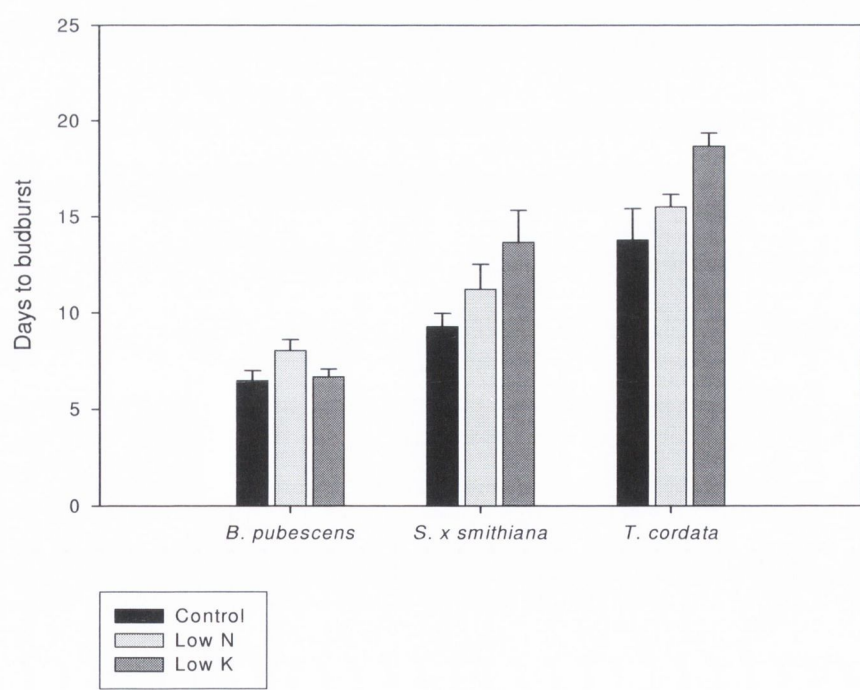


Table 15. Analysis of variance for the effect of nutrient availability on the time to budburst of fully chilled one-node budsticks of *B. pubescens*, *S. x smithiana* and *T. cordata*. Data are from experiment 1.b. (effect of nutrient deficiency).

	df	SS	MS	F	p
<i>B. pubescens</i>	2	119.0	59.5	21.53	<0.001
<i>S x smithiana</i>	2	315.9	157.9	20.29	<0.001
<i>T. cordata</i>	2	284.7	142.3	14.10	<0.001

Table 16. Post-hoc test for the effects of the three different levels of nutrients applied in experiment 1.b. on the time to budburst fully chilled one-node budsticks of (a) *B. pubescens*, (b) *S. x smithiana*, (c) *T. cordata*

<i>(a) B. pubescens</i>		
	Control	Low N
Low N	<0.001	-
Low K	0.149	<0.001
<i>(b) S. x smithiana</i>		
	Control	Low N
Low N	0.026	-
Low K	<0.001	<0.001
<i>(c) T. cordata</i>		
	Control	Low N
Low N	0.038	-
Low K	<0.001	0.009

DISCUSSION

1. Effects and interactions of temperature and photoperiod during dormancy and quiescence

All species responded to increasing chilling duration by decreasing their time to budburst, with the largest effects in the early stages of chilling. This response supports the use of a negative exponential relationship between the amount of accumulated chilling units and the number heat units required for budburst in phenological models, as in the alternating model (Cannell and Smith, 1983; Murray *et al.*, 1989; Chuine *et al.*, 1999).

Whereas in *B. pubescens* and *F. sylvatica* LD was a complete or almost complete substitute for chilling *S. aurita* *S. x smithiana* and *T. cordata* had a minimum chilling requirement for dormancy release, which was reduced by the exposure to LD. In general, forcing in LD advanced budburst, and this effect was especially evident after partial chilling, confirming that the effect of photoperiod decreases as chilling progresses, as previously shown by Myking and Heide (1995), and Heide (1993a).

Chilling temperatures of 2, 4 and 6°C were more effective than 10°C in promoting dormancy release in *B. pubescens* and *S. x smithiana* while in *T. cordata*, all the tested chilling temperatures (2, 4, 6 and 10°C) had a similar effect on budburst timing. These differences in the responses to chilling temperature highlight the species-specific nature of chilling requirements and active chilling range thresholds, and recommend caution when applying phenological models to species different than those they were developed for.

The next section will discuss in detail the effects of temperature and photoperiod during dormancy induction, dormancy and quiescence for each of the species used in this study. The effects of forcing temperature, light intensity and nutrients will be dealt with in sections 2, 3 and 4, respectively.

1.1 *B. pubescens*

Dormancy induction

During dormancy induction (the phase that corresponds to the end of summer in natural conditions) the most important environmental cue appeared to be short photoperiods, which acted cumulatively and whose effect was enhanced by low temperatures. The dormancy inducing effect of short days (SD) in *B. pubescens* (birch) has already been demonstrated in a number of studies (see for example Kawase, 1961; Rinne *et al.*, 1994; Welling *et al.* 1997; Myking and Heide, 1995; Li *et al.*, 2003) in which dormancy was triggered through the application of SD.

The results from experiment 3.c (effect of dormancy induction temperature and duration and chilling photoperiod and duration) suggested a cumulative action of SD, as a longer period of exposure to dormancy inducing conditions lead to earlier budburst in chilled plants and later budburst in unchilled plants. The quantitative character of the effect of short photoperiod on dormancy induction in *B. pubescens* was shown by Kawase as early as 1961 and is in agreement with the subsequent findings of Welling *et al.* (1997) who demonstrated that birch had a minimum SD requirement of 21 days in order to attain dormancy. According to Kawase (1961), the depth of dormancy attained depends on the number of photoperiods that are shorter than a critical threshold.

In the current work, the dormancy inducing action of SD appeared to be enhanced by low temperature. This effect was observed in experiment 3.c in which plants that received a low dormancy induction temperature flushed earlier than plants that had received a high dormancy induction temperature, suggesting that low temperatures advanced the attainment of full dormancy induction and the subsequent start of dormancy release. Low constant temperatures (Downs and Borthwick, 1956; Junttila, 1980), and low night temperature associated with high day temperatures (Håbjørg, 1972; Downs and Bevington, 1981) have been found to advance dormancy onset in birch. The budburst delaying effect of high temperatures during dormancy induction in chilled *B. pubescens* was also reported by Heide (2003).

Indeed, the observed interactions between presence/absence of chilling and dormancy induction temperature and duration could be interpreted as dormancy reversibility in its

initial stages, similar to the findings by Kawase (1961). Buds receiving short dormancy induction at high temperature were not deeply dormant when they were transferred into forcing condition without previous chilling and had not completely lost their growth competence. On the other hand, buds receiving either long dormancy induction duration and/or low dormancy induction temperatures developed a deeper state of dormancy and were unresponsive to forcing conditions unless dormancy was released by chilling.

The difference in the dormancy intensity attained after different dormancy induction conditions in experiment 3.c could also explain the fact that the absence of chilling did not result in any significant main effect on budburst timing. Unchilled, non dormant plants could resume growth quicker once in forcing conditions when compared to unchilled, fully dormant plants. When plants were chilled the situation reversed, as non dormant plants became fully dormant during chilling, but lagged behind plants that were already dormant at the beginning of the chilling treatment. This delay subsequently resulted in a longer time to budburst for plants receiving short dormancy induction and/or high temperature during this period.

Effect of chilling duration and temperature

During dormancy, high temperatures delayed subsequent bud development and decreased budburst percentage. The dormancy releasing effect of chilling temperatures in the range 2-10°C was confirmed by the results of experiment 2.a (effect of chilling temperature and duration). As expected, the exposure to chilling temperatures released dormancy in birch twigs and enabled a fast and complete budburst. Interestingly, we did not find an optimal chilling temperature but only a threshold temperature (10°C) below which all chilling temperatures had a similar effect, which is contrary to the findings of Sarvas (1974) for *B. pubescens*. These results support the hypothesis that under a certain threshold chilling accumulation is not a function of temperature (Samish, 1954). However, this effect might also be due to the fact that after the longest chilling durations, the accumulation of thermal time at 4, 6 and 10°C compensated for the partial growth competence resulting from these sub-optimal chilling temperatures.

The effect of chilling duration was conspicuous in all but one of the experiments testing this effect. Similar to previous studies (Myking and Heide, 1995; Cannell and Smith, 1983;

Murray *et al.* 1989), there was a decrease in the time to budburst as chilling progressed. The fact that in experiment 3.a, 3 out of 4 plants flushed even when they had not received any chilling treatment (Figure 37) might be explained by the exposure to chilling temperatures in the period preceding the start of the experiment. In fact, while temperature never decreased below 12°C during this period, the active chilling range of *B. pubescens* may have a higher threshold. Based on their findings, Myking and Heide (1995) hypothesised the upper threshold for chilling in *B. pubescens* to be slightly above 12°C. Our result show that 18°C was too high a temperature to release dormancy, so for this particular clone of birch the upper threshold for the active chilling range must be located somewhere in the mid-teens.

Figure 37. Growth in unchilled *B. pubescens* rooted cuttings exposed to LD forcing at 18°C (experiment 3.a). Only 1 of the 4 plants receiving this treatment in did not flush (left).



An alternative explanation might be the difference in the age of the cloned birches used in the two experiments, freshly rooted cuttings in experiment 3.a and one year old plants in experiment 3.c. A study by Partanen *et al.* (2005) provided evidence for age-specific rest patterns in *Picea abies*. While twigs taken from young trees responded to LD during

autumn, old material did not resume growth, regardless of the photoperiod until the vernal equinox. This observed transient rest completion during autumn suggests that responsiveness to autumn photoperiod could decrease with the age of the tree. On the other hand, the clones used in the present work did not show the secondary rest phase observed by Partanen *et al.* (2005), and it is known that rooted cuttings retain characters typical of mature trees, such as fruiting and plagiotropic growth. This effect, called “physiological ageing”, is more pronounced as the parent material grows older, as has been demonstrated in many tree species, including birch (Cameron and Sani, 1994). The plant material used in the current work was cloned from a 30 year old birch tree, which makes it likely that it retained mature characters, as could be seen from the horizontal growth pattern of the new shoots (Figures 37 and 38). Thus it is unclear whether this phenological difference could be attributed to the age of the clone.

A final explanation could involve the synthetic auxin (NAA) applied to promote rooting a few months earlier, which was possibly still active at the start of the experiment. Auxins are known to delay leaf senescence and have been shown to regulate floral bud development in *Arabidopsis* (Taiz and Zeiger, 2002). IAA levels at the shoot apex in *B. pendula* have been shown to decrease concurrently with dormancy development (Li *et al.*, 2003). The NAA contained in the rooting powder might have altered the phenological behaviour of the cloned birches and decreased the depth of dormancy.

What was clearly demonstrated by these experiments, however, was that in a northern temperate climate the minimal chilling requirement of this *B. pubescens* clone is likely to be fulfilled before the start of winter (21 December). In fact, experiment 3.c and 4.a showed that once dormancy had been fully attained at the end of September, the cloned birches flushed at most after 30-50 days of chilling temperatures, depending on the chilling temperature (30 days at temperatures between 2-6°C and 50 days if the chilling temperature was sub optimal, such as 10°C).

Figure 38. Plagiotropic growth in recently propagated *B. pubescens* (top) and *F. sylvatica* (bottom)



Chilling photoperiod

While photoperiod significantly affected the course of dormancy at the moment of dormancy induction and forcing, the present results suggest that it does not affect chilling fulfilment at 10°C. In both experiments testing this factor (3.c, comparing an 8h photoperiod with total darkness, and 4.a, comparing an 8h with a 16h chilling photoperiod) neither the timing nor the percentage of budburst changed as a result of its manipulation. Ontogenetic development during this period seemed to rely exclusively on temperature and the state of development already reached.

The observation that partially chilled plants exposed to LD during the forcing treatment (16 h photoperiod and 18°C) advanced their timing of budburst (experiment 3.a) or were released from dormancy earlier than plants exposed to SD, suggests that at a partial stage of chilling, LD effectively enhances bud growth (or is a substitute for chilling) only at temperatures above the active chilling range.

Effect of forcing photoperiod

In accordance with the responses observed in *B. pubescens* (Myking and Heide, 1995) and *Picea glauca* (Nienstaedt, 1966), long photoperiods during quiescence compensated for lack of chilling. However, when chilling was complete, there was hardly any difference in budburst timing and percentage between plants that received long and short photoperiods. This result suggests that the effect of photoperiod on dormancy release could become especially important in the scenario of milder winters not fulfilling plant's chilling requirements. This effect is not integrated into predictive models of budburst and may substantially change their prediction of budburst timing under climate change scenarios. The photoperiod sensitivity of birch trees has already been noted by various authors (Kawase, 1961; Håbjørg, 1972; Li *et al.*, 2003; Welling *et al.*, 1997).

Experiment 3.b suggested that increasing chilling gradually lowers the threshold photoperiod allowing bud growth during quiescence. This threshold decreased from 14 h in plants that had not received experimental chilling to less than 10 h in plants that had received 95 days of natural chilling. Unlike in experiment 3.a, in which a significant difference was observed between the times to budburst of clones exposed to SD and LD forcing, in experiment 3.b this difference was very small and non significant. In fact, the

most evident response to the variation in photoperiod and chilling duration was given by percentage budburst, increasing as these two factors increased.

The reason for the uniform response of budburst timing across forcing photoperiods above the critical threshold might lie in the fact that one-node budsticks as opposed to rooted plants were used for the experiments. The budburst delay of plants exposed to shorter photoperiod might have been missed due to the quick decay of the cut twigs. Also, the range of tested photoperiods was narrower than in experiment 3.a, which could have decreased the difference in budburst timing between SD and LD. In fact while in experiment 3.a, we compared an 8 with a 16 h photoperiod, in experiment 3.b we compared 4 photoperiods in the range 10 to 16 h. A final explanation could be that one-node budsticks have a different response to environmental triggers of dormancy release. The lack of correlative inhibitions in single buds could elicit a more synchronous response in buds once above a critical threshold photoperiod.

Effect of warm and cold spells during quiescence

Warm and cold spells applied during quiescence at gradually increasing photoperiod did not significantly affect the timing of budburst, when compared to plants receiving constant temperature and photoperiod. However, because the two factors were manipulated simultaneously, the interpretation of the results was confounded. In fact, it is not clear whether the lack of difference can be attributed to a lack of effects of warm and cold spells, or an interaction between photoperiod and temperature regimes. Previous studies have shown that fluctuating day/night temperature during forcing promotes budburst in *Pseudotsuga menziesii* (Campbell and Sugano, 1975) and *Picea abies* (Partanen *et al.*, 1998), even though this effect could be due the result of additional chilling received during the cold nights (Partanen *et al.*, 1998). However, the difference in the length of the temperature cycles applied in our study and that of Partanen *et al.* (1998) (8h and 16h days vs. diurnal cycle), makes it difficult to compare them.

1.2 *F. sylvatica*

Effect of chilling duration and forcing photoperiod

Beech has been demonstrated to be very sensitive to photoperiod by studies testing its response to LD exposure at various stages of chilling (Wareing, 1953; Heide, 1993). Wareing (1953) showed that unchilled beech seedlings were able to burst in October when exposed to continuous light. He found the same response in beech that had been chilled outdoor until March, and concluded that the only requirement for beech budburst was a long photoperiod (Wareing, 1953). Falusi and Calamassi conducted a similar experiment, exposing chilled and unchilled beeches to two different photoperiods, 9 h and 13 h (1990). The absence of significant differences between the two photoperiod treatments lead them to conclude that the dormancy releasing effect of photoperiod is negligible when compared to the effect of winter chilling (Falusi and Calamassi, 1990).

Heide (1993), against the background of these conflicting results, systematically tested the daylength requirements of beech buds exposed to various degrees of chilling throughout the fall and the winter. He found that beech twigs sampled from outdoor trees at various stages of winter chilling responded to LD only in October, prior to chilling, and in January, after receiving outdoor chilling for a couple of months (Heide, 1993). On the other hand, twigs exposed to SD never showed percentages of budburst >40%, even after extensive chilling lasting until mid March. He concluded that twigs sampled in October respond to photoperiod because they are in a state of “predormancy”, and that both chilling and long photoperiod are required for dormancy release (Heide, 1993).

The results reported here are in contrast with the conclusions of Heide (1993). It was found that beech exposed to LD after various durations of artificial chilling (in the dark at 3°C) were always able to flush provided that they received LD for a long enough period. LD forced plants in experiment 3.a (effect of chilling duration and photoperiod) that had received 0 and 11 days of chilling flushed after an average of 58 and 61 days respectively. This suggests a strong photoperiodic response of beech to photoperiod that, at least in this study, was able to override the metabolic constraints imposed by dormancy without requiring extensive chilling. In fact, even accepting that the minimum temperature received by the plants during dormancy induction (12°C) is included in the range of active chilling temperature for beech, the number of days with average daily temperatures equal or close

to 12°C received during this period was minimal and not comparable to the extensive chilling requirements described for beech by Murray *et al.* (1989).

In addition, we found that beech exposed to SD was able to flush after receiving 105 days of artificial chilling, in contrast to the conclusion of Heide (1993) that beech has an LD requirement for budburst. Even though budburst was delayed when compared to LD forced plants, budburst percentage in SD forced plants was quite high (85%), implying that dormancy release was complete. The finding that beech does not necessarily require LD for budburst was further supported by the casual observation that potted cloned beeches left in the cold room at 6-8°C in the darkness, flushed in July after 6 months of chilling in the dark.

The contrast between our findings and those of Heide (1993) could be due to the fact that while he tested cut twigs we used whole plants. The problem with cut twigs is that, as Heide (1993) pointed out, they quickly use up their carbohydrate reserves and thus cannot be monitored reliably for long periods. For this reason, Heide (1993) terminated his experiments after 50 days from the transfer into forcing conditions. However, our unchilled and slightly chilled (11 days) plants flushed after about 60 days, while the plants that had received 30 days of chilling flushed after about 51 days, which is longer than the duration of the forcing treatment in the experiments of Heide (1993) which suggests that he might have missed the late flushing of the beech buds due to the rapid decay of the twigs.

We should also note that the longest exposure to chilling received by the twigs during the study of Heide (1993) corresponded to natural chilling lasting throughout the winter until the 15th March, after which SD forced plants showed only partial budburst. The end of our longest chilling treatment corresponded to the 4th March, but the constant artificial chilling in the dark that we applied might result in a faster dormancy release. Another possible explanation involves the direction of the change of the photoperiod occurring at the time of the transfer from chilling to forcing condition: while in Heide (1993) the SD forced plants experienced a decrease in photoperiod (from about 12 h of the vernal equinox to 8 h of the forcing treatment) our SD forced plants always experienced an increase in photoperiod (from 0 h to 8 h). Shortening the photoperiod delayed budburst in chilled *Picea abies* suggesting that growth initiation was affected by the direction of change rather than its absolute length (Partanen *et al.*, 1998). This effect could be present also in beech.

Thus, our results suggest that dormancy release in *F. sylvatica* is regulated by an interaction between chilling and photoperiod and that a substitutive effect occurs between the two factors, such that a long enough period under the effect of LD can substitute for chilling and a long enough period under the effect of chilling can substitute for LD (dormancy release in beech requires either chilling or long days).

1.3 *S. aurita* and *S. x smithiana*

Effect of chilling duration and temperature

Relatively few studies have been conducted on the environmental triggers of dormancy release in *Salix* (Lenartsson, 2003; Paus *et al.*, 1986; Jones *et al.*, 1997; Murray *et al.*, 1989). Growth cessation and dormancy in *Salix* appear to be induced by short photoperiods and low temperatures (Lenartsson, 2003). Unchilled *S. viminalis* exposed to a dormancy induction temperature as high as 15°C eventually flushed, even if forced in SD (Lenartsson, 2003). A more or less extended period of chilling is then needed to break dormancy and allow bud development (Lenartsson, 2003). The chilling requirements of *S. polaris* were quantified by Paus *et al.* (1986) as between 14 to 42 days at 0.5°C.

Experiment 3.a, testing the effect of chilling duration and forcing photoperiod, confirms the necessity of chilling exposure for dormancy breaking and flushing in both *S. aurita* and *S. x smithiana*. The minimum chilling requirements for *S. aurita* and *S. x smithiana*, as found in experiment 3.a were of a similar magnitude to those found by Paus *et al.* (1986) for *S. polaris*, assuming a similar effect of 0.5 °C and 3 °C (applied in our chilling treatment) on dormancy release. Plants that did not receive sufficient chilling in experiment 3.a did not flush for the whole 90 days period of monitoring, and even after transfer outdoor (in summer conditions, with naturally long days and warm temperatures) did not show significant budburst. Thus, these two species, contrary to birch and beech demonstrated a very strict necessity of chilling for dormancy release that could not be replaced by LD or a high sum of heat units.

In both *Salix* spp. time to budburst decreased with increasing chilling duration as found for *S. viminalis* by Murray *et al.* (1989). The fact that *S. aurita* showed a comparatively lower

percentage budburst after 30 and 55 days of chilling, suggests that it has a higher chilling requirement than *S. x smithiana*.

Although our results clearly show that *S. x smithiana* has a requirement for chilling exposure for dormancy release, when we tested cut twigs at a range of chilling temperatures (2-10°C) we could not detect any optimal chilling temperature. All temperatures below 10°C appeared to exert a similar effect on the state of chilling and dormancy release, especially after long chilling durations. This might suggest a threshold type response to chilling temperatures in *S. x smithiana*. However, these results should be considered with caution, due to the variance heterogeneity of the observations, which might have caused type I errors (non significant results erroneously found to be significant).

Effect of forcing photoperiod

While forcing photoperiod had a significant effect on the percentage of budburst in both *Salix* species, only in *S. x smithiana* was the timing of budburst affected by this factor. However it should be remembered that the “timing” of budburst of non-flushing plants could not be analysed, and this probably affected the outcome of the analysis. In fact, while in the graph (Figure 21.c) non flushing plants have been represented as flushing after 90 days to facilitate its interpretation, these arbitrary values could not be included in the ANOVA. Thus, despite the fact that it is clear from the pattern of decrease in forcing days required for budburst that LD hastens dormancy release, this could not be detected by the analysis for budburst timing. The photoperiodic effect on budburst timing in *S. x smithiana* decreased notably after 105 days of chilling, which suggest that photoperiod is important only for partially chilled willows.

1.4 *T. cordata*

Effect of chilling duration and temperature

Relatively few studies have been conducted on the environmental triggers of dormancy in *T. cordata*. However, it is known that *T. cordata* requires a chilling period in order to break dormancy and be able to grow in spring (Wilson *et al.*, 2002; Wilson and Sibley, 2001; Pigott, 1991). Our results from experiment 3.a (effect of chilling duration and forcing

photoperiod) confirm that a period with chilling temperatures is required by lime for budbreak. The length of the chilling period required for dormancy release in our experiment coincides with that reported by Pigott (1991) who found that 6-8 weeks of temperatures $<5^{\circ}\text{C}$ were needed by lime for normal budburst. In fact even if we observed some budburst in LD forced limes after only 4 weeks of chilling at 3°C , high percentages of budburst and response to SD forcing were observed only upon the exposure to at least 55 days (8 weeks) of chilling. On the other hand, Wilson *et al.* (2002), reported somewhat minor chilling requirement (200-400 hours $< 7.2^{\circ}\text{C}$) for lime, in a trial involving 4 different lime cultivars. However, the variation in chilling requirements among cultivars showed by the same study, suggests that a certain degree of intraspecific variation is to be expected.

