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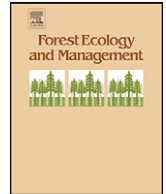
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Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Modeling the effects of winter environment on dormancy release of Douglas-fir

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ARTICLE INFO

Article history:

Received 1 February 2009

Received in revised form 23 May 2009

Accepted 14 June 2009

Keywords:

Chilling
Forcing
Dormancy
Climate change
Global warming
Modeling

ABSTRACT

Most temperate woody plants have a winter chilling requirement to prevent budburst during mid-winter periods of warm weather. The date of spring budburst is dependent on both chilling and forcing; modeling this date is an important part of predicting potential effects of global warming on trees. There is no clear evidence from the literature that the curves of chilling or forcing effectiveness differ by species so we combined our data and published information to develop new curves on the effectiveness of temperature for chilling and forcing. The new curves predict effectiveness over a wide range of temperatures and we suggest both functions may be operating at the same time. We present experimental data from 13 winter environments for 5 genotypes of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) and use them to test various assumptions of starting and stopping dates for accumulating chilling and forcing units and the relationship between budburst and the accumulation of chilling and forcing units. Chilling started too early to be effective in one treatment but the other 12 environments resulted in budburst from many combinations of chilling and forcing. Previous reports have suggested benefits or cancellations of effects from alternating day/night or periodic temperatures. Our simple models do not include these effects but nevertheless were effective in predicting relationships between chilling and forcing for treatments with a wide range of conditions. Overall, the date of budburst changed only slightly (+1 to –11 days) across a wide range of treatments in our colder test environment (Olympia, WA, USA) but was substantially later (+29 days) in the warmest treatment in our warmer environment (Corvallis, OR, USA). An analysis of historical climate data for both environments predicted a wide range in date to budburst could result from the same mean temperature due to the relative weightings of specific temperatures in the chilling and forcing functions. In the absence of improved understanding of the basic physiological mechanisms involved in dormancy induction and release, we suggest that simple, universal functions be considered for modeling the effectiveness of temperature for chilling and forcing. Future research should be designed to determine the exact shape of the curves; data are particularly lacking at the temperature extremes. We discuss the implications of our data and proposed functions for predicting effects of climate change. Both suggest that the trend toward earlier budburst will be reversed if winter temperatures rise substantially.

Published by Elsevier B.V.

1. Introduction

Many temperate zone woody plants have a chilling requirement which prevents budburst during a warm period mid-winter. The chilling requirement is usually described as some time period (i.e., days or hours) of temperatures in a specific range (e.g., $\geq 0^\circ\text{C}$ and $\leq 5^\circ\text{C}$) that is necessary for budburst or reduces the time to budburst in the spring. Past researchers have

studied chilling for many species; some have tested models with long-term phenological records and others have conducted controlled experiments. In spite of great interest in this topic for more than 100 years, the biological mechanisms involved in perceiving and “remembering” temperatures effective for releasing winter dormancy (bud dormancy, seed dormancy, flowering and vernalization) and forcing (warm temperatures that accelerate budburst) are not well understood (Amasino, 2004; Sung and Amasino, 2005). There have been several recent advances in physiology, biochemistry and molecular markers which may hold promise for understanding the underlying mechanisms (cf. recent reviews by Horvath et al., 2003; Arora et al., 2003; Rohde and Bhalerao, 2007). Currently, however, empirical studies and modeling are necessary for addressing questions of how plants respond to warmer winter environments

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(e.g., Murray et al., 1989; Hänninen, 1995; Raulier and Bernier, 2000; Rea and Eccel, 2006).