Effect of forcing photoperiod

The effect of photoperiod on budburst in *T. cordata* was evident, as only LD forced plants were able to flush after 30 days of chilling and still after 55 days of chilling there was a difference in budburst timing among LD and SD treatments. However this photoperiodic effect decreased notably after 105 days of chilling, which suggest that photoperiod is important only for partially chilled limes. Despite the fact that in experiment 3.a this photoperiod sensitive state of partial dormancy release occupied only a narrow time window during the progress of chilling, in natural outdoor conditions, this window could be larger due to fluctuating temperatures and slower fulfilment of chilling.

2. Effect of forcing temperature during quiescence

Sarvas (1972) provided conclusive experimental evidence for a logistic relationship between temperature and the rate of development of flower buds of several tree species. These findings were later supported by Campbell (1978), and are consistent with the hypothesis that during quiescence, bud growth capability is restored and responds to temperature equally to during the growing season (Sarvas, 1974).

The results from experiment 2.b, testing the effect of different forcing temperatures, confirmed that time to budburst decreases as temperature increases in all of the species studied (*F. sylvatica*, *B. pubescens*, *S. x smithiana*, *T. cordata*). This result is consistent with the previous studies of Skuterud and Dietrichson (1994), Myking (1997), Mahmood *et*

al. (2000), Hunter and Lechowicz (1992), and Cannell and Smith (1983). The response of budburst rate (a measure of bud growth) to increasing temperature could be described by a logistic curve, as previously shown by Sarvas (1974) and Campbell (1978). Despite the fact that the curve fits were based on the response to only 6 different temperatures, in all 4 species a logistic growth pattern was clearly detectable, with bud growth adjusting to an optimum at high temperatures. This supports the use of a logistic response of bud growth during quiescence in phenological models (Campbell, 1978; Landsberg, 1974; Hänninen, 1990; Chuine, 2000). However, the effect of extremely high temperatures, i.e. more than 30°C, on bud growth during quiescence has rarely been tested so that it is unsure whether this response is truly logistic or unimodal.

The comparison between the curve and parameters describing the response of budburst rate to temperature revealed affinities between *T. cordata* and *F. sylvatica* on one hand and between *B. pubescens* and *S. x smithiana* on the other. In fact, *T. cordata* and *F. sylvatica* showed a rapid increase of budburst rate at low temperatures with inflexion points of the fitted logistic curves around 11.5°C for both species, followed by a slower adjustment to the optimum. Their rates of increase (slope) of the curves were also numerically close. On the other hand the budburst rates of *B. pubescens* and *S. x smithiana* rose slowly at low temperatures and more rapidly above 15°C (the inflexion point of the fit logistic curve was at 23.4 and 15.4°C for *B. pubescens* and *S. x smithiana*, respectively) and reached their optima at higher temperatures. Hänninen (1990) fitted a similar logistic curve using the observations recorded in an experiment by Sarvas (1972) and also found that for *B. pubescens* budburst rate increased rapidly at relatively high temperatures, with the inflexion point of the forcing curve occurring at 18.4°C. This similarity is illustrated most clearly in Figure 15, plotting the relation between forcing temperature and rate of forcing for the 4 species. The parameters of the curves fitted to *F. sylvatica* and *T. cordata* are very similar, and the lower temperature of their inflexion points (c parameters) reveals a more rapid response at lower temperatures.

These patterns of response suggests a higher sensitivity to high temperature in *S. x smithiana* and *B. pubescens*, which are the faster developing and earlier flushing species. Likewise, the responses of *F. sylvatica* and *T. cordata* suggest a proportionally higher sensitivity to temperatures below 15°C and a relatively minor response to heat waves at the

time of growth onset. Their similarity might be related to their common survival strategy, that of dominant, slow growing and long lived species.

3. Effect of light intensity during quiescence

Light intensity has received relatively minor attention in studies investigating dormancy release, compared with other environmental factors such as temperature and photoperiod. In general, it seems that the effect of increased light intensity on bud phenology is indirect, through a warming caused by the absorbed radiation energy (Repo *et al.*, 1991, as cited in Partanen *et al.*, 2001). Among the species we tested the most marked response to light intensity was that of *F. sylvatica*, that advanced its budburst onset by approximately 4 days (from 11 to 7 days, a 30% advance) in high light intensity. *T. cordata* also responded to high light intensity by advancing its time to budburst, but only by one day. On the contrary, *B. pubescens* and *S. x smithiana* were not significantly affected by light intensity; in willow the times to budburst were exactly the same for the 2 treatments and flushing was synchronous, while for birch there was an advance in budburst timing by about 1 day (from 3 to 2 days) that was not significant.

If the advance in budburst onset was actually a result of a more elevated temperature of the bud due to absorbed radiation energy, it might be expected that the difference would be more detectable in plants requiring a higher thermal sum for budburst. However, as the observations were taken only once a day the measurement resolution was much lower for fast flushing species like birch than for slow flushing species like beech, and this might have reduced the accuracy and comparability of the ANOVA results for *B. pubescens*.

On the other hand the results from experiment 2.b testing the effect of forcing temperature suggest that the observed differences in budburst timing between the two light intensity treatments are not due only to temperature. Puckacki *et al.* (1980) reported that in natural conditions, on a sunny day, the temperature in the buds of Norway spruce may rise by 7.5°C above air temperature. Our growth cabinets were ventilated, and the radiation energy produced by the applied light intensity treatments was far weaker than outdoor on a sunny day. But even if the high light intensity treatment had raised bud temperature by 8°C above the air temperature of the cabinet (24°C), the decreases in time to budburst in high light

intensity treatment are far more consistent than would be expected if they were only caused by temperature. In fact, in experiment 2.b an increase in temperature from 24 to 32°C produced a decrease in the time to budburst of beech of only the 11% (from 13.5 to 12 days) as opposed to 30%, and had no effect on the budburst timing of lime.

Thus it can be concluded that light intensity does have a significant effect on the phenology of *F. sylvatica* and *T. cordata*, and that this effect is not attributable to bud warming due to radiation absorption. Instead it might be a result of a higher photosynthetic rate in the buds of *F. sylvatica* and *T. cordata*, two notably shade tolerant species that can take advantage of very low light irradiances (Ellenberg, 1988, Harbinson and Woodward, 1984; Kazda *et al.*, 2000). In fact, it has been shown that bud scales are not totally opaque to light and that they selectively transmit the incident light spectrum, so that only red and far red light reach the apical dome (Puckaki *et al.*, 1980). At high light irradiances, 0.11% of incident light reaches the centre of the buds of *F. sylvatica* (Puckaki *et al.*, 1980). Larcher and Nagele (1992), observed photosynthetic activity in beech buds and twigs during the whole year, even though it was lowest from January to March. An increase in photosynthetic activity then occurred in concomitance with rising temperatures, and was more consistent for bud primordia than in the cortex of the twigs (Larcher and Nagele, 1992).

The low light intensities reaching the bud primordia during experiment 1.a, might have been exploitable only by beech and lime, which responded by increasing photosynthetic rate and the rate of bud development in the high light intensity treatment.

4. Effect of nutrient deficiency

Potassium (K) and Nitrogen (N) are essential nutrients, which, if in inadequate supply, can lead to reduced growth rate and disrupt plant metabolism and function. N is required by plants for many cell components such as amino acids and nucleic acids (Taiz and Zeiger, 2002). K plays an important role in the regulation of the osmotic potential of plant cells (Taiz and Zeiger, 2002). Relatively few studies have focused on the effect of nutrient availability on phenology but they generally agree on the fact the phenophases most affected by nutrient levels are growth cessation and leaf senescence, and that improved nutrient availability delays them (Sigurdsson, 2001; Mugasha *et al.*, 1999; Roberntz, 1999).

In the present study, the applied severe nutrient deficiencies affected the timing of budburst onset by delaying it by a few days. The most pronounced delays were caused by K deficiency in *T. cordata* and *S. x smithiana* and N deficiency in *B. pubescens*. In addition, lime and willow were both affected by both nutrients resulting in a budburst onset delay, whereas birch was only affected by N deficiency.

These results confirm the fundamental importance of nutrients for plant growth. In fact, it is known that K is important for meristematic growth, a process during which it enables protein synthesis in the ribosomes (Wyn Jones and Pollard, 1983) and exerts an activating effect on the plasmalemma ATPase (Shingles and Mc Carthy, 1994). Kliwer and Cook (1971) found that during the winter, the vine stores arginine and K in the wood to be remobilized in spring for the rapid growth of shoots and new leaves. N, like K, is also stored in the bark during the winter, although the largest storage of N in this period is represented by the roots (Tagliavini *et al.*, 1999). N is also of foremost importance during vegetative growth, and plant nitrate concentration have been shown to increase sharply from growth onset until anthesis (Mengel and Kirkby, 2001).

Thus, it is expected that a severe deficiency of these nutrients could lead to a later budburst, as a result of a reduced bud growth rate. The fact that birch was especially affected by N deficiency is apparently in contrast with this species' capacity to tolerate nutrient poor and moist soils with low N availability. However, the small budburst delays observed in the three tested species are, in themselves, unlikely to affect their survival, which is instead most likely to be determined by the subsequent physiological adjustment to the low nutrient level during the vegetative season. The sensitivity of birch buds to N deficiency had already been documented in a study by Horgan and Wareing (1980), in which lateral buds of birch seedlings exposed to low N availability failed to form shoots as in the control group (Horgan and Wareing, 1980).

Despite the fact that the effects of nutrient deficiency on budburst timing were statistically significant, the observed delays in nutrient deficient treatments were small (a few days) when compared to the effects of temperature and photoperiod. Also, nutrient effects would be expected to be rather stable over time because of the buffering of nutrient in the soil. Their secondary role suggests that nutrients should be included in models of budburst only

once an adequate level of resolution in the calculation of budburst date has been reached, and especially for comparisons between sites with very different edaphic characteristics.

5. The control of budburst in trees

The physiological mechanisms underlying dormancy have not been clarified yet, and until now, it has been possible to base predictive models only upon whole plant-responses to changes in environmental condition (Chuine *et al.*, 2003). Our results certainly point to the complexity of these mechanisms: the same environmental conditions resulted in different “budburst” responses according to the phase of winter rest at which they were applied. Most importantly, the course of these phases did not appear to be clear-cut, but gradual, highlighting a continuous and quantitative nature of dormancy. Furthermore, the phases in which we divided the application of the experimental treatments (dormancy induction, chilling and forcing) appeared to be interdependent, the duration and conditions of one phase affecting the course of the following ones.

The effects induced by chilling depended on its timing (experiment 3.c), duration (experiments 2.a, 3.a, 3.b and 3.c) and temperature (experiments 2.a and 4.a). Similarly, the effects induced by SD during dormancy induction depended on its temperature, photoperiod and duration (experiments 3.c and 4.a). Finally, the effects induced by temperature during forcing depended on its timing, photoperiod and the state of chilling attained by the bud (experiments 2.b, 3.a, 3.b and 4.a). These multiple effects by external drivers were further complicated by the observed differences among experiments testing the same factors (such as lack of chilling in experiment 3.a and 3.c), which suggested that the physiological state of the bud can also affect the timing and the depth of dormancy. These results support the view of dormancy as a complex, dynamic phenomenon, involving many parallel and concomitant pathways and that can be influenced by conditions both internal and external to the plant (van der Shoot, 1996; Arora *et al.*, 2003, Ross, 1996; Horvath *et al.*, 2003)

5.1 External effects: photoperiod and temperature

It appeared evident that while in all species dormancy release was controlled by both photoperiod and chilling, the degree to which the two triggers contributed to the progress of dormancy varied among species. In *F. sylvatica* and *B. pubescens* daylength could substitute for lack of chilling completely or almost completely. *F. sylvatica* was particularly affected by LD that could replace a very large chilling requirement. In *B. pubescens* growth cessation and resumption were regulated by both temperature and photoperiod, which had interacting and overlapping effects.

The observed photoperiodic control particularly in *B. pubescens* highlights the adaptive significance of photoperiod as a timekeeper at northern latitudes, where the ample annual fluctuation in daylength is the only environmental factor providing a clear and absolute time signal from year to year. The reliance on more than one environmental signal maximizes the length of the growing season while limiting frost damage in the variable boreal climate (Li *et al.*, 2003), establishing birch as one of the most northerly tree species tolerating extreme growing conditions. The requirement of beech for long photoperiods, ensures budburst in late spring/early summer in the case where the large chilling requirement of this species had not been met, and provides a stabilizing signal to its spring phenology. While our results show that beech does not necessarily require chilling for growth resumption, it is clear that 16 h photoperiods do not occur in natural conditions in the middle of the winter or even in early spring, thus the sensitivity to this environmental signal does not expose beech to frost risk.

5.2 Internal effects: the physiological state of the bud

According to Crabbé and Barnola (1996) dormancy in woody species is the result of the interplay of external environmental triggers and internal correlative effects. In the summer, organogenesis, internodal elongation and leaf expansion interact strongly, and any limiting factor for any of these subprocesses can ultimately inhibit growth as a whole (Crabbé and Barnola, 1996). Summer correlative dormancy is quickly overcome by a new cycle of growth unless particular environmental conditions trigger the passage to longer, environmentally regulated dormant phases, such as winter dormancy (Crabbé, 1994).

The view of dormancy as being regulated by the interaction of internal and external influences agrees with some of the effects observed in the present study. For example, the enhancing effect that low temperatures exerted on the rate of dormancy induction in *B. pubescens* suggested that dormancy, besides being triggered by short photoperiods was also influenced by the rate of growth cessation and bud formation, which was higher at low temperatures. Similarly, the shallow dormant state that was observed in freshly rooted *B. pubescens* cuttings in experiment 3.a, was probably due to the effect of the synthetic auxin (NAA) contained in the used rooting powder, suggesting a dormancy-inhibiting effect of this hormone. These observations suggest that endodormancy cannot be considered solely as the result of external triggers, but the endpoint of a gradual evolution starting with eco or paradormancy that changes the physiological state of the bud, as described by Crabbé and Barnola (1996).

It is then clear that any event altering the context of meristematic domains (such as defoliation, or decapitation) or the physiological state of the plant could also modify the course and the depth of environmentally regulated dormancy (Bailly and Mauget, 1989). These effects need to be carefully considered when the effect of environmental factors alone cannot explain budburst observations.

6. Indicators of climate change

The results clearly show that, species like *S. aurita*, *S. x smithiana* and *T. cordata* require a period of exposure to chilling temperatures in order to restore growth competence and eventually flush. LD acted on these species by decreasing the minimum chilling requirement and increasing the rate of bud development in partially chilled plants. However, the influence of photoperiod decreased after extended periods of chilling; around 55 days for the two willow species and 105 days for lime. This suggests that if their small chilling requirements are met, during late winter/early spring these species are sensitive only to temperature. As the climate affecting the current distributional ranges of *S. aurita*, *S. x smithiana* and *T. cordata* at present largely satisfies their chilling requirements, their timings of budburst are probably the best indicators of climate change, and they will reflect changes in temperature in the months preceding them without any confounding effect of photoperiod. In addition, their responses to forcing temperature suggests that while the

timing of budburst of *T. cordata* could be a good indicator of the March-April average temperatures the two willow species could be a good indicator of the temperature of the months of January, February and March with a particular responsiveness to higher than average spring temperature ($>15^{\circ}\text{C}$).

A comparison with a correlation study analyzing the relationships between average monthly temperature and the timing of budburst of these clones at four IPG sites in Ireland (Sweeney *et al.*, 2002) confirms that the timing of leaf unfolding in *T. cordata* is significantly correlated with the average February-April temperature, while *S. x smithiana* and *S. aurita* do not show high correlation coefficients with the average temperature over the same period. However, this could be due to the fact that average monthly temperatures are not good indicators of unusual heat waves lasting a few days, which were shown to have immediate and pronounced effects on the timing of growth onset of *S. x smithiana* and *S. aurita*.

7. Phenology and life strategies

Phenology is a major determinant of tree species range (Chaine and Beaubien, 2001) because it influences the reproductive success and survival of trees. The present results confirm the adaptive value of the mechanisms that differently regulate dormancy and budburst in trees. For example, photoperiod sensitivity was particularly developed in *B. pubescens* that grows at northern latitudes where the differences in the light signal are strong and are reliable indicators of the time of the year. However the current study also suggests that phenological adaptations might be related to the particular life strategy of each species, in agreement with Kramer *et al.* (2000), who recognized different types of phenological responses among temperate tree species to variation in temperature.

The response of beech and lime to environmental triggers that release them from dormancy confirms the conservative life strategy of these two tree species. While *F. sylvatica* counts on a dual regulating system that minimizes frost risk while ensuring budburst, both species respond to temperature “conservatively”, with large chilling requirements and relatively low budburst rates at high temperatures. This system makes budburst less sensitive to early

spring heatwaves, which might be followed by late frosts. This strategy may be advantageous in a global warming scenario with extreme climate events during spring.

On the other hand, opportunistic pioneer species, such as *B. pubescens* and *S. x smithiana* showed generally smaller chilling requirements, and fast bud growth rates sharply rising at high temperatures. These characteristics agree with their ecological role of rapidly colonizing species of open and disturbed environments (Verwijst, 2001; Sennerby-Forsse *et al.*, 1992; Atkinson, 1992). While in *B. pubescens* these responses were balanced by a photoperiodic control delaying budburst in SD until dormancy was almost totally released, in *S. x smithiana* the photoperiodic effect was more limited. These mechanisms might allow seedlings and juvenile trees of these shade-intolerant species to trade a higher risk of frost damage for the opportunity of vigorous growth at the beginning of spring, before the closure of the canopy.

INTEGRATING PHOTOPERIOD INTO MECHANISTIC MODELS OF BUDBURST

INTRODUCTION

1. Mathematical models of budburst

1.1 Empirical, mechanistic and teleonomic mathematical models

Mathematical models can be generally classified according to their structure and how they address the phenomenon they want to represent. Thornley and Johnson (1990) defined model types in terms of the organizational levels they consider for the explanation of the observed data. In plant science, the different organizational levels follow a hierarchical structure in which the lower levels are represented by the cells and organelles and the higher by the plant and the crop. According to Thornley and Johnson (1990), models can explain the behaviour observed at “level i ” in terms of attributes observed either at the same level, or at lower ($i-1$, $i-2$, ...) or higher ($i+1$, $i+2$, ...) levels.

The simplest models are **empirical**: direct descriptions of observational data are linked to environmental variables through a fitting procedure. Empirical models describe level i behaviour in terms of level i attributes alone, without any constraints from higher levels or knowledge of lower order mechanisms (Thornley and Johnson, 1990). Although they provide means of summarizing data and are of practical value, they do not improve our understanding of the system (Box *et al.*, 1978).

Mechanistic models attempt to construct a description of the behaviour observed at the i -level through the mechanisms that are assumed to occur at $i-1$ (or lower) organizational level (Thornley and Johnson, 1990). Through analysis and reduction, the mechanistic modeller breaks the system down into components and assigns processes and properties to these components, in order to introduce extra variables at the lower levels. The observational (or experimental) data that are available for these lower levels are then used, and finally the set of equations that define the system are integrated. Obviously this type of model is far more complex than an empirical one, and, due to the many constraints built into its structure provides a less good fit for the data. However its contents apply to a wider range of phenomena, and it offers more possibilities for manipulating the system (Thornley and Johnson, 1990). More importantly, if a mechanistic model is adequately verified, it

confirms our scientific understanding of the system, while if it is not, it can suggest new sets of experimental conditions that are worthy of investigation (Box *et al.*, 1978).

Finally, **teleonomic** models are formulated in terms of “goals”. These models refer the responses at the *i*-level to the constraint provided by the *i*+1 level (Thornley and Johnson, 1990). They are built assuming that the plant has certain requirements (such as optimizing its growth rate or its survival probabilities), and that the mechanisms occurring at the lower levels are able to satisfy those requirements. These models however only play a minor role in plant modelling and are not as widely used as empirical and mechanistic models.

1.2 Models of budburst: a classification

The above classification by Thornley and Johnson (1990) also applies to phenological models. Indeed, the proposal by Chuine *et al.* (2003) of dividing the models for the prediction of budburst into statistical (comparable to empirical), mechanistic and theoretical (comparable to teleonomic) models, reflects the view that they can be classified in terms of the “organizational levels” they consider to explain the behaviour of the observed data. However, since the cellular mechanisms triggering dormancy and dormancy release are not known, the processes occurring at the *i*-1 levels explaining budburst timing are intended more as experimentally established whole-tree responses to changes in the environmental triggers of dormancy during each of the sub-phases of winter rest, than actual biochemical events.

1.2.1 Theoretical models

These types of models, are concerned with a “goal”, which in the case of leaf phenology has been indicated as the optimization of the cost/benefit tradeoff of producing new leaves (Chuine *et al.* 2003; for an example see Kikuzawa and Kudo, 1995).

1.2.2 Statistical models

Statistical phenological models relate the timing of phenological events to environmental factors. The timing of the observed event is examined and represented as a response of the assumed driving factors (usually temperature), and then an equation (usually a linear relationship) is fitted to the data. The simplest statistical models of budburst establish a

correlation between its timing and average temperature of some period of the year (see for example, Chmielewsky and Rotzer, 2000; Beaubien and Freeland, 2000). However there are also more complex models that consider more than one environmental factor (Schwartz, 1997). While statistical models of budburst/flowering/leaf unfolding have been used to represent the recently observed changes in the timing of these phenophases in relation to temperature, it is not possible to extrapolate any future trends in budburst timing as a response to predicted global warming, as statistical models do not account for any functional relationships between temperature and budburst (Kramer *et al.*, 2000).

1.2.3 Mechanistic models

Mechanistic models formally describe known or assumed cause-effect relationships between biological processes and driving environmental factors (Chuine *et al.*, 2003). As the physiology of dormancy is largely unknown, it is especially complicated to introduce new relationships explaining budburst timing in terms of events occurring at the cellular or organ levels. Indeed, only a few mechanistic models have been based on hypotheses of the actual biochemical events triggering dormancy (Schaber and Badeck, 2003; Erez and Fishman, 1998). To date, most mechanistic models of tree phenology have been based on whole-bud or whole-tree responses to temperature or photoperiod.

Experimental studies have shown that the response of the dormant plant to different environmental triggers (such as photoperiod or temperature) changes as dormancy progresses, and its responses are in some cases proportional to the amount of time during which a particular condition occurs. These findings have resulted in the elaboration of mechanistic models that describe budburst timing as a consequence of two different processes: dormancy release upon the cumulative effect of chilling temperatures and ontogenetic development upon the cumulative effect of forcing temperatures (Hänninen, 1990). These models account for the effects of temperature in terms of developmental units: different functions relate temperature with the rate of growth or the rate of dormancy release of the buds, and the resulting “forcing” or “chilling” units are accumulated until the fulfillment of a critical threshold, that marks the transition to the next stage (full dormancy release or budburst).