Most past research has determined that temperatures slightly above freezing are most effective in satisfying the chilling requirement for bud dormancy. Depending on species, the temperature ranges considered to be optimum have differed (e.g., ≤ 4.4 °C for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), van den Driessche, 1975; 0–12 °C for loblolly pine (*Pinus taeda* L.), Garber, 1984; ≤ 7 °C for fruit trees, Samish, 1954; or ≤ 10 °C for sugar maple (*Acer saccharum* Marsh.), Raulier and Bernier, 2000) but with general agreement that temperatures of 3–4 °C are effective. Many researchers have apparently assumed that freezing temperatures are ineffective; however, some have suggested subfreezing temperatures may be effective in satisfying chilling (cf. Omsted, 1951; Ritchie, 1984; van den Driessche, 1977). There is also disagreement as to the upper end of temperatures which are effective in satisfying chilling requirements. Researchers have suggested that temperatures above 10 °C are not effective in satisfying chilling (Sarvas, 1974), 10 °C is the break between effectiveness of chilling and forcing temperatures (Raulier and Bernier, 2000), and that temperatures around 10 °C are only half as effective as temperatures somewhat colder (Richardson et al., 1974). Garber (1983) proposed that temperatures 0–12 °C are equally effective and that even warmer temperatures are somewhat effective in satisfying chilling.

Forcing or the promotion of budburst is usually assumed to be a simpler process to model than chilling and researchers have used step functions (every hour above a set temperature is effective), linear, or sigmoid functions (cf. Thomson and Moncrieff, 1982; Hänninen, 1995; Raulier and Bernier, 2000). Although researchers have differed in the date when they start accumulating forcing, most have assumed that forcing occurs after the chilling requirement has been met.

Although the basic physiological processes involved in sensing or accumulating chilling are not completely known (see review articles referenced above), we suggest that the biochemistry involved in sensing chilling is likely to be the same for many species. Thus, we hypothesize that “universal” temperature response functions (i.e., the relationship between specific temperatures and their relative effectiveness in satisfying chilling or forcing) exist for many species. Although researchers have reported different results for multiple species, the differences are not large and we suspect that the reported differences in apparent chilling or forcing effectiveness by temperature are not due to fundamental differences between species but instead may be due to factors such as the design or interpretation of studies, the range in temperatures used, or the correlations between numbers of hours in different temperature bands. Past researchers have often focused on determining the optimum range for chilling effectiveness (as opposed to determining the shape of the response function) or on evaluating temperature ranges important for specific commercial practices (e.g., chilling in cold storage) and thus may not have been interested in the broader question of the shape of the curve in the temperature ranges which have less than optimum effectiveness.

We first present results from the literature on chilling and forcing for several species and develop two functions which relate the relative effectiveness of specific temperatures to dormancy release and promotion of budburst. We then test our proposed functions for dormancy release and forcing with data from 13 treatments which utilized 5 seed sources of Douglas-fir. We also use our response functions to: (1) predict date of budburst from historical temperature data from two locations and show how these predictions fit with our experimental treatments and (2) show how date of budburst from an independent study compares to predicted values. We also discuss the implications of these results for predicting response to alternative future climate scenarios.

Past researchers have developed several classification systems to describe dormancy. Since most of them cannot be determined from visual examination of the bud and their relationship to the specific physiological functions involved in dormancy induction and release has not been clearly demonstrated, we will simply use the term dormancy to refer to what others have labeled as winter dormancy (Sarvas, 1974), rest (Chandler, 1925), endodormancy (Lang et al., 1987) or true dormancy (Vegis, 1964).

2. Methods

2.1. Literature survey

We surveyed the literature for examples of studies which reported the effectiveness of various temperatures for dormancy release or forcing. We preferred to find studies where results were expressed in terms of days to budburst, but other measures such as growth after budburst were used. In this first phase of literature summary, we also included some examples which reported the effects of temperature on release of seed dormancy or vernalization even though these processes may be under control of different factors. We started with the concept that the effectiveness of dormancy release would vary across a fairly wide range of temperature; we based this on the temperature responses proposed for vernalization of rye (Hänsel, 1953 as shown in Seeley, 1996), dormancy release of apple seeds (Schander, 1955 as shown in Seeley, 1996), promoting of flowering in apple (Seeley, 1996), dormancy release versus temperature for *Betula pubescens* seedlings and seeds (Sarvas, 1974), and for peach (Richardson et al., 1974) and cherry trees (Anderson et al., 1986). All of these functions showed the effectiveness of chilling to peak at an intermediate value (the 2.5–9.1 °C maximum effectiveness suggested by Richardson et al., 1974 encompassed the peak values suggested by others) and that temperatures above or below this maximum value had some effectiveness but at a lower level (Fig. 1A).