The simplest models only consider the effects of forcing temperatures for the prediction of budburst timing. In the **Thermal Time model** (Cannell and Smith, 1983; Chuine *et al.*, 1999; Kramer, 1994b; Pop *et al.*, 2000), also called **Spring Warming model** (Hunter and Lechowicz, 1992) temperature is linearly related to the rate of growth of the developing bud and the derived thermal units are accumulated from t_0 until a critical threshold is reached and budburst occurs. Thermal time models do not consider the timing of dormancy fulfillment to be an important factor in determining budburst timing, and thus assume that forcing starts at a fixed date, when all chilling requirements are fulfilled (Chuine *et al.*, 2003).

More sophisticated models also consider the effect of chilling temperatures, but differ as to the timing of the periods during which chilling and forcing temperatures are active. While some models (*e.g.* **Sequential model**, Utah chill model) consider ontogenetic development to start only after the fulfillment of a fixed chilling requirement (Sarvas, 1974; Richardson *et al.*, 1974; Hänninen, 1987, 1990), after which chilling has no more effects on bud growth, some others have a more dynamic vision of dormancy transition and do not consider dormancy as a sequence of steady states separated by boundaries.

The **Parallel model** (see Landsberg, 1974; Hänninen, 1987, 1990) considers forcing temperatures to be active simultaneously with chilling temperature. The double effect of temperature at advanced stages of dormancy release has been supported by experimental evidence (Seeley, 1996) and implies that at some temperatures dormancy release occurs at the same time as bud growth. The **Alternating model** goes a step further and considers chilling and thermal time requirements to be interrelated (Cannell and Smith, 1983; Murray *et al.*, 1989; Chuine *et al.*, 1999; Kramer 1994b). The Alternating's model assumptions are also based on experimental evidence: woody plants that were moved into a forcing environment after increasing durations of chilling showed an inversely exponential decrease in thermal time to budburst (Landsberg, 1974; Cannell and Smith, 1983).

The **Four phases model** was formalized by Hänninen (1990) on the premise that the range of environmental conditions in which plant development is possible narrows and widens during the annual cycle (Vegis, 1964). Based on the idea of Vegis (1964), Hänninen divided winter rest in pre-rest (dormancy induction, during which growth capability decreases), true-rest (dormancy or endodormancy) and post-rest (dormancy release, during

which growth capability increases again) and quiescence (ecodormancy), and formalized these assumption by gradually changing the temperature threshold above which bud development could take place.

Similarly to the four phases model, the **Deepening rest model** (Kobayashi *et al.*, 1982) considers dormancy to be the result of a gradual decrease in growth competence (deepening rest phase) and quiescence to be the result of a gradual increase in growth competence, but it does not consider the true-rest stationary phase in which growth capability is 0. The Four model phases and the Deepening rest models are the only models reflecting the gradual and quantitative character of full dormancy attainment shown by experimental studies such as those by Kawase (1961), Downs and Borthwick (1956) and Welling *et al.*, (1997).

All these different models are nonlinear and non-nested within each other, which makes it impossible to compare them with classical comparative tests such as the F-test (Chuine, 2000) and easily select the model that best describes a set of data (but see Kramer, 1994a). This problem was addressed by Hänninen (1990), who proposed a synthesis model for the effects of temperature on budburst, unifying the sequential, the parallel, the deepening rest and the four phases model. The synthesis model contains part of the original models as special cases and its parameters represent aspects of bud dormancy that should be verified through experimental studies (Hänninen, 1990). This synthesis model was developed further by Hänninen (1995) through the use of variables that at any moment during dormancy simulation could express the state of rest break, growth competence and ontogenetic competence of the bud. The state variables were related to environmental variables through general equations, and additional assumptions, specified by further equations, referred to submodels that could be chosen freely.

2. The Unified model

Chuine (2000) developed a fully unified model, through which she showed that the functions describing the responses of dormancy release and bud growth to temperature in the Sequential, Parallel, Alternating, Four phase and Deepening rest model are all particular cases of more general functions (Chuine, 2000). In the Unified model, two general functions relate temperature with the rates of chilling and forcing development, and another

function defines the dependency between the state of forcing and the state of chilling (see Box 1).

A final relationship defines the periods during which the buds are sensitive to chilling and forcing temperatures, accounting for all different possibilities proposed by the previous models (forcing accumulation starting only after chilling fulfillment, as in the Sequential model; forcing accumulation starting before the end of chilling accumulation as in the Parallel and Alternating models). These functions are integrated within a framework defined by a total of nine parameters. This common statistical framework allows for a direct estimation of the response of bud growth to chilling or forcing temperature, to identify the period of action of chilling and forcing temperatures on bud growth and to identify the relationship between the amount of chilling units accumulated and forcing units necessary for budburst. In addition, the unified model allows for model comparison through classical comparative tests (F-test and likelihood ratio), and facilitates the identification of the phenological model which better suits a particular set of data. This selection is made on the basis of the parameter values corresponding to the best fit, which indicate the model structure and specify the character of the bud (or plant, or species) responses to temperature. According to Chuine (2000) and as pointed by Myking (1997), our lack of understanding of the processes regulating dormancy could mislead us in our interpretation of phenological data, if our *a priori* assumptions are too constrained by a fixed model structure. Indeed, the Unified model provides a powerful and flexible tool that supports an open and exploratory approach to the interpretation of dormancy events.

2.1 Parameter estimation: the Metropolis algorithm

The Unified model (Chuine, 2000) uses a simulated annealing algorithm for its parameter fitting procedure instead of traditional numerical methods such as the Downhill simplex and the Newton methods. In fact, these optimisation methods are often non-convergent, due to the many local minima of the least square functions of budburst models (Chuine *et al.*, 1999). The convergence is most likely obtained by the Metropolis algorithm (Press *et al.*, 1989), which enables a more effective exploration of the parameter space, which proceeds through a “random walk” tending to gradually decrease the residual sum of squares (Chuine, 1998; Chuine *et al.*, 1999; Press *et al.*, 1989). The parameter space is explored roughly at first by “large steps” between one set of parameter values and the next, and then

in detail with a smaller step size around the relative or absolute minimum found. It is not always the case that a set of parameters that increases the residual sum of squares is rejected: the probability of rejecting it is higher at the initial stages of the fitting procedure, when there is also a higher probability that the minimum found is a relative one. In fact, this algorithm mimics the process through which slowly cooling metals anneal and reach the minimum energy conformation, and uses the principle on which is based the Boltzmann probability distribution, $\text{Prob}(E) \approx \exp(-E/kT)$, according to which a system at higher temperature has its energy more distributed around all the different energy states (E).

Analogously, the Metropolis algorithm gradually decreases its “temperature”, so decreasing the probability of rejecting a change that increases the sum of squares. By decreasing the temperature very slowly, the convergence towards the absolute minimum will be facilitated even for the least squares function of a phenological model (Chuine *et al.*, 1999).

The Unified model (Chuine, 2000)

The Unified model rests on the assumption that temperature is the only significant driver of bud growth. Chilling temperatures act by gradually releasing dormancy and restoring the bud's ontogenetic competence, or its ability to grow in response to forcing temperature. Average daily temperature is converted into chilling or forcing units by means of non-linear relations:

- A general curve defined by three parameters quantifies chilling:

$$(1) \text{ Chilling Units} = 1/1 + e^{a(x-c)(x-c)+b(x-c)}$$

- A general sigmoid curve defined by two parameters forcing:

$$(2) \text{ Forcing Units} = 1/1 + e^{d(x-e)}$$

In both functions, the independent variable x stands for temperature. These two functions are named CF_c (1) and CF_f (2) by Chuine (2000).

According to the values fitted to the parameters, the model will account for all the types of temperature responses assumed by the sequential, parallel, alternating and thermal time model.

The states of forcing and chilling are described as daily accumulations of rates of forcing and chilling. Accumulation of chilling starts at t_0 , which is set arbitrarily as the 1st of September, while accumulation of forcing starts as soon as a minimum state of chilling is reached (C_{crit}).

Based on experimental evidence showing a negative relationship between the state of forcing and the state of chilling, the amount of forcing units required for budburst depends on the amount of accumulated chilling units CU_{sum} , through a two-parameters inverse exponential relation:

$$(3) F_{crit} = w e^{(k CU_{sum})}$$

With $k > 0$, $k < 0$.

However, for values of k tending to 0, this relationship is reduced to a horizontal line, $y = 1$, implying that there is no relation between chilling and thermal time.

According to the parameters fitted to C_{crit} (onset of quiescence and start of the forcing period, reached at day t_1) and t_c (the time when chilling temperatures cease being active) we can have different cases:

1. Chilling temperatures (CT) act from t_0 to t_1 and forcing temperatures (FT) act from t_1 to budburst (t_2), (Sequential model).
2. CT act from t_0 to $t_c > t_1$ and FT act from t_1 to t_2 (Alternating model).
3. CT act from t_0 to $t_c > t_1$ and FT act from $t_1 \sim t_0$ to t_2 (Parallel model)

Let t'_0 be the day when the state of chilling becomes strictly positive. These three cases can then be formalized as follows:

1. $t_c = t_1$ and $C_{crit} > 0$,
2. $t_c > t_1$ and $C_{crit} > 0$,
3. $t_c > t'_0$ and $C_{crit} \sim 0$.

Thus, the parameters defining the Unified model are nine in total: a , b , c , d , e , w , k , C_{crit} and t_c .

3. Aims of the study

The experiments conducted during this study offered some insights into the mechanisms through which photoperiod and temperature interact to promote budburst in *B. pubescens*. Photoperiod was the main factor triggering dormancy induction, although temperature was shown to speed up the rate of dormancy induction (experiments 3.c and 4.a). Once endodormancy had been attained, the photoperiod received during the exposure to chilling temperatures did not affect the subsequent rate of budburst (experiments 3.c and 4.a). However, once a minimum chilling requirement was fulfilled, photoperiod above a critical threshold enabled growth at forcing temperatures and enhanced percentage budburst as well as advancing budburst timing (experiment 3.a and 3.b). The effect of photoperiod decreased as chilling progressed and the buds reached full growth competence. Increasing chilling increased the growth rate at a given forcing temperature, and decreased the time to budburst (experiments 2.a, 3.a, 3.b, 3.c).

The aim of this work is to integrate photoperiod into a mechanistic model of budburst, so to account for the the experimentally observed responses to this environmental factor, and increase the realism of budburst models for photoperiod-sensitive species. This may help in gaining more insight into the functional relationships between environmental drivers and dormancy release, and pinpoint areas of this field where knowledge is lacking. A more detailed knowledge of the triggers of dormancy and their interaction will ultimately lead to more accurate predictions of budburst timing.

METHODS

The effects of photoperiod on budburst timing have been previously modelled as a change in chilling rate through a multiplication coefficient K (Campbell and Sugano, 1975) or an additive factor (Kramer, 1994b), or through the use of a fixed calendar date (Häkkinen *et al.*, 1998). These attempts have given conflicting results (Kramer, 1994b, Häkkinen *et al.*, 1998). Our finding that photoperiod does not affect the timing of budburst if coupled with low, chilling temperatures suggested that photoperiod directly affected growth rate rather than chilling rate.

Here the responses observed during the experimental part of this study have been formalized and integrated into the framework of the Unified Model (Chuine, 2000), to develop a mechanistic model accounting for both photoperiod and temperature in *B. pubescens* and other tree species showing similar photoperiodic responses.

1. Formalization of the effects of photoperiod and temperature on the progress of dormancy

Assuming that the date of the start of chilling accumulation is similar from year to year, the date of budburst timing is defined by both the *rate of chilling* and the *rate of forcing*.

1.1. Rate of chilling

The rate of chilling is conveniently modelled through the CF_c function (a_c , b_c , d_c , x_t) of the Unified Model (Chuine, 2000). This general sigmoid function accounts for all types of responses previously hypothesised by the most used phenological models as has been shown by Chuine (2000). It can assume very different shapes according to the values fitted to the three parameters (Figure 39). It can account for both a uniform temperature-response with no optimal temperature eliciting a faster dormancy release, and a very steep temperature response with only a narrow range of chilling temperatures triggering dormancy release. It can also account for intermediate responses, such as that previously observed by Sarvas (1974), who found that while the optimal temperature for dormancy

release in *B. pubescens* was 3.4°C, all temperatures in the range from -3 to 10°C contributed to this transition. Our experiments did not highlight an optimal chilling temperature nor a precisely shaped chilling-curve and the generality and adaptability of this curve suits our lack of precise information on this response.

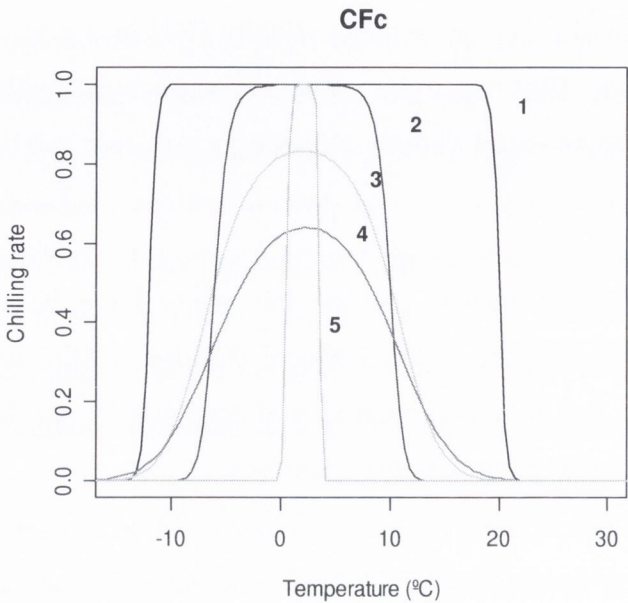


Figure 39. Relation between temperature and chilling rate as described by the CFc function (ac, bc, dc, xt), with xt the average daily temperature at the julian day t . The shape of the function can vary according to the parameters value choice. (1) $ac = 0.1$; $bc = 3.2$; $cc = 20$; (2) $ac = 0.1$; $bc = 1.6$; $cc = 10$; (3) $ac = 0.025$; $bc = 0.4$; $cc = 10$; (4) $ac = 0.0175$; $bc = 0.2$; $cc = 8$; (5) $ac = 3$; $bc = 9$; $cc = 3.5$.

1.2 Rate of forcing

The experiments conducted indicated that the rate of forcing in *B. pubescens* depends on three different factors:

1. Forcing temperature.

The results confirmed the previously shown sigmoidal relationship between forcing temperature and rate of budburst (Campbell, 1978; Hänninen, 1990; Sarvas, 1974). The sigmoidal pattern in the increase of the rate of budburst at increasing temperatures can be seen in Figure 29.a.

2. Chilling duration

The results indicated that for *B. pubescens*, there is a negative relationship between chilling duration and number of days to budburst, confirming previous studies of Cannell and Smith (1983) and Murray *et al.* (1989). Figure 40 shows that the relationship between chilling

duration and days to budburst for *B. pubescens* in experiment 3.a can be described by a negative exponential relationship.

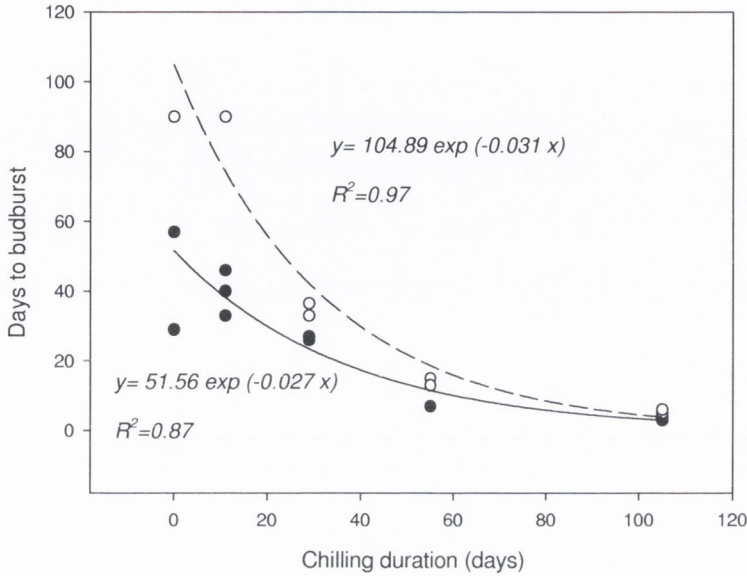


Figure 40. Relation between chilling duration and number of days to budburst for *B. pubescens* at a 16 h (filled symbols) and 8 h (open symbols) photoperiod. Non flushing plants have been represented as flushing after 90 days, which was the duration of the forcing treatment. Data are from experiment 3.a (effect of chilling duration and forcing photoperiod (8 and 16 h)).

3. Forcing photoperiod

Forcing photoperiod above a critical threshold was shown to enhance the rate of forcing, especially for short chilling durations and at temperature above 10°C. The exposure to a range of forcing photoperiods after varying chilling durations suggested that for a given temperature the critical photoperiod enabling budburst is not constant, but decreases after the exposure to increasing chilling durations (experiment 3.b, result section, Figure 23). The threshold photoperiod, corresponding to the attainment of a minimum of 40% budburst progressively decreased from 13 ± 1 to <10 hours (Figure 41).

We hypothesised that the effect of forcing photoperiod on the forcing rate was expressed through a horizontal shift in the xy plane of the sigmoid curve CFf (0, b_f, c_f, x_t) relating temperature with forcing rate, so that a long photoperiod made the forcing curve shift towards the y-axis and a short photoperiod made it shift away from the y-axis. The shift of the curve towards the y-axis results in an increase in the forcing rate elicited by a given forcing temperature, without altering the optimum forcing rate. This shift can be obtained with a decrease in the value of the c_f parameter of the CFf curve, that controls the x-

coordinate of the inflexion point of the sigmoid. C_f corresponds to the temperature at which half a forcing unit is accumulated (T50) (Figure 42).

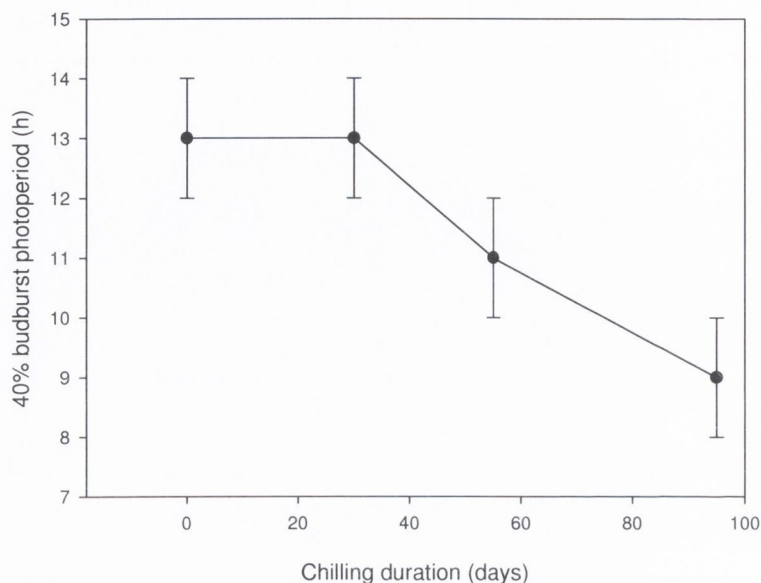


Figure 41. Relation between chilling duration and threshold photoperiod above which budburst > 40%. The error bars (± 1) indicate a 2-h interval between the tested photoperiods. Data are from experiment 3.b (effect of chilling duration and forcing photoperiod (10, 12, 14 and 16 h)).

The other way to control the forcing rate is by introducing a coefficient K , which is a factor of multiplication of the forcing rate. However, this does not account for our experimental results suggesting that the increase in forcing rate due to photoperiod is not uniform at all temperatures (experiments 3.c and 4.a). Thus, the shift of the CFF curve appears to be a more suitable method to express the influence of photoperiod on forcing rate.

The enhancing effect of photoperiod on forcing rate was expressed through a relationship linking c_f (expressing T50) with Pht_t , the photoperiod at the julian day t . This relationship, called CFT50, is negative (higher photoperiods correspond to a lower T50) and sigmoidal, as it is assumed that T50 cannot decrease below 0 (the state of maximum growth competence, with forcing units being accumulated at all temperatures $>0^\circ\text{C}$) or increase above T_{max} , (the highest temperature at which forcing rate is 1/2 or the state of minimum growth competence). Experiment 2.a testing the effect of a range of forcing photoperiods on timing and percentage of budburst, shows that the action of photoperiod on growth depends on the duration of the exposure to chilling temperatures, and that the threshold photoperiod for budburst decreases as chilling progresses. We hypothesised that this effect

was the cause of the observed increase in forcing rate of plants exposed to long chilling durations at a given photoperiod, and that it indirectly affected the position of T50 through a shift in the curve CFT50. This effect was thus expressed through another relationship, called CFDI50, linking the number of chilling units accumulated at the julian day t with the threshold photoperiod at which $T50=1/2(T_{max})$, which we called DI50, representing the x-coordinate of the inflexion point of CFT50. This relationship is negative (high sums of chilling units correspond to lower DI50) and as DI50 stands for day length, it is assumed that it cannot decrease below 0 or increase above 24.

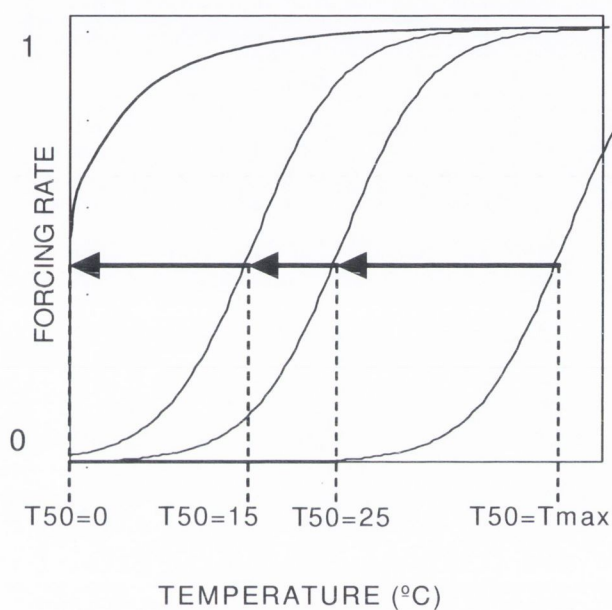


Figure 42. Shift in the position of the CFf curve due to a change in cf (expressing $T50$). A decrease in the value of $T50$ corresponds to an increase of the forcing rate at a given temperature.

As a consequence, differently from the Unified model, the model proposed does not utilize the threshold C_{crit} (minimum state of chilling after which forcing temperatures are active) nor the relationship $F_{crit} = w e^{kCU_{sum}}$ linking the state of chilling with the critical number of forcing units needed for budburst. In fact, the critical state of forcing F_{crit} is constant, while the forcing rates assigned to each forcing temperature change with chilling duration and photoperiod. For example, after little chilling and in short photoperiods, the forcing curve will be positioned towards high temperature values, with consequent accumulation of none or little forcing at normal winter-spring temperature. However, after a long chilling duration, DI50 will have decreased, and even for low photoperiods, the value of $T50$ identifying the position of the forcing rate sigmoid on the x-axis will be low, so that and that almost all forcing temperatures $>0^{\circ}\text{C}$ will elicit high forcing rates. Correspondingly, a

small amount of chilling will be partly compensated by long photoperiods, as observed in experiments 3.a and 3.b.