In our second phase of literature review we looked for additional studies reporting data on chilling effectiveness at two or more temperatures. For each study, the values were plotted against temperature with the most effective treatment assigned a value of 1 (maximum effectiveness) and other treatments scaled appropriately (Fig. 1B). Our initial function was adjusted to fit the available data by stretching or reducing the limits (adjusting the values of the function with zero or maximum levels). The data sources used were: for Douglas-fir—Wommack (1964), Sugano (1971), Ritchie (1984), McCreary et al. (1990); for birch—Sarvas (1974), Myking and Heide (1995); for fruit trees—Erez and Lavee (1971), Sugiura et al. (2002).

Based on these suggested relationships, we then constructed a chilling function (based on the three-parameter Weibull distribution) which was centered on the values of maximum effectiveness and had tails which showed effectiveness declining to zero at the widest range shown in past studies (Fig. 1C). The upper limit for chilling was set at 15/16 °C based on the suggestion from Nishiyama (1972) that 15 °C is a key physiological temperature based on several physical (e.g., surface tension of water) and biological observations. Several authors indicated that 0 °C was less effective than slightly warmer temperatures but it seemed logical to us that at least several degrees of subfreezing temperatures should be considered as having some effectiveness.

2.2. Experimental data for Douglas-fir

Two experiments on winter dormancy were conducted during the winter of 2007–2008 using five open-pollinated seed sources of Douglas-fir from western Washington and Oregon (Fig. 2). These

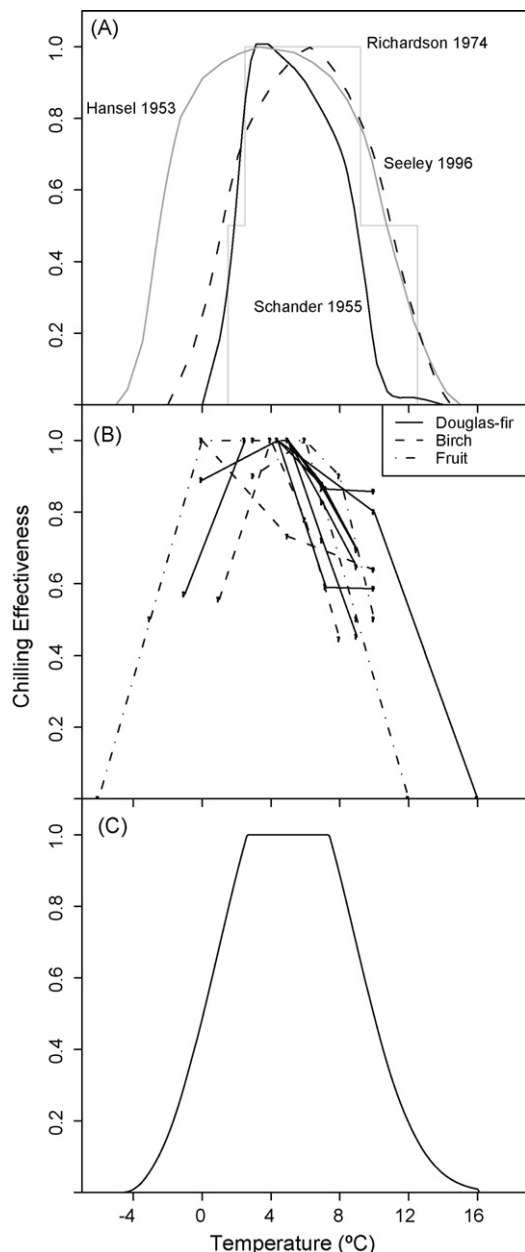


Fig. 1. Relationship of temperature to chilling effectiveness. (A) Published relationships for: vernalization of winter rye (Hänsel, 1953), dormancy release in apple seeds (Schander, 1955), step function for flowering of peach trees (Richardson et al., 1974) and the resulting smooth curve presented by Seeley (1996). We did not include the portion of the Richardson or Seeley graphs where they suggest temperature has a negative effect. (B) Relative chilling effectiveness based on data from published sources where multiple temperatures were tested (see text for sources). (C) The final form of the function relating chilling effectiveness to temperature (based on published results and our data). Note that the function is not symmetrical; the right-hand side has a longer tail.