2. The UnPhot model

From the above considerations, a model, derived from the unified model (Chuine, 2000), with 9 parameters (a_c , b_c , c_c , $bDI50$, $cDI50$, $bt50$, $Tmax$, b_f , F_{crit}) that we call UnPhot model, is defined as follows:

$$(1) CU = 1 / (1 + e^{-(a_c(x_t - c_c) + b_c(x_t - c_c))}) \quad (CFc)$$

As in the unified model, the response of chilling rate to chilling temperature is quantified by a general sigmoid curve defined by three parameters called $CFc(a_c, b_c, c_c, x_t)$, with x_t the mean daily temperature at the julian day t .

$$(2) DI50 = 24 / (1 + e^{-(bDI50(x_t - cDI50))}) \quad (CFDI50)$$

The response of DI50 to chilling accumulation is quantified by a negative sigmoid curve called DI50 ($bDI50$, $cDI50$, x_t), with x_t the sum of chilling units (CUsum) accumulated at the julian day t and $bDI50 > 0$. DI50 can vary between 0 and 24.

$$(3) T50 = Tmax * (1 / (1 + e^{-(bt50(x_t - DI50_t))})) \quad (CFT50)$$

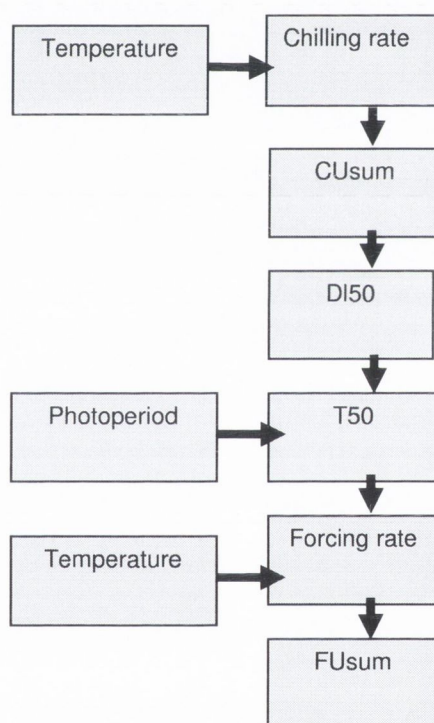
The response of the T50, the x-coordinate of the inflexion point of the forcing curve CF_f (or the temperature at which half a forcing unit is accumulated) to photoperiod is quantified by a negative sigmoid curve called T50 ($bt50$, $DI50_t$, x_t , $Tmax$), with x_t is the photoperiod Pht_t at the julian day t , $bt50 > 0$ and $DI50_t$ the DI50 at the julian day t , calculated through (2).

$$(4) FU = 1 / (1 + e^{-(b_f(x_t - T50_t))}) \quad (CFf)$$

The response to forcing temperature is quantified by a positive sigmoid curve defined by two parameters, as the one used by the unified model (Chuine, 2000) called $CF_f(b_f, T50_t, x_t)$, with x_t the mean daily temperature at the julian day t and $T50_t$, the T50 at the julian day t , calculated through (3). The nine parameters defining it are a_c , b_c , c_c , $bDI50$, $cDI50$, $bt50$, $Tmax$, b_f , F_{crit} .

The model starts to calculate chilling and forcing units from t_0 , that has arbitrarily been fixed as the 30th of September, as suggested by the experimental observation that plants exposed to naturally decreasing photoperiod (at 52° lat) and an average daily temperature of 18°C were dormant towards the end of September (experiment 4.a). A conceptual model of the effects of temperature and photoperiod in the UnPhot model is represented in Figure 43.

Figure 43. Conceptual model of the effects of temperature and photoperiod in the UnPhot model. Temperature affects forcing rate and chilling rate, photoperiod affects the forcing rate indirectly through a change in the temperature corresponding to the accumulation of $\frac{1}{2}$ forcing unit. Budburst occurs when the sum of forcing units FU_{sum} is equal to F_{crit} , the critical sum of forcing units required for budburst.



2.1 Examples of temperature and photoperiod responses accounted for by the UnPhot model

The UnPhot model is dynamic (forcing rate changes as chilling is accumulated and depends on photoperiod) and accounts for a gradual transition from dormancy to quiescence, or according to the values assigned to its parameters, for a fast dormancy release leading to maximum growth competence ($T_{50}=0$). Some examples of the types of responses accounted for by the UnPhot model are given below.

2.1.1. *The duration of rest is constant, the rate of forcing is not affected by further chilling*

($a_c=0.03$; $b_c = 10$; $c_c=25$; $bDI_{50}=3$; $cDI_{50}=50$; $bt_{50} = 3$; $T_{max} = 95$; $bf=-0.2$).

With $c_c = 25$, and $b/a \gg 25$, the response to chilling temperature is uniform over a wide range of temperatures below 25°C (Figure 44, graph 1), which means that any day during winter elicits the accumulation of 1 chilling unit. The accumulation of forcing units starts when cDI_{50} chilling units have accumulated (Figure 44, graph 2), and the photoperiod is > 0 h (Figure 44, graph 4.2.2). However, as each day corresponds to a chilling unit, the start of forcing will always be on the same date every year (about cDI_{50} days after t_0). These responses (constant duration of rest and start of forcing on the same date every year) could account for the Thermal Time model (Cannell and Smith, 1983; Robertson, 1968), with a base temperature a base temperature $< 0^\circ\text{C}$ and the accumulation of $\frac{1}{2}$ forcing unit at 0°C .

2.1.2. *The duration of rest is not constant, the rate of forcing is not affected by further chilling*

($a_c = 0.08$; $b_c = 1$; $c_c = 10$; $bDI_{50} = 1$; $cDI_{50} = 40$; $bt_{50} = 3$; $T_{max} = 90$; $bf = -0.2$)

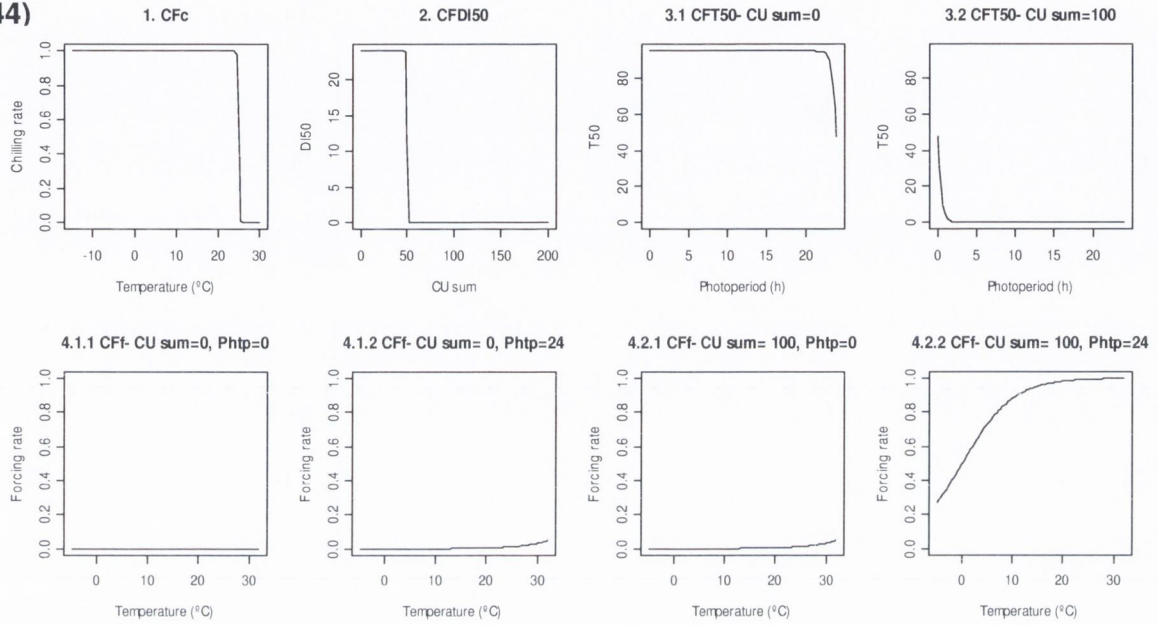
The response to chilling temperature is not uniform, and has an optimum around 4°C with lower rates of chilling at suboptimal temperatures. The rate of chilling is ≈ 0 for temperatures $> 15^\circ\text{C}$ and $< 6^\circ\text{C}$ (Figure 45, graph 1). As long as CU sum is $<$ than cDI_{50} , the forcing rate is ≈ 0 , at all photoperiods. However, around the day when cDI_{50} chilling units have been accumulated, the forcing rate quickly increases (Figure 45. graph 4.2.2) and forcing temperatures become effective. These responses (forcing temperatures become effective after a fixed amount of chilling has been fulfilled) correspond to the Sequential

Figures 44 and 45. Responses to temperature and photoperiod predicted by the UnPhot model for (5) $a_c=0.03$; $b_c=10$; $c_c=25$; $bDI50=3$; $cDI50=50$; $bt50=3$; $T_{max}=95$; $bf=-0.2$.

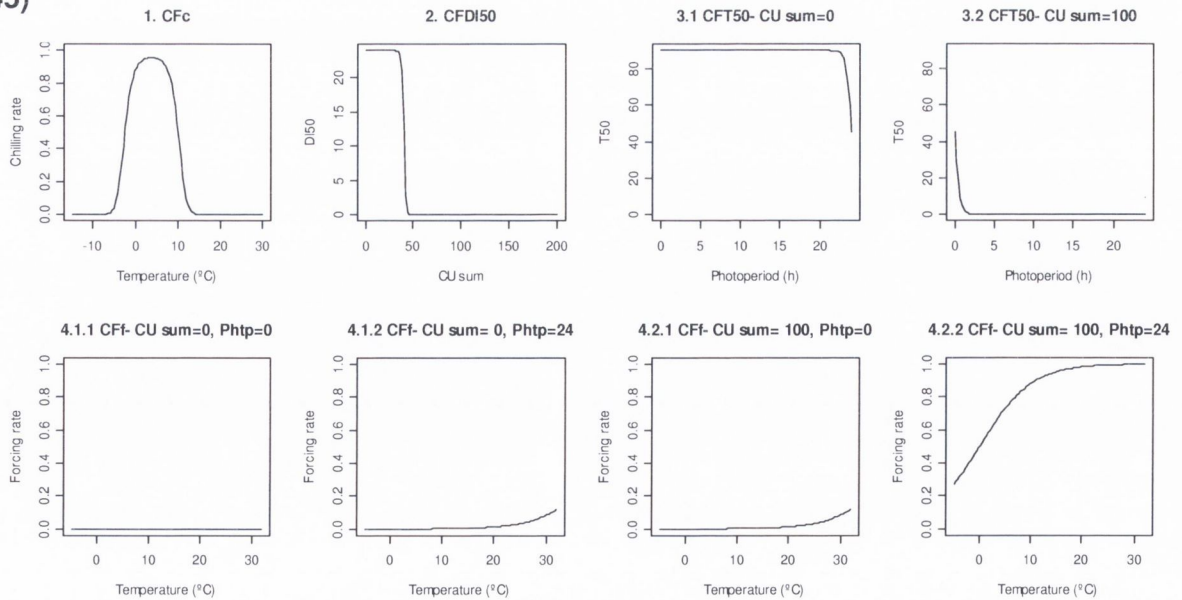
(6) $a_c=0.08$; $b_c=1$; $c_c=10$; $bDI50=1$; $cDI50=40$; $bt50=3$; $T_{max}=90$; $bf=-0.2$.

In each figure: (1) Response of chilling rate to temperature; (2) Response of DL50 to the sum of accumulated chilling units (CU); (3) Response of T50 to photoperiod, (3.1) after the accumulation of 0 CU, (3.2) after the accumulation of 100 CU; (4) Response of forcing rate to temperature, (4.1.1) after the accumulation 0 CU, at a 0 h photoperiod, (4.1.2) after the accumulation 0 CU, at a 24 h photoperiod, (4.2.1) after the accumulation 100 CU, at a 0 h photoperiod, (4.2.2) after the accumulation 100 CU, at a 24 h photoperiod.

(44)



(45)



model (Richardson *et al.*, 1974; Sarvas, 1974; Hanninen 1987, 1990) with a base temperature $<0^{\circ}\text{C}$ and the accumulation of $\frac{1}{2}$ forcing unit at 0°C .

2.1.3. The duration of rest is not constant, the rate of forcing is affected by further chilling

$$(a_c = 0.05; b_c = 0.8; c_c = 8; bDI50 = 0.1; cDI50 = 50; bt50 = 0.1; T_{max} = 90; bf = -0.2.)$$

The response to chilling temperature is not uniform, and has an optimum around 0°C , with lower rates of chilling at suboptimal temperatures. The rate of chilling is ≈ 0 for temperatures $> 12^{\circ}\text{C}$ and $< -12^{\circ}\text{C}$ (Figure 46, graph 1). The relationship between CU sum and forcing rate, for a given photoperiod and temperature may be described by a sigmoidal curve, and thus, the relationship between CU sum and time to budburst ($1/\text{forcing rate}$) by an inverse exponential curve. In fact CFf is a function of the temperature, the photoperiod and the amount of chilling units accumulated at julian day t .

$$FU = 1/1 + e^{(b_f (T_t - T_{50_t}))} \quad (4)$$

$$\text{But } T_{50_t} = T_{max} (1/1 + e^{(bt_{50} (Ph_t - DI_{50_t}))}) \quad (3), \text{ so}$$

$$FU = 1/1 + e^{(b_f (x_t - (T_{max} (1/1 + e^{(bt_{50} (x_t - DI_{50_t}))}))))}$$

$$\text{But } DI_{50_t} = (24/1 + e^{(bDI_{50} (CU_{sum} - cDI_{50}))}) \quad (2), \text{ so}$$

$$FU = 1/1 + e^{(b_f (T_t - (T_{max} (1/1 + e^{(bt_{50} (Ph_t - (24/1 + e^{(bDI_{50} (CU_{sum} - cDI_{50}))}))))))})}$$

(5)

with T_t , the mean daily temperature, Ph_t the photoperiod and x_t the CU sum at julian day t (Figure 47.a). The number of days to budburst can thus be expressed as the inverse of the daily rate of forcing (Figure 47.b), so that:

$$DTB \text{ (days to budburst)} = 1/FU$$

$$DTB = 1 + e^{(b_f (T_t - (T_{max} * (1/1 + e^{(bt_{50} (Ph_t - (24/1 + e^{(bDI_{50} (x_t - cDI_{50}))}))))))})} \quad (6)$$

The simultaneous action of chilling and forcing temperatures, and the negative exponential relationship between CU sum and time to budburst account for the main assumptions of the Alternating model (Cannell and Smith, 1983; Murray *et al.*, 1989).

Figure 46. Responses to temperature and photoperiod predicted by the UnPhot model for $ac = 0.05$; $bc = 0.8$; $cc = 8$; $bDI50 = 0.1$; $cDI50 = 50$; $bt50 = 0.1$; $T_{max} = 90$; $bf = -0.2$.

(1) Response of chilling rate to temperature; (2) Response of DI50 to the sum of accumulated chilling units (CU); (3) Response of T50 to photoperiod, (3.1) after the accumulation of 0 CU, (3.2) after the accumulation of 100 CU; (4) Response of forcing rate to temperature, (4.1.1) after the accumulation 0 CU, at a 0 h photoperiod, (4.1.2) after the accumulation 0 CU, at a 24 h photoperiod, (4.2.1) after the accumulation 100 CU, at a 0 h photoperiod, (4.2.2) after the accumulation 100 CU, at a 24 h photoperiod

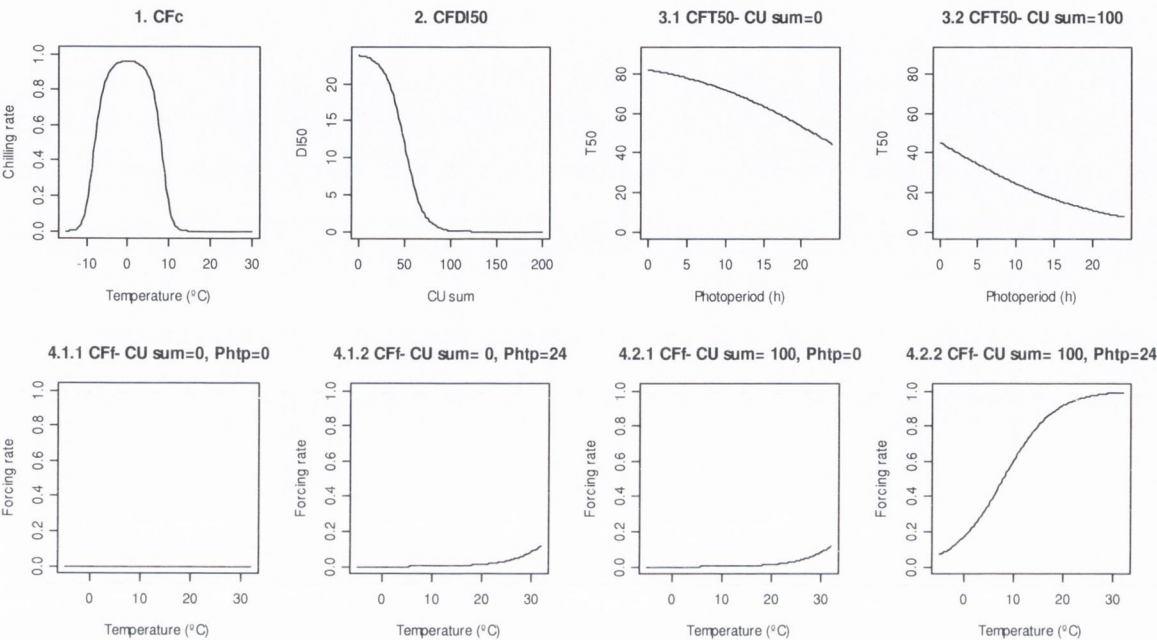
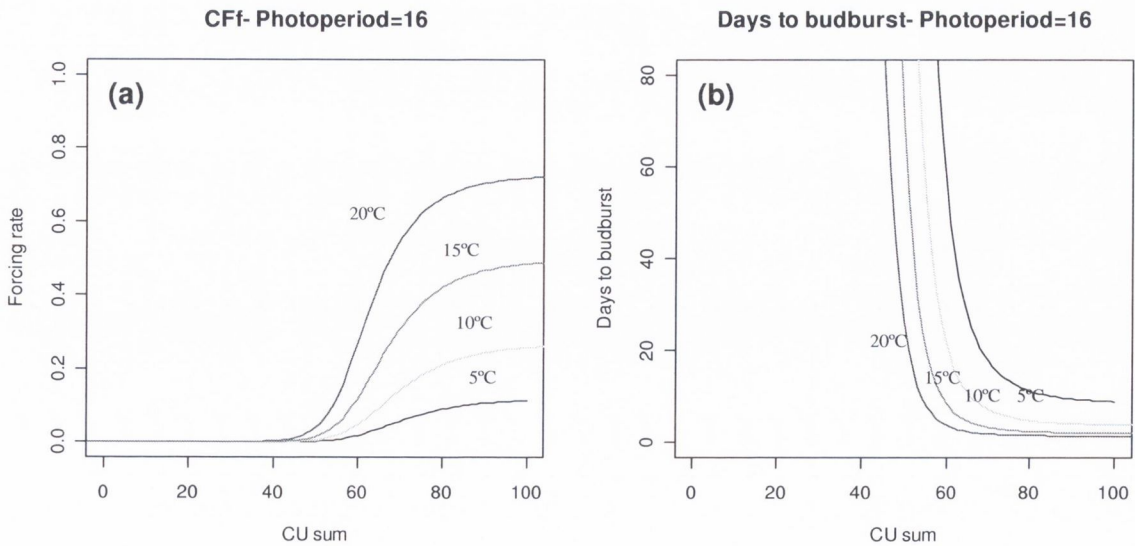


Figure 47. (a) Relationship between CU sum and forcing rate at a 16 h photoperiod and different temperatures and (b) between CU sum and days to budburst (1/forcing rate) at a 16 h photoperiod and different temperatures. Parameter values: $a = 0.05$; $b = 0.8$; $c = 8$; $bDI50 = 0.1$; $cDI50 = 50$; $bt50 = 0.1$; $T_{max} = 90$; $bf = -0.2$.



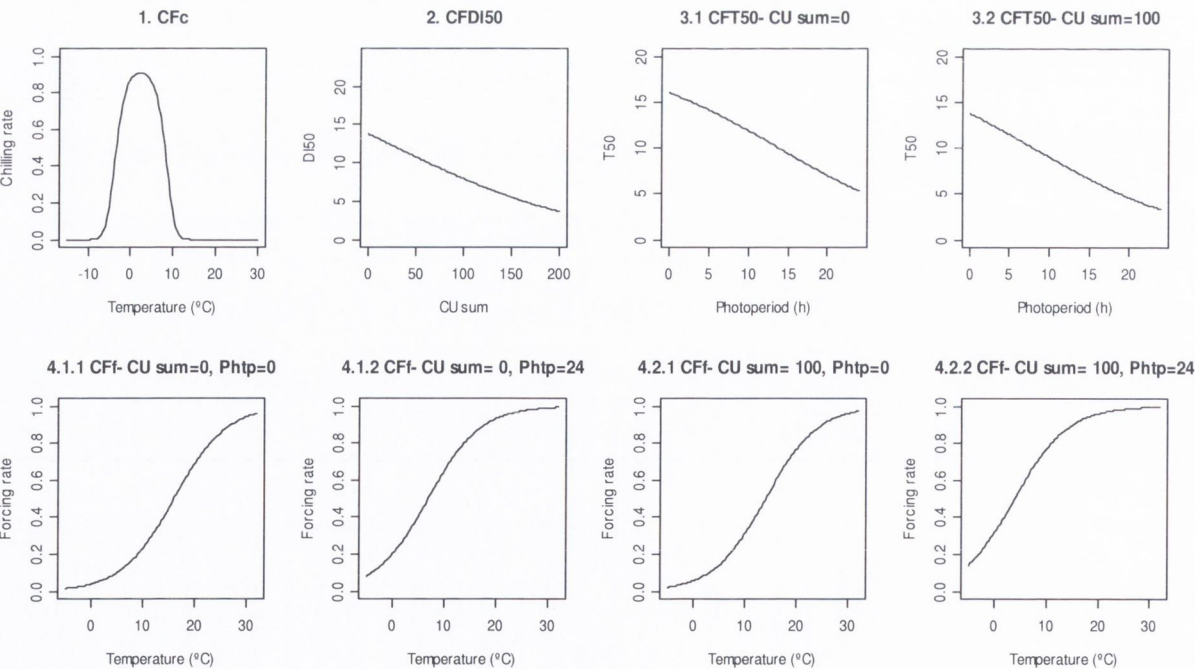
2.1.4. The rate of forcing is affected by chilling, chilling temperatures are active simultaneously with forcing temperatures from t_0 .

($a_c = 0.07$; $b_c = 0.8$; $c_c = 8$; $bDI50 = 0.01$; $cDI50 = 30$; $bt50 = 0.1$; $T_{max} = 20$; $bf = -0.2$)

The response to chilling temperature is not uniform, and has an optimum around 2°C, with lower rates of chilling at suboptimal temperatures. The rate of chilling is ≈ 0 for temperatures $> 12^\circ\text{C}$ and $< -10^\circ\text{C}$ (Figure 48, graph 1).