seed sources were a small subset of those used in an earlier seedling study of geographic variation in adaptive traits including vegetative phenology, cold hardiness, and growth (St. Clair et al., 2005). The sources were selected from those used in the previous study as having a similar number of days to budburst (having similar phenology was expected to reduce one source of variation) yet being geographically diverse (increasing the likelihood that the results would be applicable to multiple genotypes). Additional seed sources covering a wide geographic area were used in Experiment 1 and that data will be presented in a future paper. For both experiments, seed were taken out of storage in January 2007,

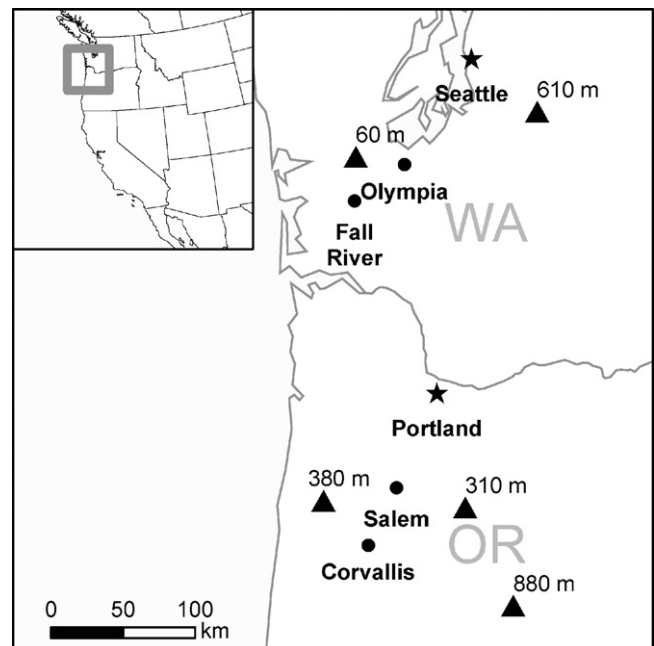


Fig. 2. Locations of Douglas-fir seed sources (triangles with elevations above mean sea level), study sites (Olympia and Corvallis), sources of supplemental data (Fall River and Salem), and major cities in western Washington and Oregon, USA.

stratified for 45–55 days, then sown in styroblocks (20 cells per block, 710 ml per cell) filled with a peat–perlite mix that included a starter fertilizer. Seed in Experiment 1 was sown in a lathhouse at Olympia, WA, April 16–18, 2007 and in a greenhouse in Corvallis, OR the following week. There were four treatments at each location with ten seedlings from each family in each treatment. Seed in Experiment 2 was sown on April 10, 2007 in a greenhouse in Olympia, WA and then moved to a lathhouse in early summer; there were three styroblocks for each of five treatments (four cells in each styroblock were randomly assigned for each family).

In Experiment 1, seedlings were subjected to four temperature regimes during the winter of 2007–2008 at each of the two locations (Table 1). The regimes were defined as: Ambient (kept outside all winter), Ambient-400, Ambient-800, and Ambient-1200. In the Ambient-400 treatment seedlings were moved once a month during November, December, January, and February into a greenhouse kept at or above 10 °C for the period of time required for the Ambient treatment to experience 100 h ≥ 0 °C and ≤ 5 °C during each month; this was 400 h over the time of the trial. The Ambient-800 seedlings were kept in the greenhouse so they had 200 fewer hours per month (800 fewer hours ≥ 0 °C and ≤ 5 °C overall), and the Ambient-1200 treatment had 300 fewer hours per month (1200 fewer hours ≥ 0 °C and ≤ 5 °C overall). Trees in all treatments accumulated forcing in the greenhouse or ambient environments; i.e., special forcing treatments were not applied. All seedlings in Experiment 1 were at ambient photoperiod for the duration of the study. The ambient and greenhouse temperatures at Olympia and Corvallis differed (averaging 0.4 and 3.1 °C warmer, respectively, in Corvallis), thus resulting in eight combinations of chilling and forcing temperatures for Experiment 1.