Forcing temperatures are active simultaneously with chilling temperatures all along dormancy, with less effectiveness at the start of chilling accumulation and for short photoperiods. This model is only apparently similar to the Parallel model (Landsberg, 1974; Hänninen, 1990), because the lack of a threshold expressing the minimum chilling requirement for budburst results in the model predicting budburst in unchilled plants.

Figure 48. Responses to temperature and photoperiod predicted by the UnPhot model for $a_c = 0.07$; $b_c = 0.8$; $c_c = 8$; $bDI50 = 0.01$; $cDI50 = 30$; $bt50 = 0.1$; $T_{max} = 20$; $bf = -0.2$. (1) Response of chilling rate to temperature; (2) Response of $DL50$ to the sum of accumulated chilling units (CU); (3) Response of $T50$ to photoperiod, (3.1) after the accumulation of 0 CU, (3.2) after the accumulation of 100 CU; (4) Response of forcing rate to temperature, (4.1.1) after the accumulation 0 CU, at a 0 h photoperiod, (4.1.2) after the accumulation 0 CU, at a 24 h photoperiod, (4.2.1) after the accumulation 100 CU, at a 0 h photoperiod, (4.2.2) after the accumulation 100 CU, at a 24 h photoperiod



The above examples show that the UnPhot model is quite general, and can approximate some of the phenological models nested in the Unified model, as shown by Chuine (2000). It accounts for the Alternating model, which was the phenological model that most resembled the responses observed in *B. pubescens* during the present study. It also has similarities with the approach of Vegis (1964), describing dormancy as a state in which the temperature range promoting growth is narrowed, and dormancy release as the gradual widening of this range. In the UnPhot model, the threshold temperature for forcing decreases as a result of chilling, as hypothesized by Vegis (1964) for the phase of post-rest. However, the UnPhot model loses its flexibility when accounting for phenological models not including a negative relationship between the state of chilling and the state of forcing (such as the sequential model and the thermal time model), due to the fact that the relationship between forcing rate and temperature is fixed when growth competence is at optimum ($T_{50}=0$). It also does not account for the Parallel model (Landsberg, 1974; Hänninen, 1990), as the lack of a minimum chilling requirement allows that the accumulation of forcing units during dormancy results in budburst without any chilling accumulation. This indicates that the UnPhot could be limited when applied to species that do not respond to increasing chilling duration and photoperiod by gradually decreasing their time to budburst, as was found for *B. pubescens* during the current study. This suggests its use for species that are sensitive to photoperiod and show a gradual release from dormancy.

3. Application of the UnPhot and Unified models

The UnPhot model was fitted using the dates of budburst of *B. pubescens* recorded during the experimental part of the present study and field observation recorded on the same clone of *B. pubescens* grown at several IPG sites in Europe. The Unified model was also fitted to the same datasets, in order to evaluate whether the integration of photoperiod had improved the model's accuracy. In fact, the Unified model has the same number of parameters as the UnPhot model (9), which makes the comparison of their accuracy immediate and straightforward.

The parameter adjustment was obtained by minimizing the least-squares function in the parameter space by means of a simulated annealing method, which used the Metropolis

algorithm (as described by Chuine *et al.*, 1998). This algorithm explores the parameter space through a random walk which tends to avoid local minima and converges towards the global minimum (Chuine, 2000). This search was repeated several thousands times, to make sure that the best-fit sets of parameters were found.

The dates of budburst in the experimental datasets were considered as number of days between the start of the experiments and budburst, and plants with less than 50% budburst were considered as dormant and indicated as flushing after 365 days.

3.2 Datasets

3.2.1 Experimental observations

The UnPhot model was fitted using the dates of budburst of *B. pubescens* from relevant experiments conducted during this study (Table 17). As the UnPhot model predicts budburst on the basis of photoperiod and temperature, the experiments selected excluded those in which factors other than photoperiod and temperature were manipulated, i.e. experiment 1.a (effect of light intensity) and 1.b (effect of nutrient availability). Similarly, as the model does not take into account the effect of dormancy induction photoperiod, the data from experiment 4.a, which tested the effect of this factor (showing it was significant) were split into two datasets with homogeneous dormancy induction photoperiod, i.e. 8h (4.a-8 h) and naturally decreasing photoperiod (4.a-Nat). In order to increase the amount of information for the model fit, the datasets of the experiments receiving similar dormancy induction photoperiods were grouped together to increase the sample size.

Table 17. Datasets used for the model fit. Each dataset corresponds to the phenological observations recorded during the experiments. The datasets were combined together or split according to the dormancy induction photoperiod to which the plants were exposed

Experiment	Factor Tested	N. of observations
2.b+3.a	Chilling duration, forcing photoperiod, forcing temperature	15
2.a+3.b	Chilling duration, forcing photoperiod, chilling temperature	22
3.c	Dormancy induction temperature and duration, chilling temperature and photoperiod	12
4.a -8 h	Chilling and forcing temperature and photoperiods	15
4.a -Nat	Chilling and forcing temperature and photoperiods	15

Experiment 2.b (testing the effect of forcing temperature) was grouped with experiment 3.a (testing the effects of chilling duration and 2 forcing photoperiods), and experiment 2.a (testing the effect of chilling temperature) was grouped with experiment 3.b (testing the effects of chilling duration and 4 different forcing photoperiods).

3.2.2. Field observations

As the studied clone of *B. pubescens* is grown in many phenological gardens in Europe belonging to the IPG network, a comparison between the two models was possible by comparing their fits to field data. The datasets used for the fits are from 5 phenological stations, Valentia in the south west of Ireland, Wulfsdorf in the north of Germany, Freiburg in the south of Germany and a combined dataset from München (south of Germany) and Zürich (Switzerland). The datasets relative to the last two stations (München and Zürich) were combined into a single one, to increase sample size and have a uniform number of observations in all datasets. These two stations are located at similar altitudes and latitudes (see Table 18).

Table 18. IPG number, coordinates and number of observations for each of the stations used for the model fits. The datasets of München and Zürich were combined into a single one, to increase sample size and have a uniform number of observations in all datasets.

	IPG n.	Latitude	Longitude	N. of observations
Valentia	13	51°56'	10°15' W	25
Wulfsdorf	22	53°39'	10°12' E	25
Freiburg	32	48°01'	07°59' E	27
München	36	48°11'	11°10' E	17
Zürich	46	47°20'	08°48' E	9

3.3 Internal and external validity

The internal validity of each model was measured by the percentage of explained variance (R^2) and tested using F-tests. The internal validity is a measure of how well the model fits the data on which it was adjusted. It is defined as:

$$(SS_{tot}-SS_{res})/SS_{tot}$$

where SS_{tot} is the total variance in the observations, and SS_{res} is the residual sums of squares of the prediction. Even though this measure was assimilated to an R^2 by Chuine

(1998, 2000) and Chuine *et al.* (2000), it can assume negative values, if the SSres is larger than the total variance of the observations.

The external validity of the models was tested by using the model fitted on a particular dataset to predict the observations of a second external dataset. This could be done only for the field datasets, as the experimental datasets could not be used to predict different experiments, due to their heterogeneous dormancy induction conditions and were too small to be split in two.

The field observations from 2 IPG sites (Valentia and Freiburg) were combined and used to fit the Unified and UnPhot models. Subsequently the models fitted on the Valentia+Freiburg dataset were used to predict the external observations of the combined dataset of Wulfsdorf+ München-Zürich.

The external validity was measured by the percentage of variance explained of external observations (Chuine *et al.*, 1998). We also included a measure the improvement by the model prediction relative to the mean of the data used for the model fit (null model), given by the additional percentage of variance explained by the model prediction compared with the null model. This percentage of additional variance was defined as:

$$(SSres1-SSres2)/SSres1$$

where SSres1 is the residual sums of squares of the prediction by the null model and SSres2 is the residual sums of squares of the prediction by the fitted UnPhot and Unified models. The external predictions by the Unified and UnPhot models were statistically compared with the null model using F-tests.

As one of the aims of the UnPhot model was to gain more understanding of the functional relationships between environmental signals and the progress of dormancy, we also evaluated whether the model outputs reasonably agreed with our knowledge of the system behaviour. This approach involved the visual analysis of the plots of the responses fitted to the various datasets.

RESULTS

1. Experimental observations

Percentages of variance explained by the Unified model varied between 15 and 99% (Table 19), while the percentage of variance explained by the UnPhot model was always very high, between 97 and 99% (Table 20).

Table 19. Parameter estimates of the Unified model for each dataset and percentages of variance explained. *** $p<0.001$

	a_c	b_c	c_c	d_f	e_f	w	k	Ccrit	tc	R^2
2.b+3.a	1.443	-21.43	-3.025	-39.972	-18.984	2.842	-0.5	13.095	269.428	0.35
2.a+3.b	0.501	-4.616	-1.202	-0.066	-16.971	572.138	-0.247	6.323	84.722	0.15
3.c	2.134	-17.178	-9.968	-2.82	-16.275	1695.827	-0.066	57.093	97.251	0.99***
4.a-8 h	2.424	-24.445	-7.611	-0.217	-19.964	103.126	-0.361	1.4	64.124	0.99***
4.a-Nat	1.335	-26.651	5.117	-2.558	-5.406	1701.499	-0.059	57.153	289.327	0.99***

Table 20. Parameter estimates of the UnPhot model for each dataset and percentages of variance explained. *** $p<0.001$

	a_c	b_c	c_c	b_f	bt50	bDI50	cDI50	Fcrit	Tmax	R^2
2.b+3.a	0.243	-17.422	-59.603	-0.585	0.071	0.127	77.717	11	31.879	0.99***
2.a+3.b	1.591	-15.668	3.327	-0.248	2.118	0.01	110.857	10.701	40.286	0.99***
3.c	0.64	-5.99	8.834	-0.386	0.31	3.449	72.722	32.653	27.925	0.97***
4.a-8 h	4.087	13.636	13.211	-0.215	0.086	10	40.926	20.485	59.985	0.99***
4.a-Nat	0.626	-19.918	-22.165	-0.675	1.075	7.817	0.136	11.181	53.08	0.99***

The Unified model did not explain significantly more variance than the null model for datasets 2.b+3.a and 2.a+3.b. It is worth noting that these two datasets included those experiments testing the effect of forcing photoperiod while keeping all other environmental factors constant (experiments 3.a and 3.b).

For some datasets (2.b+3.a, 4a-8 h, 4a-Nat) the local minima of the least square functions of both models were very close to the global minimum, despite the fact that the sets of values fitted to the parameters were very different. This means that different models had similar accuracies. Only the parameters of the best-fit models are reported in the tables, even though the differences between their R^2 was often $<1\%$ or even $<0.1\%$, and the parameter values sets assigned in each case accounted for very different responses.

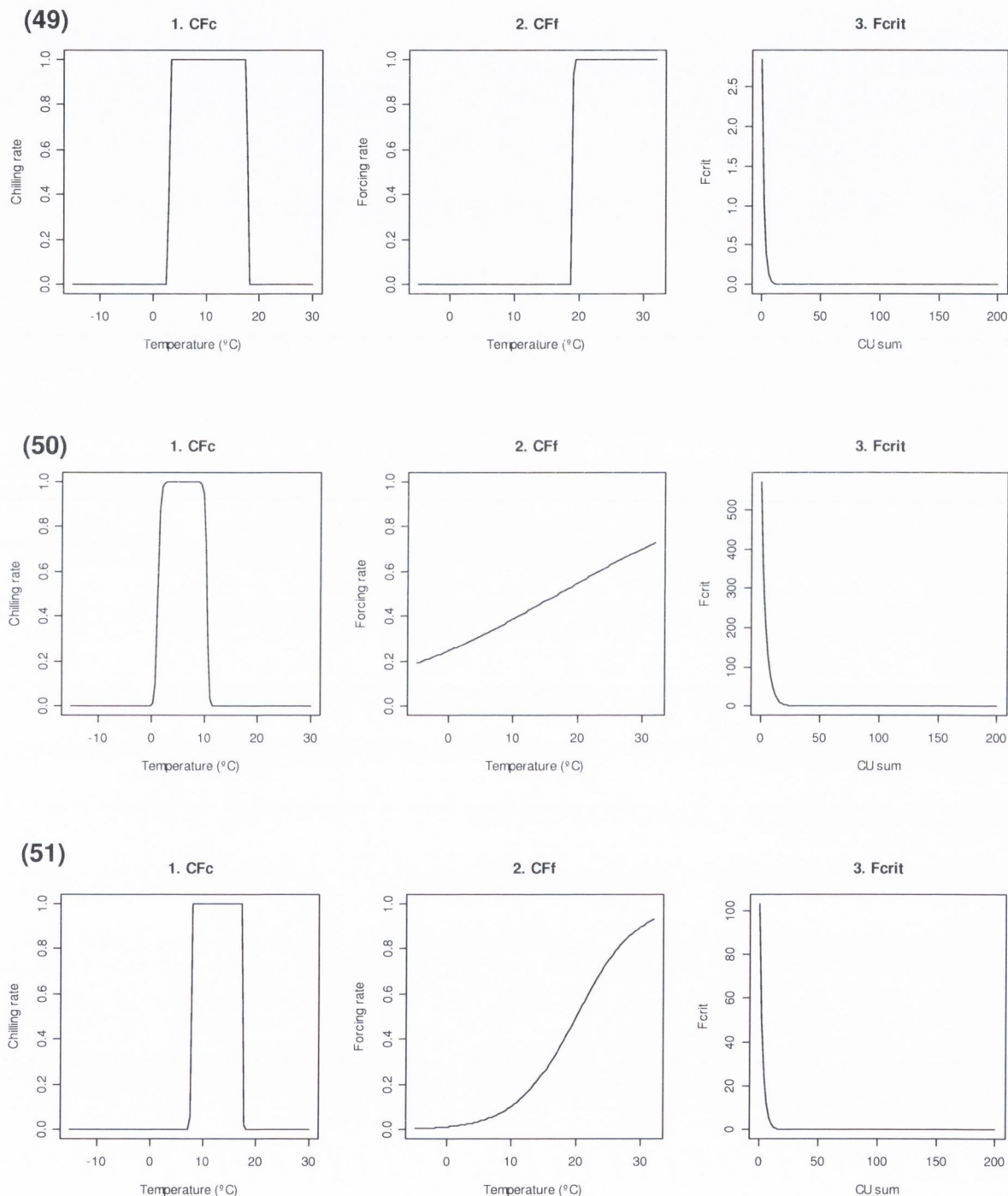
By visual inspection of the graphs of the fitted models, it was possible to identify the type of temperature responses in each of the datasets. These graphs are displayed in Figure 49, 50, 51, 52 and 53 for the Unified model and 54, 55, 56, 57 and 58 for the UnPhot model. Visual inspection of the graphs and evaluation of the scientific realism of the fitted model are the preliminary steps to statistical comparisons between models (Motulsky and Christopoulos, 2004). From visual analysis and comparison of the parameter values it was possible to observe that:

1. The responses fitted to chilling and forcing temperatures differed widely between models and experiments. This was revealed by the heterogeneity of the parameters fitted to the models (See Tables 19 and 20) and by the differences in the rates of forcing and chilling among experiments, which can be observed in Figures 49-58.
2. While the ranges of active chilling temperatures differed among datasets, the response to chilling temperature was always abrupt for both models (the values assigned to b_c are always $\gg 1$, making for steep slopes to the CFC function), reaching a plateau close to unity in the range of chilling temperatures, thus indicating a threshold response (Figures 49-58, first graph of each figure)
3. The Unified model fitted heterogeneous forcing rates to the datasets, with a threshold response above 15°C for 2.b+3.a (Figure 49.2), very smooth responses for 2.a+3.b and 4.a-8 h (Figures 50.2 and 51. 2, respectively), and intermediate responses for 4.a-Nat and 3.c (Figures 52.2 and 53.2; respectively).
4. The UnPhot model fitted more or less smooth forcing rates to all datasets, showing less heterogeneity. Despite the fact that it does not include a C_{crit} threshold (minimum chilling requirement for budburst) it was able to account for non-flushing plants, by adjusting the position of the sigmoidal curve describing the forcing rate at very high

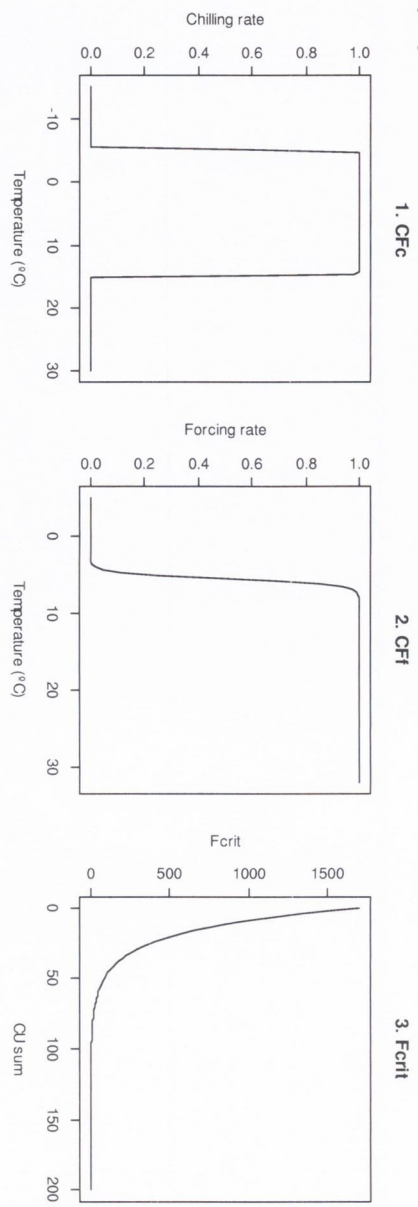
temperatures for low CU sums and photoperiods below 12 h (Figures 55.4.1, 56.4.1 and 57.4.1).

Figures 49-53. Responses to temperature fitted by the Unified model to datasets (49) 2.b+3.a, (50) 2.a+3.b, (51) 4.a 8-h, (52) 4.a-Nat, (53) 3.c.

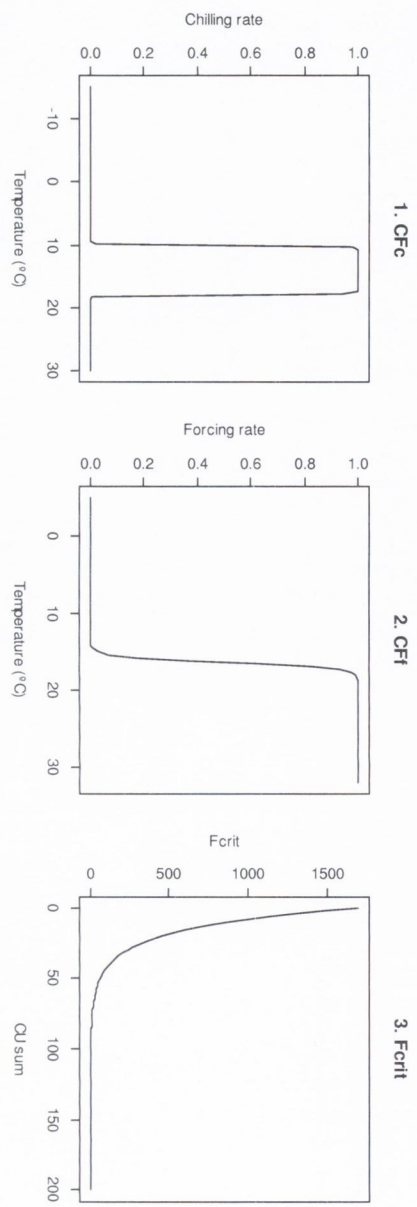
In each figure: (1) Response of chilling rate to temperature; (2) Response of forcing rate to temperature; (3) Relationship between amount of accumulated chilling units and amount of forcing units required for budburst (F_{crit}).



(52)

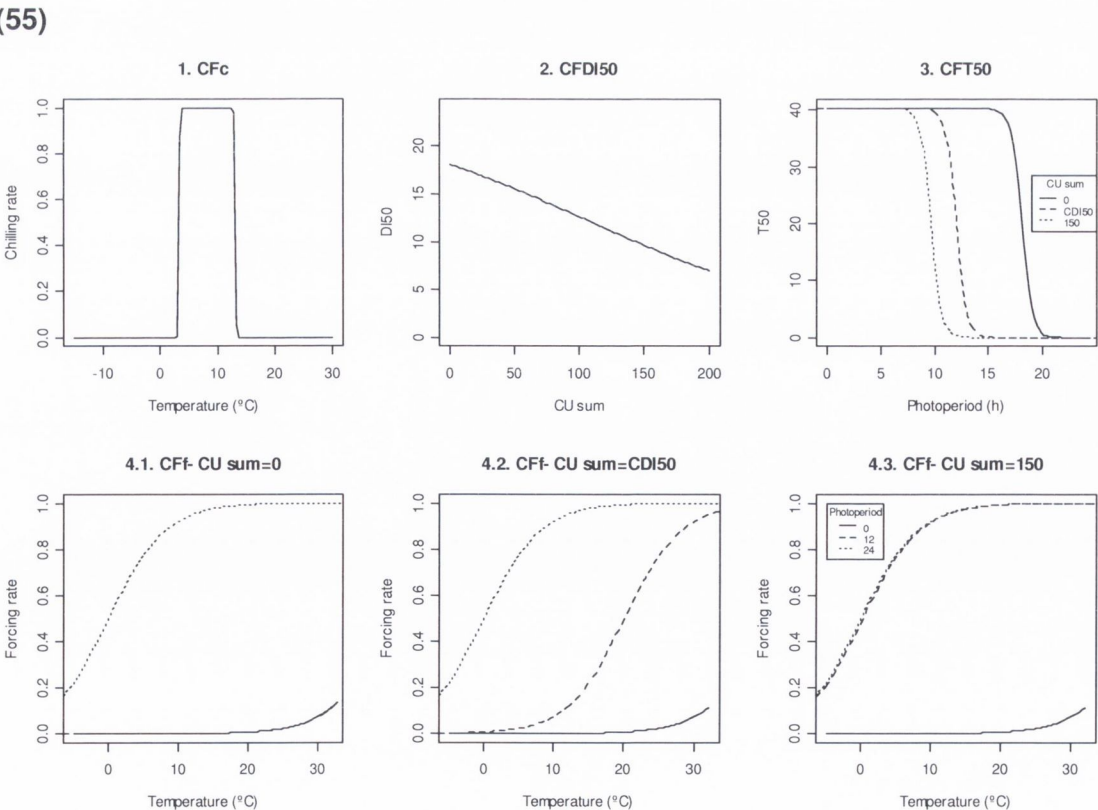
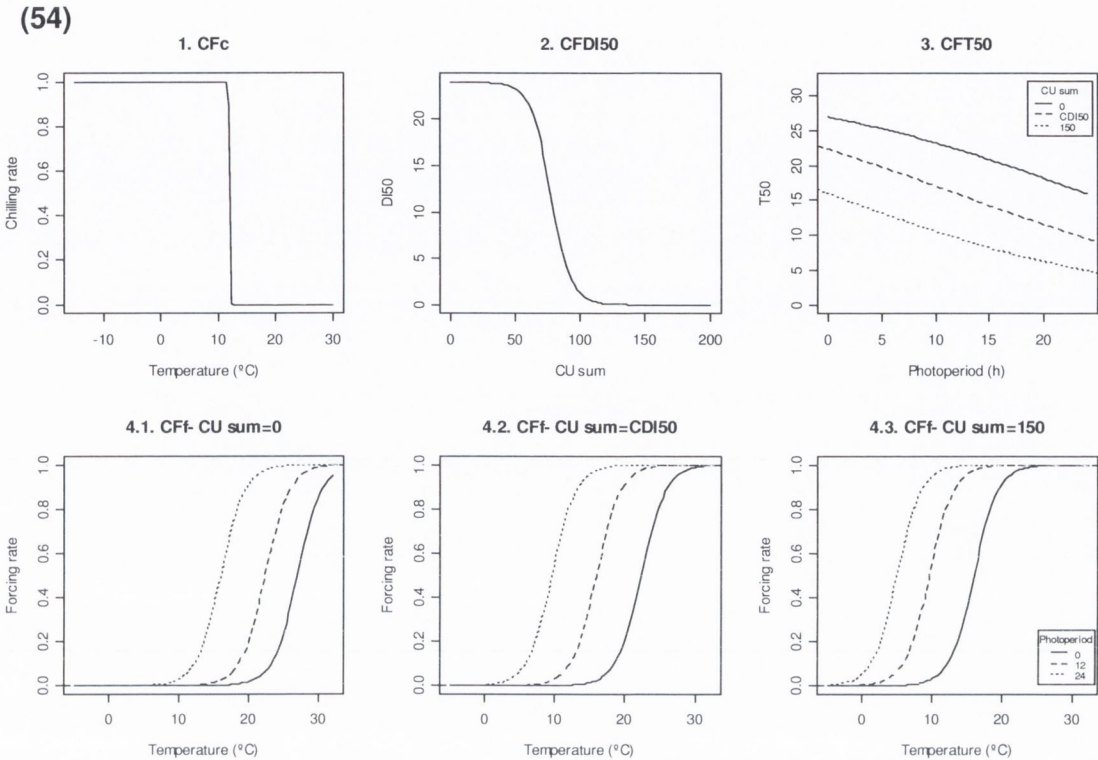


(53)

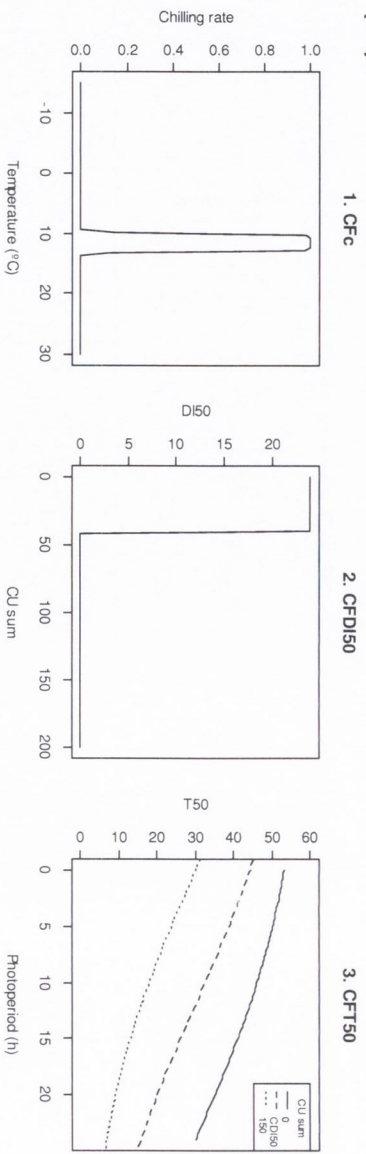


Figures 54-58. Responses to temperature and photoperiod fitted by the Unified model to datasets (54) 2.b+3.a, (55) 2.a+3.b, (56) 4.a 8-h, (57) 4.a-Nat, (58) 3.c.

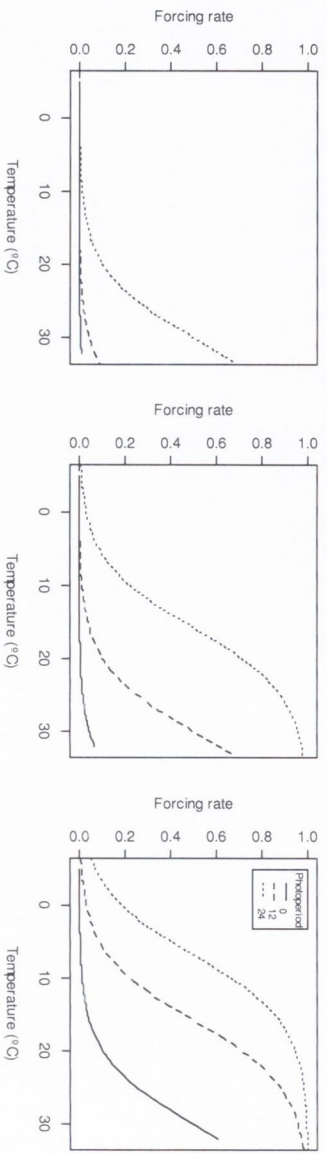
In each figure: (1) Response of chilling rate to temperature; (2) Response of DI50 to the sum of accumulated chilling units (CU); (3) Response of T50 to photoperiod; (4) Response of forcing rate to temperature after the accumulation of 0 (4.1), CDI50 (4.2), 150 (4.3) chilling units. The responses represented correspond to forcing at 0 h(solid line), 12 h(dashed line) and 24 h(dotted line) photoperiod. DI50



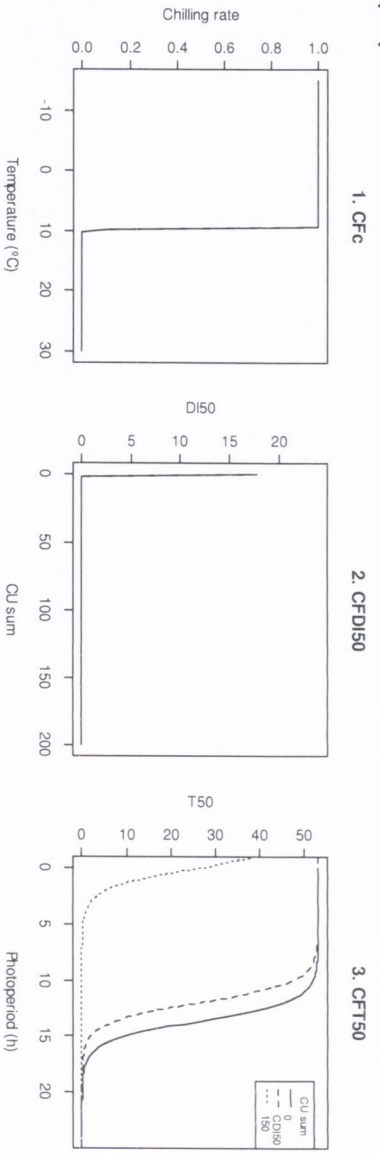
(56)



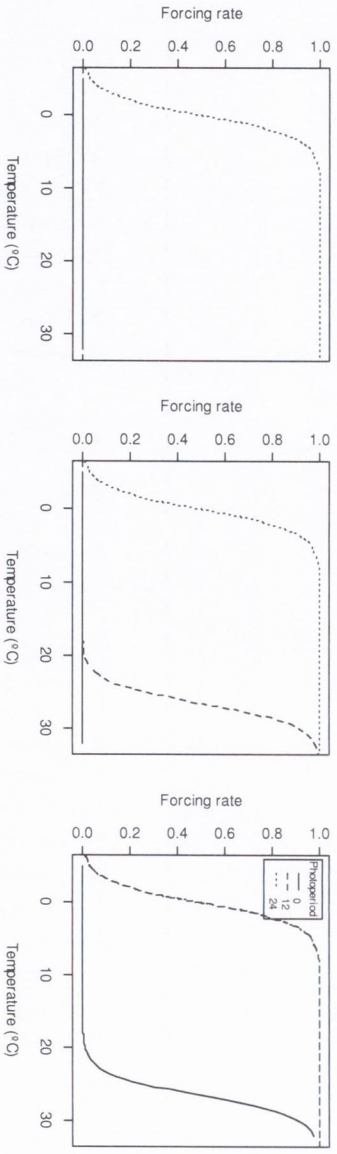
4.1. CFT- CU sum=0



(57)

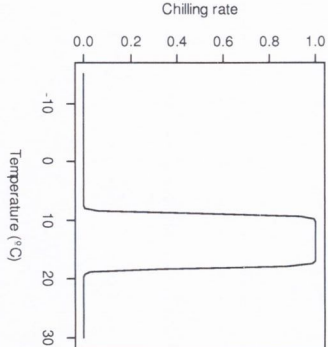


4.1. CFT- CU sum=0

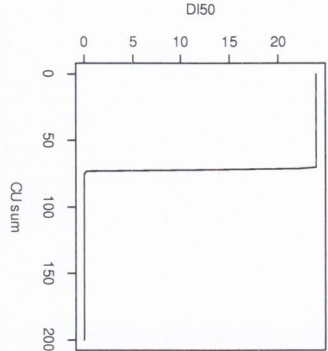


(58)

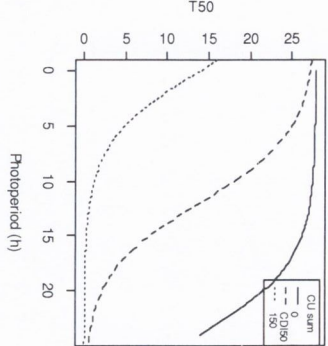
1. CFc



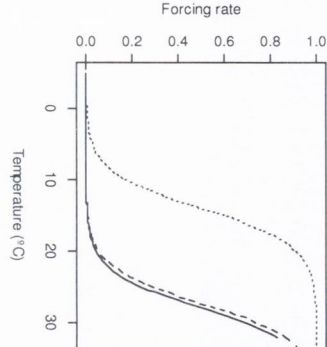
2. CFDI50



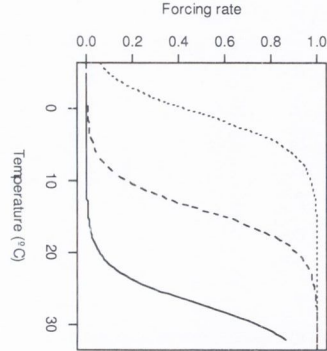
3. CFT50



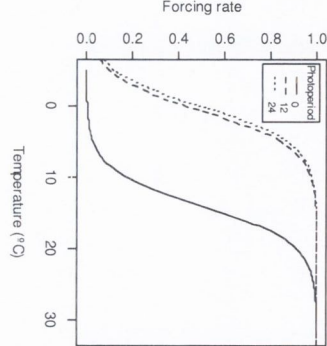
4.1. CFf- CU sum=0



4.2. CFf- CU sum=CDI50



4.3. CFf- CU sum=150



2. Field observations

Percentages of variance explained by the Unified model varied between 80 and 88% (Table 5) with an average of 81%, while the percentage of variance explained by the UnPhot model was between 81 and 89% (Table 6) with an average of 86%. Both models always significantly improved the prediction of budburst, compared with the null model. Unlike with the experimental datasets, the global minimum found for each of the station datasets clearly improved the goodness of fit compared with the local minima, and different parameter set values corresponding to very similar minima of the least square function were not found.

Table 21. Parameter estimates of the Unified model for each dataset and percentages of variance explained. *** $p<0.001$

	a_c	b_c	c_c	d_t	e_t	w	k	C_{crit}	t_c	R^2
Valentia	0.439	-8.915	2.745	-0.210	-19.272	334.546	-0.017	106.488	213.782	0.80***
Wulfsdorf	0.881	-29.938	12.596	-0.219	-18.500	1094.951	-0.030	171.775	192.188	0.78***
Freiburg	0.528	9.275	-10.725	-5.427	-10.638	513.031	-0.023	87.705	231.507	0.78***
Munc-Zur	0.851	-18.168	2.675	-6.913	-10.485	1332.877	-0.024	61.074	263.856	0.88***

Table 22. Parameter estimates of the UnPhot model for each dataset and percentages of variance explained. *** $p<0.001$

	a_c	b_c	c_c	b_t	$bt50$	$bDI50$	$cDI50$	F_{crit}	T_{max}	R^2
Valentia	9.627	-16.612	4.992	-1.316	0.133	0.039	26.073	82.120	13.173	0.81***
Wulfsdorf	3.136	-6.838	5.238	-0.789	0.193	0.019	5.620	15.507	24.267	0.88***
Freiburg	0.313	-15.994	-43.240	-0.377	0.040	0.088	77.809	33.658	24.457	0.85***
Munc-Zur	0.971	-19.428	-4.894	-0.465	0.061	10.000	148.029	18.443	27.345	0.89***

Table 23. Average julian day for t_1 (day at which the state of chilling $> C_{crit}$), minimum and maximum julian days of t_1 , and standard deviation for each dataset.

	Valentia	Wulfsdorf	Freiburg	Munc-Zur
Average t_1	108.1	172.2	145.9	65.3
min	107	172	134	62
max	113	174	154	76
Stdv	2.01	0.69	7.42	3.39

By visual inspection of the graphs of the fitted models and their comparison with the parameter values, it was possible to identify the type of temperature responses in each of the datasets (Figure 59, 60, 61 and 62 for the Unified model and 63, 64, 65 and 66 for the UnPhot model).

1. The responses fitted to chilling and forcing temperatures differed widely between models and experiments. This was revealed by the heterogeneity of the parameters fitted to the models (see Tables 21 and 22) and can be seen in the Figures 59-66.
2. The responses fitted to chilling rates by the UnPhot model were either describing a very narrow (Valentia, Figure 63.1; Wulfsdorf, Figure 64.1) or a wide range of active temperatures (Freiburg, Figure 65.1; München-Zürich, Figure 66.1). In the first case only temperatures equal or very close to 6°C elicited the accumulation of chilling units, in the second case, all temperatures below a certain threshold (close to 10°C for Freiburg and 15°C for München-Zürich) elicited the accumulation of one chilling unit. The plateau showed by the curves describing the chilling rates excluded chilling temperatures below 5°C for München-Zürich. However, given the small number of days with a mean daily temperature < 5°C, only a few days each year did not contribute to the accumulation of chilling units.
3. The Unified model fitted threshold responses to chilling temperatures for all datasets. The curves describing the chilling rates rose sharply and reached a plateau close to unity in the range of active chilling temperatures, which varied among stations (Figures 59-62, first graph of each figure).
4. The curves describing the forcing rates were generally smooth for the UnPhot model fits. The Unified model fitted a threshold response to forcing temperatures (accumulation of one forcing unit above 10°C) for Freiburg and München-Zürich (Figures 61.3 and 62.3, respectively)

External validity

For the combined Valentia+Freiburg dataset, the percentages of variance explained (R^2) by the fitted Unified and UnPhot models were 68% and 69%, respectively (Table 24).

Using the parameter values fitted on the Valentia+Freiburg dataset for the prediction of the dates of budburst of the combined Wulfsdorf +München-Zürich dataset, decreased the SSres when compared with the null model (mean date of budburst of Valentia+Freiburg used as single prediction parameter for Wulfsdorf+München- Zürich) (Table 25). However this decrease was significant only for the prediction of the Unified model. In addition, the predictions of both models gave a SSres higher than the total variance of the data of Wulfsdorf and München-Zürich, which means that their external validity was negative.

Table 24. Parameter estimates of the UnPhot and Unified model for Valentia and Freiburg (combined dataset, n=56) and percentages of variance explained. *** $p<0.001$.

	a_c	b_c	c_c	d_t	e_t	w	k	Ccrit	tc	R^2
Unified	0.632	14.823	5.809	-0.833	9.657	1943.856	-0.020	100.034	283.283	0.68***
	a_c	b_c	c_c	b_t	bt50	bDI50	cDI50	Fcrit	Tmax	R^2
UnPhot	0.153	0.276	9.914	-10.00	0.403	0.014	21.613	35.817	18.404	0.69***

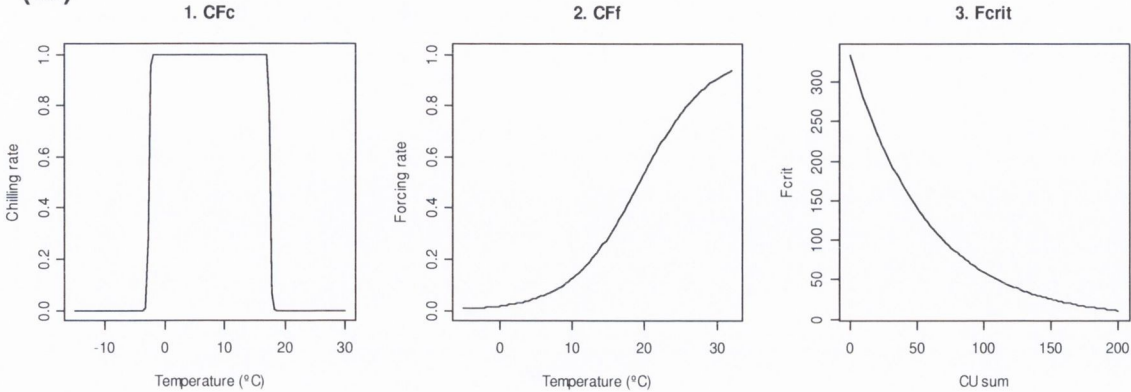
Table 25. Sum of squares (SSres) of the residuals for the fits and predictions of the Unified and UnPhot (Unified+photoperiod) models. The models, each with 9 parameters, are compared with the Null model, using mean as a single predicting parameter. %AV is the percentage of additional variance explained by the prediction of the models compared with the null model. *** $p<0.001$.

Model	Fit (Valentia+Freiburg, n=56)		Prediction (Wulfsdorf+München-Zürich, n=54)	
	SSres	R^2	SSres	% AV
Null	8200		19544	
Unified	2600	0.68***	10443	0.47***
UnPhot	2512	0.69***	15355	0.21

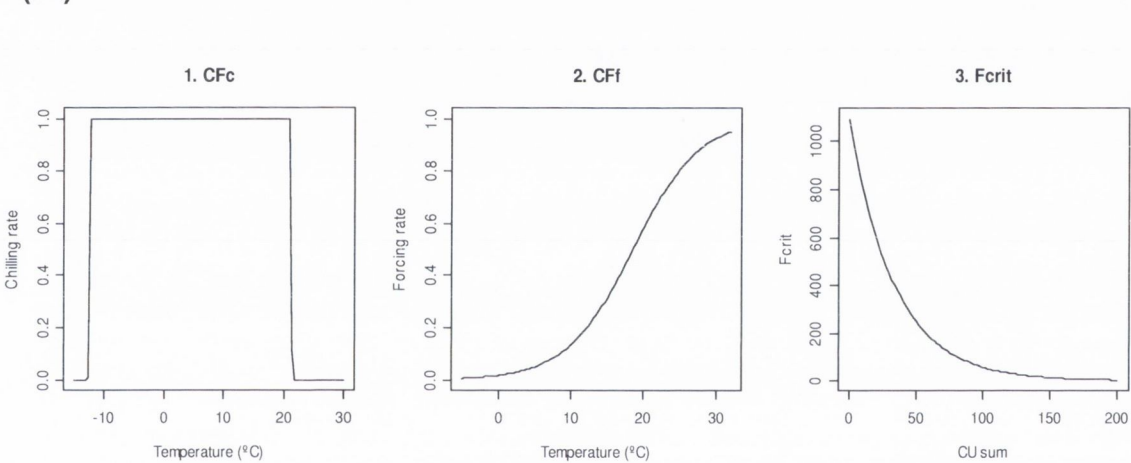
Figure 59-62. Responses to temperature and fitted by the Unified model to datasets (59)Valentia, (60) Wulfsdorf, (61) Freiburg, (62) München-Zürich.

In each figure: (1) Response of chilling rate to temperature; (2) Response of forcing rate to temperature; (3)Relationship between amount of accumulated chilling units and amount of forcing units required for budburst (Fcrit).

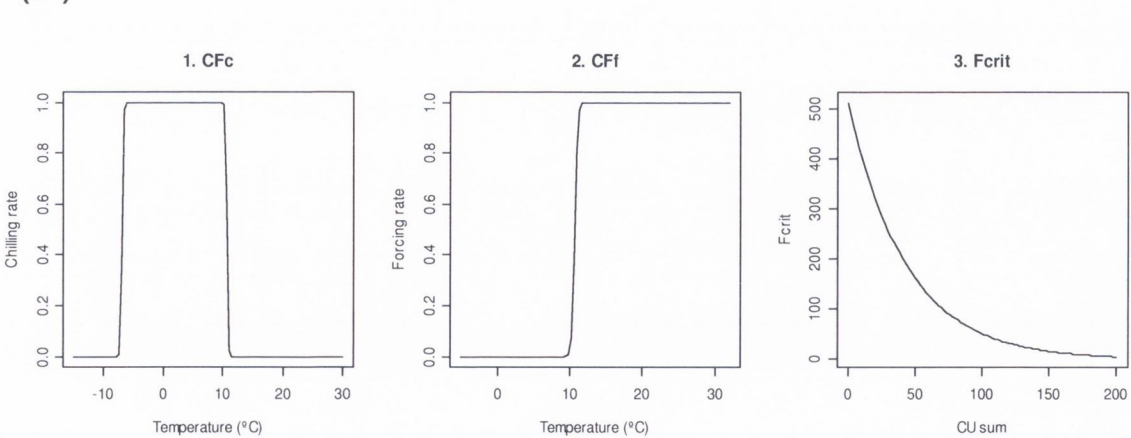
(59)



(60)



(61)



(62)

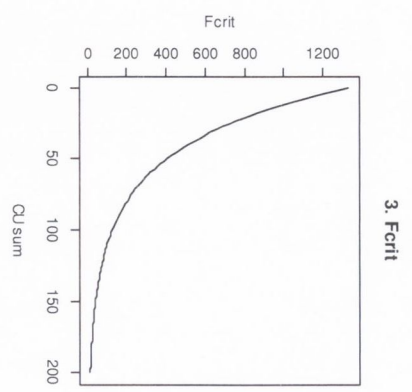
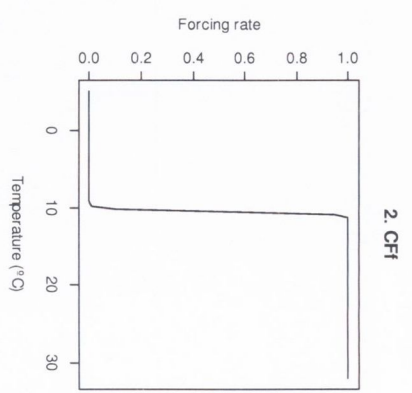
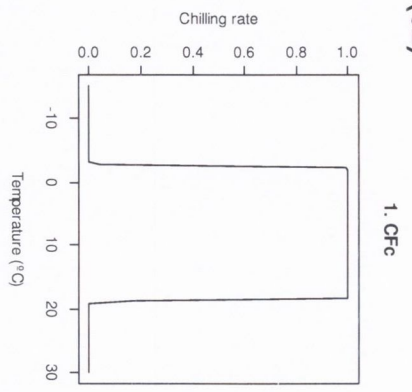
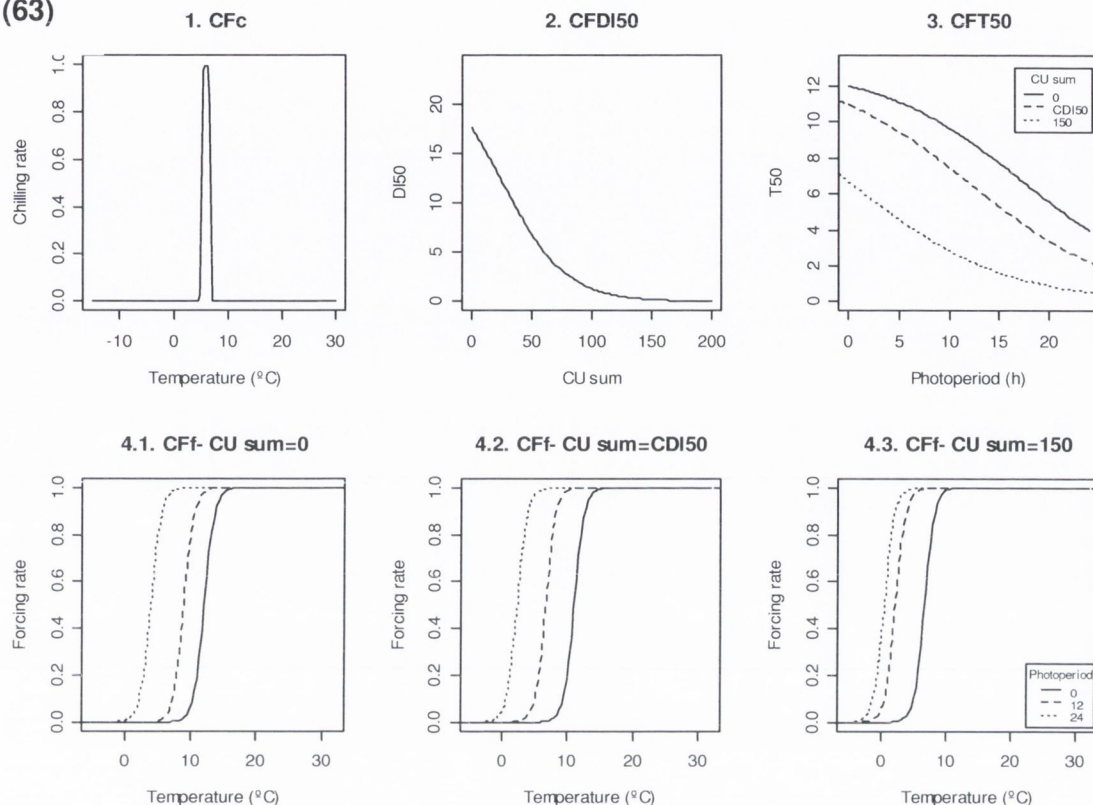


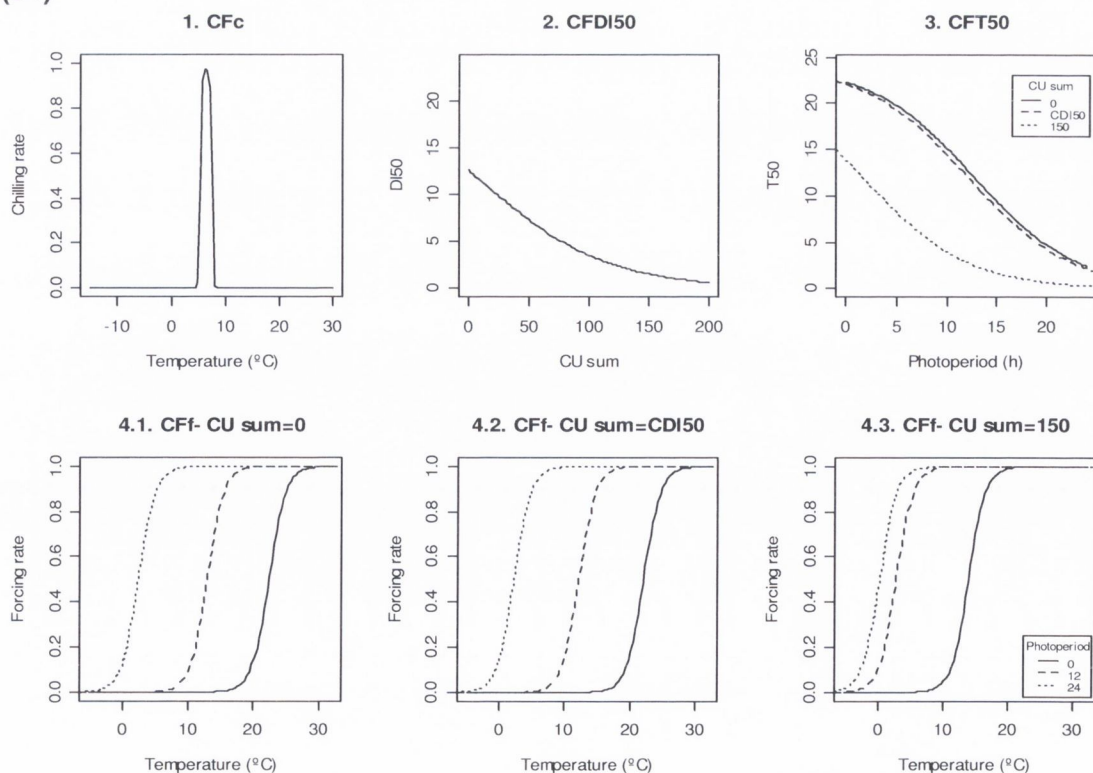
Figure 63-66. Responses to temperature and photoperiod fitted by the UnPhot model to datasets (63)Valentia, (64) Wulfsdorf, (65) Freiburg, (66) München-Zürich.

In each figure: (1) Response of chilling rate to temperature; (2) Response of DI50 to the sum of accumulated chilling units (CU); (3) Response of T50 to photoperiod; (4) Response of forcing rate to temperature after the accumulation of 0 (4.1), CDI50 (4.2), 150 (4.3) chilling units. The responses represented correspond to forcing at 0 h(solid line), 12 h(dashed line) and 24 h(dotted line) photoperiod.

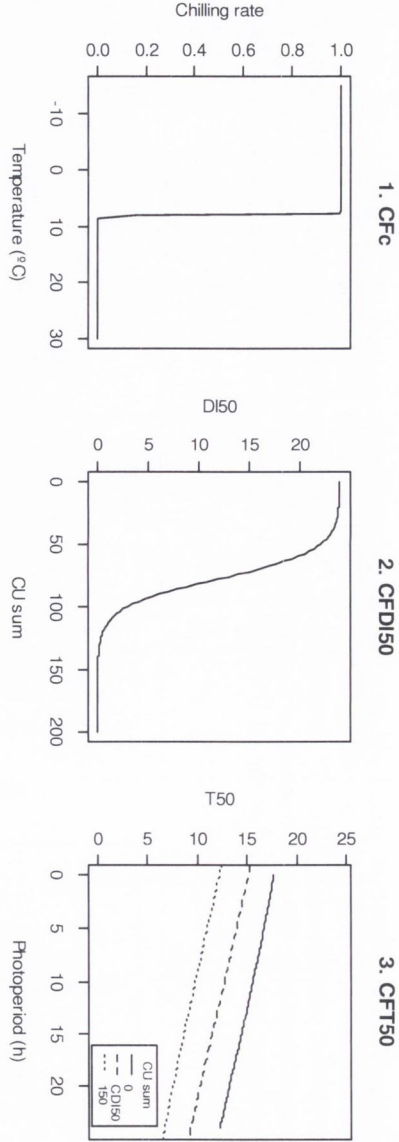
(63)



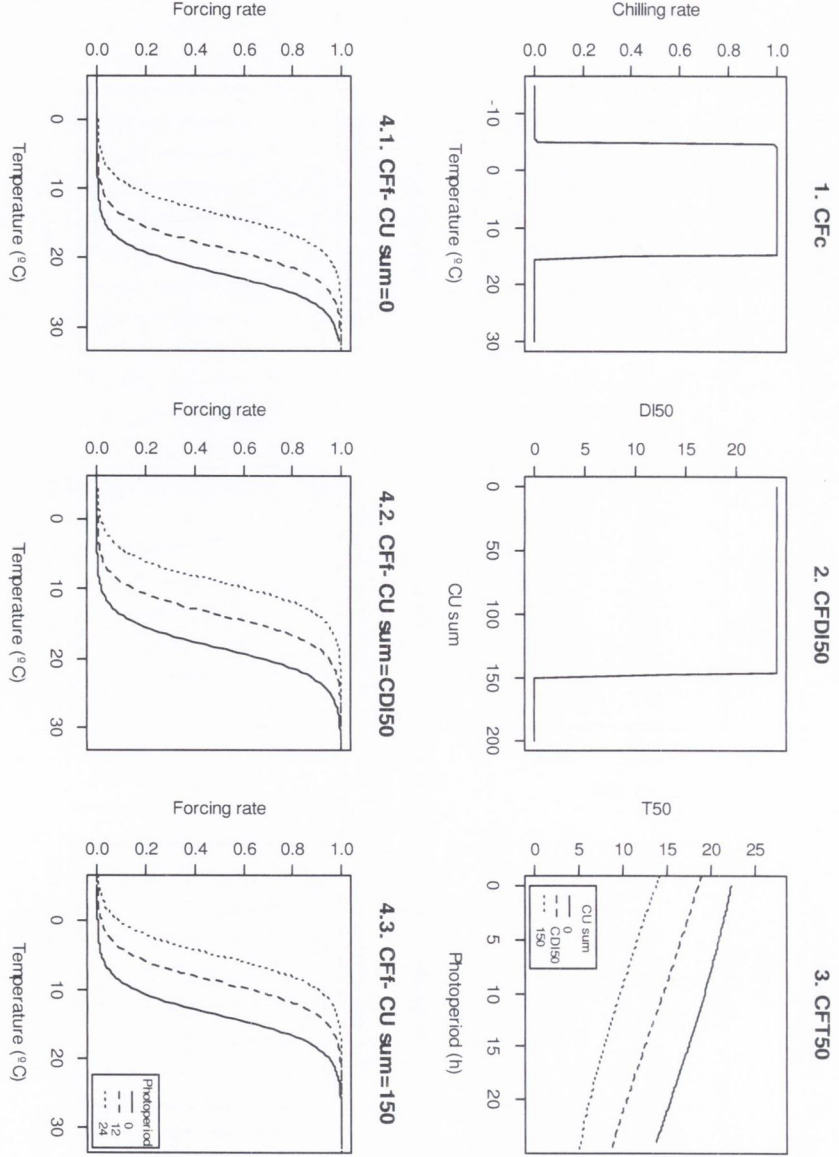
(64)



(65)



(66)



DISCUSSION

1. Experimental datasets

1.1 Internal validity

The UnPhot model showed a high internal validity in all of the datasets, which was not shown by the Unified model. These results show that the integration of photoperiod in the Unified model enabled us to explain a portion of variance previously unaccounted for in the datasets in which photoperiod was manipulated aside from temperature

The UnPhot model was successful in explaining photoperiod-driven budburst in partially chilled plants or buds in which LD acted as a substitute for chilling (2.b+3.a, 2.a+3.b) while the Unified model, considering only temperature, was unable to account for these differences. The integration of photoperiod through a “fixed calendar date” (a fixed photoperiod after which forcing temperatures are active) would not have explained the progressive decrease in the threshold photoperiod for budburst after increasing chilling durations, as observed in experiment 3.b, nor the difference in forcing rate observed between LD and SD forced plants observed in experiment 3.a.

The very high percentages of explained variance by the UnPhot model for all datasets and by the Unified model for all datasets but 2.b+3.a and 2.a+3.b are due to the presence of non-flushing plants among the treatments, whose dates of budburst were indicated as 365 days after t_0 . These “dates of budburst” greatly increased the total variance (SS_{tot}) of the observations, and consequently increased the percentage of explained variance in the models that were able to adjust to non-flushing observations.

1.2 Parameter heterogeneity

The variety of these responses and the lack of consistency among parameter values called for some considerations on model fitting, rather than further comparisons between the two models. In fact, while it was clear that the UnPhot model, contrary to the Unified model, was able to predict the dates of budburst in experiments undergoing forcing photoperiod

manipulation, it was also evident that both models fitted very different types of responses in different experiments, most of which are likely to be unrealistic, since we are dealing with the same clone.

Part of the reason of this heterogeneity might be the small size of the datasets used to fit the model. The dataset from Experiment 3.c was composed of only 12 observations, and the datasets from experiments 4.a-8 h and 4.a-Nat were composed of only 15 observations. A large sample size increases the precision of the estimates of a statistical model and decreases their confidence interval (Underwood, 2002).

Also, the limited number of experimental treatments and the fact that in most experiments constant chilling and forcing temperatures were applied, limited the “information” available for a realistic fit of temperature responses. The UnPhot model fit to the dataset of exp 2.b+3.a, which included experiments testing the effect of a wide range of forcing temperatures and chilling durations, probably shows more realistic responses to these factors, as the parameter values were adjusted on a larger amount of information. On the other hand, the chilling curve fit to the dataset of exp 2.a+3.b, which included data from experiment 2.a testing the effect of different chilling temperatures, did not show a smooth response to this factor in the investigated range (2-10°C), as the effects it elicited were actually small. In this case, a more realistic representation of the response to chilling temperature might have been obtained if the observations concerning a wider range of chilling temperatures had been used to fit the model.

The small size of the datasets and the uniformity of the conditions to which the plants were exposed might also be responsible for the fact that very different sets of parameter values corresponded to similar accuracies. This would be a result of the high number of parameters used to account for a relatively simple dataset, leading to overfitting. The observation that very similar proportions of explained variance were obtained by very different sets of parameters (accounting for very different phenological responses) certainly casts some doubts on the use of goodness of fit as the selecting factor for the “best model”. In fact, as Hänninen (1995) pointed out, a model with high internal accuracy is not always the most realistic.

1.3 Dormancy induction

One final consideration regards the fact that both models were able to accurately fit the dates of budburst in experiment 3.c, testing the effect of dormancy duration and temperature. The experiments showed that dormancy induction duration and temperature significantly affected budburst, and incomplete dormancy attainment could result in partial budburst in unchilled plants. However, the average percentage of budburst in this situation was lower than 50%, so the plants were considered dormant (the budburst date was considered as 365 days). Thus the models could not pick up the partial dormancy reversibility affecting unchilled plants receiving short dormancy induction at high temperature, which might have resulted in a decrease in their accuracy, since dormancy induction rate is not considered by the model. The action of low temperatures during dormancy induction (advancing dormancy attainment, and consequently advancing the start of dormancy release through the accumulation of chilling units) was instead explained by the models as chilling accumulation, and, as chilling temperatures during dormancy induction and dormancy resulted in having the same final effect on budburst timing, this did not decrease their internal validity.

2. Field observations

1.1 Internal validity

Both models showed very high percentages of explained variance for all stations, confirming the high internal validity of both the Unified and UnPhot models. However, the responses and the parameters fitted by both models were heterogeneous, indicating that the model fits lacked realism, as found for the experimental datasets.

1.2 Response to chilling temperatures

The responses that appeared to be the less realistic are chilling rates. In fact, while the fitted Unified model described threshold responses, with all temperatures within a wide range eliciting the same chilling accumulation, the UnPhot model described a very narrow chilling curve for Valentia and Wulfsdorf and a wide and uniform response for München-Zürich and Freiburg. Both these extremes are not likely to reflect reality, as previous

experiments (Sarvas, 1974) show that birch budburst responds to chilling smoothly, with an optimum chilling rate around 3.4°C and decreasingly effective chilling temperatures around this optimum in the interval -3 to 10°C. Our experiments show that while temperatures in the range 2-10°C trigger dormancy release (with temperatures between 2-4°C being slightly more effective than 10°C in the first stages of dormancy) a temperature of 18°C does not. Similarly, Myking and Heide (1995) report an upper threshold for active chilling temperatures in birch slightly above 12°C and a differential effect of chilling temperatures on budburst rates during early chilling stages.

The lack of scientific meaning of the chilling responses fitted by both models suggests that in the present climate chilling temperatures are less important than forcing temperature in the determination of budburst timing, confirming previous studies (Chuine *et al.*, 1998; Chuine *et al.*, 1999). In fact, the heterogeneous and unrealistic responses fit by the models, were most likely just adjusted to fit the most convenient (in terms of SSres) start for the accumulation of forcing units, year by year. On the other hand the mechanistic problem of the realistic and accurate representation of this phase of dormancy remains unresolved. The presence of a chilling requirement is an established fact and it is necessary to integrate it correctly into mechanistic models of budburst. This might be obtained by fixing some of the parameters of the chilling response in the model, even though this is likely to decrease the accuracy of the model fits, and it is not clear how the temperature dependence of chilling rate evolves during dormancy. There is evidence that the active range of chilling temperatures might change as chilling progresses (Overcash and Campbell, 1955) and the experiments conducted in the present work suggest that the moment of attainment of dormancy changes depending on the rate of dormancy induction, which in turn is influenced by temperature. A deeper understanding of the mechanisms through which chilling releases dormancy will certainly lead to a more accurate and realistic model of this phenophase.

1.3 Response to forcing temperatures

Forcing responses fitted by the Unified model similarly conflicted with experimental evidence for some of the datasets (Freiburg and München-Zürich, Figures 61 and 62, respectively), in which sharply rising sigmoidal curves suggested an all or nothing response to forcing temperature. On the contrary, previous investigations suggest a smooth effect of

forcing temperatures on forcing rate (Sarvas, 1974), and it has been established that bud growth during quiescence responds to temperature in a similar way to during the vegetative period, i.e bud growth increases logistically at increasing temperatures (Sarvas, 1974; Campbell, 1978; Hänninen, 1990).

In addition, for all datasets, the UnPhot model predicts that forcing accumulation could take place at t_0 , given a long enough photoperiod (see graphs 4.1 in Figures 59, 60, 61 and 62) or a very warm ($>20^{\circ}\text{C}$) temperature. While a simultaneous action of forcing and chilling temperatures is not unrealistic, it is known that flushing cannot occur in dormant plants unless they receive a period of exposure to chilling temperatures. However, this experimental evidence is contradicted by the responses fitted to the datasets by UnPhot model, which, unlike the Unified model, does not have a threshold specifying the minimum state of chilling for budburst to occur, and thus predicts that budburst will occur in unchilled buds if they are exposed to forcing temperatures for long enough. The application of these sets of parameters to any data containing cases of unchilled dormant buds would obviously produce a poor prediction.

Such unrealistic responses might again be due to the fact that both chilling and forcing parameters were unconstrained by the lack of extreme scenarios in the data, and thus free to assume whatever value could minimise the SSres. The relatively homogenous climate conditions represented in the datasets could not “force” the free parameters to take biologically realistic values. For example, at t_0 the photoperiod was always less than 12 h and daily average temperatures were almost always below 20°C , thus it was impossible for the model to fit realistically the response of bud growth to LD and high temperatures at t_0 . This would instead have been possible if the model was fit on a combined dataset containing both experimental data and field observations. Hänninen (1995) had already pointed out the importance of fitting models to a wide range of environmental conditions in order to obtain realistic fits with external validity. Another way to prevent the model from predicting budburst in unchilled plants would be by introducing a new parameter quantifying minimum chilling requirement for budburst, such as C_{crit} in the Unified model.

1.4 Response to photoperiod

The analysis of the curve fitted to CFT50 (the relationship between T50 and photoperiod, equation (3)) by the UnPhot model reveals a smooth response of forcing rate to photoperiod and multiple states of forcing between the maximum ($T50 = 0$) and minimum ($T50 = T_{max}$) growth competence. This particular response highlights that for a given temperature there is a gradual increase in growth rate as daylength increases in spring, that chilling accumulation affects either gradually (Valentia, Wulfsdorf, Freiburg, Figures 63.2, 64.2 and 65.3, respectively) or suddenly (München-Zürich, Figure 66.2), through a shift in D150.

1.5 External validity

The prediction of the dates of budburst in Wulfsdorf and München-Zürich using the models fitted on the combined dataset of Valentia and Freiburg improved the Sum of Squares of the residuals (SSres), when comparing them with those obtained using the external mean of Valentia and Freiburg as a single predicting parameter, implying that they are more accurate than the null model. However, the negative percentages of explained variance of the external data indicated that the models' high internal validity was not corresponded by an equally high external validity. This problem has already been highlighted by various authors, among them Chuine (1998) and Kramer (1994b). Chuine (1998) tested the accuracy of commonly used phenological models for the prediction of the dates of flowering of several tree species and found that for only some species accurate predictions could be obtained. These species were mostly late flowering species such as *Olea europea* and *Platanus acerifolia*. She hypothesised that the effect of the relatively constant temperature regime occurring at the time of flowering of these species might be better explained than the cold/warm spells affecting budburst of early flowering species (Chuine *et al.*, 1998). Despite the fact that budburst of *B. pubescens* is a relatively early event, in the experiments conducted during the present study we did not find a significant effect of cold/warm spells lasting several days, at the time of forcing. Thus, the reasons for the low external validity of the models of budburst for birch must be of a different nature.

1.5.1 Model complexity

Mechanistic models formally describe known or assumed cause-effect relationships between biological processes and driving environmental factors (Chuine *et al.*, 2003). Obviously this type of model is far more complex than an empirical one, and, while it offers more possibilities for manipulating the system, provides a worse fit for the data due to the many constraints built into its structure (Thornley and Johnson, 1990).

Our results confirm that obtaining a mechanistic model of budburst with a good external validity is indeed a complicated task. The experiments performed during the current study suggested some possible ways in which photoperiod could interact with temperature in the release of dormancy. On this basis a new important environmental variable (photoperiod) was introduced into models of budburst. However, when applied to field observation, while improving the internal validity of the models, this new variable and the assumptions through which it had been integrated into the model decreased the accuracy of the model for external predictions. Part of the reason for the UnPhot model performing worse than the Unified model on external data might be that the integration of photoperiod, while not increasing the number of parameters of the model, introduced a new state variable into the model and thus increased its complexity. In addition, the UnPhot model offered a limited description of datasets in which forcing rates do not change with changing photoperiod and chilling duration, due to the fact that the relationship between forcing rate and temperature is fixed when growth competence is at optimum ($T_{50}=0$). This constraint might have limited the external validity of the UnPhot model.

1.5.2. Model testing on field observations is necessary but not sufficient for model testing

Another reason for the poor performance of the UnPhot and Unified models in the prediction of external data, might be the fact that these predictions used parameters adjusted to observations in natural conditions. Hänninen (1995) concluded that budburst observations in natural conditions alone are not sufficient for the testing of mechanistic models of budburst. Our results support his view.

The major issue of adjusting mechanistic models of budburst to field observation is that mechanistic models are derived in experimental conditions, in which plants are subjected to

a variety of extreme environmental conditions such as lack of chilling or abrupt photoperiod extension. Such extreme conditions are almost never experienced in natural conditions at temperate latitudes, where chilling always largely fulfills chilling requirements and average temperatures are similar from year-to-year. Thus, field observations almost never contain enough information to constrain the parameters of mechanistic models and make them assume realistic values, or test their assumptions.

The fact that the Unified model based only on temperature provided a better prediction for the external data might be due to the difficulty of assigning realistic values to photoperiod responses, that could be then extrapolated to an external scenario. In fact, while temperature at the time of forcing is not constant, photoperiod changes with a constant pattern from year to year, which does not provide information for a correct and realistic fit of the dependency of forcing rate on photoperiod. In addition, the largest effects of photoperiod on forcing rate were observed in partially chilled plants, while the climatic condition presently affecting the IPG sites used for the model fits ensure that chilling requirements are completely met. A final issue related with the fitting of realistic photoperiod responses to field observations is that increasing forcing temperatures and day length in spring might be correlated, so that their independent effects are not easily detected.

However, the incorporation of photoperiod is necessary for a complete representation of the triggers affecting dormancy progress in birch and could substantially improve prediction in extreme environmental conditions such as those included in global warming scenarios. In fact, as bud dormancy appears to be regulated by both temperature and photoperiod, the addition of photoperiod into mechanistic models of budburst increases their realism, and thus their applicability to external conditions.

The subject of realism in models had previously been addressed by Hänninen (1995), who showed that different models, some of which unrealistic, were almost as equally accurate in their predictions. This poses a problem at the time of model selection, because models with a lower accuracy might be discarded in favour of models with a higher accuracy but lower realism. Such unrealistic models might then perform worse when applied to external datasets for predictions. In fact, the models with the highest internal validity are unlikely also to be the ones with more external validity, if the internal dataset does not include

enough information to fit realistic responses of budburst. This holds especially true for complex models including a high number of state variables and parameters that could account for the “noise” in simple and uniform datasets, and result in a very high internal validity not matched by an equally high external validity.

A model fit of low realism will predict accurately external observations only if they are affected by climatic conditions similar to those of the model fit. Thus, it is important when selecting a model for external predictions, to analyse the responses fitted to chilling and forcing temperatures and evaluate whether they have or not a biological meaning. The parameterization of realistic models could be facilitated prior to the fitting procedure, by constraining the parameters determining known responses, such as the regression rate of the logistic response to forcing temperatures. However this approach requires considerable caution, as dormancy is poorly understood, and there is a risk of transmitting our preconceived notions to the model.

1.5.3. Inconsistencies among stations in the identification of phenophases

The data recorded at the different IPG locations were collected by different people, who identified leaf unfolding through the use of visual keys provided by the central headquarters of the IPG network. However, these data collectors might have had different criteria for the identification of timing at which 50% of leaves unfolded on the tree. Consequently some stations might have recorded their data consistently earlier or later than others. These types of errors would not be detected by quality control procedures, that discard only outliers or aberrant observations. The obvious effect of this type of over or underestimation of the timing of leaf unfolding would be the increase in the SSres when different stations are fit together, or when a model fit is used to predict leaf unfolding at an other station. Thus, the inconsistencies among stations in the identification of phenophases might be a further explanation for the low external validity shown by the Unified and UnPhot models.

1.5.4. Integration of temperature and photoperiod responses into the model

One of the major issues in developing mechanistic models of budburst is the integration of experimentally observed responses to environmental variables into a conceptual framework. While a number of experimental studies has shown the relationships between these factors and budburst timing in controlled environmental conditions, it is difficult to

link these responses together, because of the dynamic nature of dormancy and the differences between the species, the provenances, the age and the type (cuttings vs. seedling or clones) of the experimental material. The environmental conditions prior to the experiments are also important (natural photoperiod vs. controlled photoperiod, different dormancy induction temperatures *etc.*). The identification of a marker of dormancy would enable the comparison between different studies and environmental conditions, and the monitoring of the evolution of dormancy. However, while such a useful indicator is not accessible, intuition and further experiments are the only ways to link the available knowledge on plant dormancy into the structure of a phenological model.

A possible explanation for the reduced external validity of the UnPhot model concerns its conceptual structure. The relationship linking DI50 with increasing CUsum (equation 2) was suggested by the experimental evidence showing a decrease in the threshold photoperiod for budburst as chilling was accumulated. However DI50 might affect forcing rate differently than through a shift of the forcing rate curve. Another issue of speculation at the time of model development was whether to link photoperiod and chilling duration to forcing rate through two independent relationships or, as we did, to express the effect of photoperiod as a function of chilling duration, then link this factor to forcing rate. From a physiological point of view, having two relationships independently linking chilling duration and photoperiod to forcing rate would be equivalent to hypothesising two parallel mechanisms of control of bud dormancy, one regulated by chilling and the other by photoperiod. The current structure of the model instead accounts for a single control mechanism whose “receptiveness” to photoperiod is influenced by the state of chilling.

However, before providing a falsification of this framework, more tests will be needed. It now appears obvious that it cannot be discarded before it is calibrated on a large dataset containing both field and experimental data including the manipulation of photoperiod. An alternative way to test the assumptions of the UnPhot model would be to perform a large experiment testing the interactions of chilling duration and photoperiod on forcing rate at different temperatures.

GENERAL DISCUSSION AND CONCLUSION

The details of the physiological processes that underlie dormancy are as yet unclear (Arora *et al.*, 2003). However, it is becoming more and more important to understand how tree growth responds to changes in environmental conditions, and predict how species will be impacted by global warming. Yearly variations in tree phenology are responsible for large differences in forest productivity (Lieth, 1970, 1997; Kellomäki and Kolström 1994), and earlier budburst due to global warming could lead to extensive frost damage (Kramer, 1994a, 2000; Hänninen, 1991, 1995). Also, the timing of phenological events is a potential bio-indicator summarising climate trends and variations, and their impact on biological systems (Parmesan and Yohe, 2003). In order to gain more understanding of the triggers of tree phenology, ecophysiological studies are necessary that investigate the relationship between environmental factors and spring phenology.

Different models of tree phenology have been proposed to predict the timing of budburst and flowering of tree species by the input of environmental variables such as temperature and day length (Sarvas, 1974; Landsberg, 1974; Richardson *et al.*, 1974; Hänninen, 1995; Chuine, 2000). The mathematical description of the effects that these factors have on bud growth may be very useful to elaborate plant protection strategies and predict the impacts that climate change will have on ecosystems. The global warming that is predicted to affect us in the coming decades, will probably have heavy impacts on ecosystems and affect plant development. In order to mitigate this impacts it is crucial to understand its effects on plant growth and develop models that describe the causal relationship between climatic drivers and the timing of phenological events. The study of the regulation of phenological events has also many more immediate applications in horticulture and agriculture. The quantification of the chilling requirements of temperate fruit species is important for the selection of cultivars to be grown in climate conditions different from those affecting their natural ranges (Fuchigami and Wisniewski, 1997; Balandier *et al.*, 1993) and the modelling of growth onset provides important information for the management of the canopy (Austin *et al.*, 2002)

However, models will be only be as accurate as our understanding of the underlying cellular mechanisms regulating dormancy release (Arora *et al.*, 2003). As long as these mechanism are unknown, the only way to facilitate the development of predictive models

and gain information on the impact of environmental changes on tree phenology, will be to investigate plant responses through ecophysiological studies

The aim of this thesis was to quantify the effect of temperature, photoperiod, light intensity and nutrient deficiency on the timing and percentage of budburst in various tree species (*B. pubescens*, *F. sylvatica*, *S. aurita*, *S. x smithiana*, *T. cordata*), and integrate this information into a mechanistic model of budburst. In order to eliminate intraspecific variation, the experiments were conducted on plant material that was vegetatively propagated from clones growing in the International Phenological Gardens network.

1. Quantifying the effect of environmental triggers on the timing and percentage of budburst of *Betula pubescens*, *Fagus sylvatica*, *Salix aurita*, *Salix x smithiana* and *Tilia cordata*

The experiments conducted during the present study highlighted varied responses to different environmental factors among the tree species under study. However, it was possible to recognize some general patterns that confirmed the presence of a common mechanism controlling dormancy, based mostly on temperature and a photoperiod signal. In all species, increasing chilling duration resulted in a decrease in the time to budburst under forcing conditions. This decrease clearly followed a negative exponential pattern in *B. pubescens*. All species, after partial chilling responded to long photoperiod by increasing their rate of budburst and their percentage budburst, even if there were differences among species as to the importance of this response. In all species, budburst rate increased logistically at increasing temperatures. These common features suggest that while dormancy release is controlled by photoperiod and chilling, the degree to which the two triggers contribute to the progress of dormancy varies among species. In *F. sylvatica* and *B. pubescens* daylength could substitute for lack of chilling completely or almost completely. *F. sylvatica* was particularly affected by LD, which could replace completely a very large chilling requirement.

The experimental results suggested that the phenological differences observed among the species under study might be related to differences in their ecology and the life strategy. Dominant, long lived species such as *T. cordata* and *F. sylvatica* were characterized by low rates of budburst, high chilling requirements and responsiveness to light intensity, while

opportunistic, pioneer species such as *B. pubescens*, *S. aurita* and *S. x smithiana* had high rates of budburst, and low chilling requirements, besides not being affected by light intensity. In addition, bud growth in *B. pubescens* and *S. x smithiana* was comparatively more responsive to high forcing temperatures ($>15^{\circ}\text{C}$) than in *T. cordata* and *F. sylvatica*. These results suggest that the timing of growth onset in *B. pubescens* and *S. x smithiana* is regulated through a less conservative mechanism than in *T. cordata* and *F. sylvatica*, and that these species trade a higher risk of frost damage for the opportunity of vigorous growth at the beginning of spring, when more light is available.

The differences in the phenological responses observed in the species under study suggest that *S. aurita*, *S. x smithiana* and *T. cordata* are the best indicators of climate change, because of their relative insensitivity to photoperiod in the late stages of chilling. Their responses to forcing temperature and the difference in their chilling requirements suggest that while the timing of budburst of *T. cordata* could be a good indicator of the February–April average temperatures the two willow species could be a good indicator of higher than average temperatures and warm spells occurring during the months of January, February and early March.

After the first year of the study, *B. pubescens* was selected among the five tree species to become the object of a more comprehensive study that would provide the information for the development of a mechanistic model of budburst. The results of these experiments showed that dormancy induction in *B. pubescens* was a gradual process reversible in its early stages, mainly triggered by short photoperiods, and enhanced by low temperatures. The alternation of warm and cold spells during forcing did not significantly affect the timing of budburst in this species, suggesting that bud growth responses depended on average temperature. The photoperiod sensitivity of *B. pubescens* was shown by various experiments which suggested that daylength affects the rate of dormancy progress mainly during dormancy induction and quiescence. Increasing chilling durations decreased the threshold photoperiod above which flushing occurred in partially chilled buds, suggesting a negative relationship between increasing chilling accumulation and critical photoperiod for forcing accumulation.

2. Integrating photoperiod into mechanistic models of budburst

The information gained during the experimental part of this study confirmed some previously established responses assumed by widely used phenological models, such as the negative exponential relationship between chilling duration and time to budburst (Murray *et al.*, 1989; Cannell and Smith, 1983), integrated into the alternating model (Murray *et al.*, 1989), or the logistic relationship between forcing temperature and rate of budburst (Sarvas, 1974; Campbell, 1978; Hänninen, 1990) used for calculating forcing units in the Deepening rest (Kobayashi *et al.*, 1982), Sequential (Hänninen, 1990), Parallel (Landsberg, 1974) and Unified (Chuine, 2000) models. The enhancing effect that photoperiod exerts on budburst rate during forcing had been previously shown by a number of studies (Wareing, 1954; Nienstaedt, 1966; Myking and Heide, 1995; Heide, 1993), however, attempts to use photoperiod in addition to temperature for the prediction of budburst have given conflicting results (Häkkinen *et al.*, 1998; Kramer, 1994b). For this reason, an attempt was made to integrate photoperiod into the Unified model (Chuine, 2000), a temperature based general model of budburst accounting for the most widely used models of spring phenology.

We hypothesised that the observed effect of photoperiod was expressed through an increase in the forcing rate for a given temperature, without altering the maximum rate of forcing, and that the magnitude of this increase depended in turn on the amount of accumulated chilling units. We formalized these hypotheses by two negative relationships, the first linking photoperiod to T50, the temperature at which half a forcing unit was accumulated, that could vary between 0 and T_{max}, a parameter to be assigned through a fitting procedure. The second relationship links the amount of accumulated chilling with DI50, the threshold photoperiod at which T50 occurred at a temperature of $\frac{1}{2}$ T_{max}. These two relationships were integrated into the Unified model, and replaced the negative relationship linking the state of chilling with the amount of forcing units needed for budburst. The minimum chilling requirement C_{crit} was also removed as it was believed that the model could adjust itself to account for minimal forcing accumulation in unchilled plants. The number of parameters in this new model, called the UnPhot model, is the same as for the Unified model, 9 in total.

The model was fitted to the experimental results and to field observation recorded on the same *B. pubescens* clone at 5 IPG stations, Valentia (Ireland), Wulfsdorf, Freiburg,

München (Germany) and Zürich (Switzerland). The UnPhot model showed very high percentages of explained variance for all datasets, including the experimental results. The Unified model showed very high percentages of explained variance for the IPG observations but failed to explain budburst timing in those experiments manipulating photoperiod aside from temperature. This suggests that the integration of photoperiod enabled the model to explain a portion of variance due to the effect of photoperiod that was previously unaccounted for. The integration of photoperiod through a “fixed calendar date” would not have explained the progressive decrease in the threshold photoperiod for budburst, nor the difference in forcing rate observed between LD and SD forced plants observed in the experimental phase of the study.

However the heterogeneity of the parameter values fitted to the models in the different datasets and the low consistency among the temperature responses suggested that the models fitted during the present study cannot be applied to external datasets for predictions of budburst without a decrease in the accuracy of prediction. This was confirmed by the lower percentages of explained variance obtained when half of the field observations were used to predict the other half. In fact, while both models improved the Sum of Squares of the residuals (SSres) of the predicted budburst dates when comparing them with the null model (the external mean used as a single predicting parameter), the predictions of both models gave a SSres higher than the total variance of the external data. Of the two models, the one that gave better external predictions was the Unified Model.

There might be different reasons for the low external validity shown by the UnPhot model. The first is that the model explains budburst timing through the input of two state variables (photoperiod and temperature) as opposed to one (temperature) and is thus more complex than the Unified model. Introducing new constraints into mechanistic models decreases their accuracy in the early stages of model development, despite the fact that it provides a means to test new hypotheses and pinpoint areas where knowledge is lacking (Thornley and Johnson, 1990).

The second reason is that the model was fitted without constraining its parameters, expecting that the parameter adjustment would reflect the actual effect of photoperiod on forcing rate. However, in natural conditions and in the current climate, field observation do not contain enough information to realistically fit this response. In fact, the experimental results

showed that photoperiod played an important role especially in partially chilled plants, while the climatic conditions currently affecting the tested IPG sites ensure that chilling requirements are completely met. This decreased the realism of the adjusted responses.

A third possible explanation is that the observations collected in the phenological gardens might not have been collected accurately and homogeneously. The observed phenophase (leaf unfolding) might have been identified differently at the different sites, resulting in a decrease in the percentage of explained variance when different sites were fitted together or when the model fits were used for external predictions.

Finally, the effect of photoperiod might not have been correctly integrated into the Unified model and that the mathematical relationships formalizing its effects on forcing rate might need to be modified. A major issue of speculation at the time of the development of the UnPhot model was whether to link photoperiod and chilling duration to forcing rate through two independent relationships or, as we did, to express the effect of photoperiod as a function of chilling duration, then link this factor to forcing rate

Therefore, further model testing is necessary to establish whether the inherent structure of the UnPhot model appropriately reflects the effects and interactions of photoperiod and temperature on dormancy progress and budburst timing. The calibration of the model on a large dataset including both experimental data and field observation would increase the realism of the fitted responses and enable us to evaluate the appropriateness of the model framework.

3. Future research

3.1. Investigating the interaction of chilling duration and forcing photoperiod on the rate of budburst at different temperatures

During the present study, we quantified the effects of 6 different forcing temperatures on fully chilled budsticks, and confirmed the previously observed (Sarvas, 1974) logistical increase in budburst rate at increasing temperatures. However, we did not test the effect of chilling duration and forcing photoperiod on the response of budburst rate to forcing temperature. This piece of information could have facilitated the development of the UnPhot model, as it would have enabled us to link the relationships describing the effect of photoperiod and chilling duration with forcing rate without any guesswork. In fact, while it was generally known that the rate of budburst increased after increasing chilling durations and at increasing forcing photoperiods, during the present study, these effects were tested only at high temperatures such as 18°C (experiment 3.a, interaction of chilling duration and forcing photoperiod), or 22°C (experiment 3.b, effect of chilling duration and different forcing photoperiods).

The literature is lacking detailed and comparable information on these effects. Håbjørg (1971) investigated the interaction between different 3 different growing temperatures and 6 different photoperiods in *B. pubescens* and found that increasing temperature resulted in a larger increase in height at photoperiods above the critical threshold for apical growth, that he reports to be included between 12 and 14 hours. However, the effects of the temperature-photoperiod interaction on bud growth may be different than those found during the growing season, and these interactions might change as the chilling requirements are progressively fulfilled.

Further investigations on the interaction of chilling duration, forcing photoperiod and a wide range of forcing temperatures on the timing of budburst may lead to a more effective integration of the effects of these environmental factors into mechanistic models of budburst, and to an accurate evaluation of the parameters describing these responses.

3.2. Quantifying more accurately the effects of summer-autumn temperature and photoperiod on the rate of dormancy induction

The experimental results obtained in the present study highlighted the significant effect that photoperiod and temperature played on dormancy induction and the subsequent timing of budburst in *B. pubescens*, confirming previous studies (Welling, 1997; Kawase, 1961). After conducting experiments on the effects of temperature during dormancy induction, Heide (2003) concluded that high autumn temperature increases the depth of dormancy and chilling requirement in *B. pubescens*. The interaction between late summer-early autumn temperatures and photoperiod is not considered by most models of budburst that assume that dormancy is fully attained on a fixed date (Utah chill model, Sequential, Alternating and Parallel models). However, a deeper understanding of these effects might improve models predictions for external datasets, as the date of start of accumulation of chilling units might vary considerably at different locations with different climates. For example, locations affected by a continental climate are subjected to a sharp decrease in temperature during autumn, while locations affected by oceanic climate experience milder autumns.

Additional experimental and field studies are needed to quantify the effects of summer temperature and its interaction with photoperiod in inducing bud dormancy. Linking the start of dormancy with summer temperature will facilitate the development of a whole-year phenological model.

3.3. Integrating the percentage of budburst into mechanistic models of budburst, as a measure of dormancy release

The present study suggested a close relationship between the timing and percentage of budburst. In partially chilled plants, a longer time to budburst often corresponded to a lower percentage budburst. Despite the fact that plants with less than 50% budburst were considered as non-flushing in the budburst models fit to the experimental data, almost always these non-flushing plants showed small, varying percentages of budburst, highlighting that dormancy release is a gradual process even before the 50% budburst threshold is reached. The prediction of this response could increase the applications of mechanistic models of budburst and add valuable information to the predicted scenarios of the impacts of global warming.

The percentage of budburst is of special interest also for horticulturist and agricultural scientists. Temperate fruit trees grown in warm regions are affected by incomplete chilling symptoms, such as incomplete budburst and slow growth. These effects were observed for many species, including peach (Weinberger, 1950) and cherry (Mahmood, 2000), and have been dealt with either through the use of dormancy breaking chemical or the selection new varieties with low chilling requirement (Arora *et al.*, 2003). The latter approach may be facilitated by the application of models of budburst that, given a particular climate scenario, predict budburst percentages besides its timing.

In order to add this prediction to phenological models of budburst, future studies on ecophysiological regulation of dormancy will have to measure percentage budburst besides its timing and investigate its patterns of variation. Further quantification of the correlation between these two variables would enable us to integrate the estimation of the percentage of budburst into phenological models, therefore increasing their value.

3.4. Testing the UnPhot model with a combined dataset of experimental and field observations

The results obtained during the present study support Hänninen's (1995) conclusions that budburst observations in natural conditions alone are not sufficient for the testing of mechanistic models of budburst. This proved especially true for the UnPhot model, whose assumptions are hard to test in natural conditions and in a temperate climate fulfilling the chilling requirement of *B. pubescens* very early after dormancy induction. The logical step after making an attempt at predicting the timing of budburst in an external dataset using the parameters adjusted on a series of field observation (and verifying Hänninen's conclusion) is to test the model fitted on the experimental observations.

For this task, the model will have to be fit on experiments testing the assumptions of the model, providing enough information for a realistic fit of the photoperiodic response in partially chilled conditions. Combining a series of field observations with experimental data would provide an ideal dataset, enabling the parameters values of the model to adjust to a wide range of natural (such as a wide range of chilling and forcing temperatures) and experimental constraints (such as the effect of incomplete chilling and photoperiod on dormancy release). If the model fit explains a significant portion of variance when

compared with the null model, it may then be interesting to use it for external predictions and compare them with predictions based on the model derived in natural conditions only.

3.5. Refining the response to forcing temperature in the UnPhot model and incorporating the effects of high temperatures on bud growth

One limitation of the UnPhot model is its rigidity when it comes to describing sequential and thermal time models of dormancy not influenced by photoperiod, in which, once dormancy is released, heat accumulation occurs at a constant rate despite the additional accumulation of chilling and the extension of photoperiod. As it was seen in the examples provided in the methods section, the model could account for such non-changing response only for $T_{50}=0$, describing a sigmoidal curve with its inflexion point at 0°C . One possible improvement in the model would be to leave the minimum value of T_{50} free, as a parameter to be adjusted. This would add a T_{min} parameter to the model. In order to keep the number of parameters constant, T_{max} could be fixed, on the basis of the observation that budburst almost never occurs in dormant temperate tree species, unless a minimum state of chilling has been attained or a sufficiently long photoperiod received. This would imply fixing T_{max} as a high temperature (such as 50°C), shifting the sigmoid curve describing forcing rate towards high temperatures and resulting in no forcing units being accumulated at normal spring temperatures in unchilled plants receiving short photoperiods (in agreement with experimental observations).

Another possible improvement to the realism of the model would be including the response to very high temperatures in the description of forcing rate. It is known that plant growth increases proportionally to temperature up to an optimal temperature. At supraoptimal temperatures, growth rate declines and tissue damage occurs (Taiz and Zeiger, 2002). It has been shown that cassava plants do not flush above a threshold temperature between $36.6\text{--}39.8^{\circ}\text{C}$ (Keating and Everson, 1979). Seedling elongation and leaf extension in maize also stopped as temperature reached an upper threshold (Watts, 1971). As the rate of bud development is similar to the active growth developmental rate (Sarvas, 1974), it is expected that bud growth will be stopped at very high temperatures, leading to delays in budburst and reducing the percentage of budburst. A reduction in percentage budburst was already observed during the present study at 32°C . However, these results are not shown, as it was impossible to determine whether bud mortality was due to high temperature damage

or desiccation of the cuttings. More studies are needed to determine the response of buds to high temperatures, if budburst models are to be applied to climate change scenarios. As twigs and cuttings are particularly vulnerable to desiccation at high temperatures, similar studies should investigate whole plants (for example, rooted cuttings or seedlings).

A possible way to represent the decline of bud growth at high temperatures involves the experimental determination of the maximum temperature at which bud growth can occur and its addition into the model through a negative function. This response probably depends only on the physiology of the plant species and its adaptations to a particular climate (Raison *et al.*, 1982). As it is probably not affected by photoperiod and temperature, it would not be necessary to introduce new dynamic relationships linking it to the state variables, thus not resulting in an increase in the number of parameters.

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