In Experiment 2, we wanted to test the effects of chilling at different times during the dormant season. We assumed in designing the study that temperatures at or above 10 °C would not be effective in chilling (an assumption later shown to be invalid). The study intent was to keep seedlings in an environment where they would not receive chilling until they were moved into a growth chamber. The treatments were: Ambient (seedlings kept outside all winter followed by growth chamber forcing beginning

Table 1

Summary of experiments with Douglas-fir families in 2007–2008. OW = Olympia, WA; CO = Corvallis, OR; GH = greenhouse; CC = chilling chamber; FC = forcing chamber. See text for additional details.

Treatment ID	Location	Chilling temperature	Forcing temperature	Chilling photoperiod	Forcing photoperiod
Oly-A	OW	Ambient (A)	Ambient	Ambient	Ambient
Oly-A-400	OW	A-400 h $\geq 10^{\circ}\text{C}$	Ambient + GH	Ambient	Ambient
Oly-A-800	OW	A-800 h $\geq 10^{\circ}\text{C}$	Ambient + GH	Ambient	Ambient
Oly-A-1200	OW	A-1200 h $\geq 10^{\circ}\text{C}$	Ambient + GH	Ambient	Ambient
Corv-A	CO	A	Ambient + GH	Ambient	Ambient
Corv-A-400	CO	A-400 h $\geq 10^{\circ}\text{C}$	Ambient + GH	Ambient	Ambient
Corv-A-800	CO	A-800 h $\geq 10^{\circ}\text{C}$	Ambient + GH	Ambient	Ambient
Corv-A-1200	CO	A-1200 h $\geq 10^{\circ}\text{C}$	Ambient + GH	Ambient	Ambient
Oly-A + F	OW + FC	Ambient	20 $^{\circ}\text{C}$	Ambient	16 h
Oly-Fall	OWGH + CC + FC	2–3 $^{\circ}\text{C}$	20 $^{\circ}\text{C}$	Ambient	16 h
Oly-Winter	OWGH + CC + FC	2–3 $^{\circ}\text{C}$	20 $^{\circ}\text{C}$	Ambient	16 h
Oly-Spring	OWGH + CC + FC	2–3 $^{\circ}\text{C}$	20 $^{\circ}\text{C}$	Ambient	16 h
Oly-10C+	OWGH	$\geq 10^{\circ}\text{C}$	$\geq 10^{\circ}\text{C}$	Ambient	Ambient

March 17), Fall (1200 h of growth chamber chilling starting October 15, immediately followed by growth chamber forcing), Winter (kept in greenhouse at or above 10°C until December 20, then 1200 h of growth chamber chilling immediately followed by growth chamber forcing), Spring (kept in greenhouse at or above 10°C until March 3, then 1200 h of growth chamber chilling immediately followed by growth chamber forcing), and None (kept in greenhouse at or above 10°C all winter). All growth chamber chilling was at 2–3 $^{\circ}\text{C}$ at ambient photoperiod; growth chamber forcing was at 20/17 $^{\circ}\text{C}$ (day/night) with a 16-h photoperiod. Thus, the growth chamber light regimes were different for the fall, winter, and spring chilling treatments. The chilling and forcing chambers were Geneva Scientific/Percival growth chambers (Model PGC - 105X) which maintained temperature within 0.1 $^{\circ}\text{C}$. Chamber lighting was a combination of 25-W incandescent and 160-W fluorescent bulbs (Phillips F27T12/CW/VHO). The chambers had dual chilling units so that defrost cycles did not result in warm spikes in temperature during chilling treatments. There were two brief interruptions due to power outages which lasted only a few hours. Plants were watered as needed while in the chambers.

Date of terminal budburst was recorded two to three times a week (budburst was defined as the date when bud scales had parted sufficiently so that green needles were visible) in both experiments. Heights were measured prior to the beginning of the chilling treatments and at the end of the summer. Douglas-fir can switch from fixed growth to free growth within the growing season (Cline and Harrington, 2007); thus, seedling height growth for the season is not necessarily an indication of conditions during bud development.

Temperatures in each environment (greenhouses, ambient, chilling and forcing chambers) were monitored with at least two temperature or temperature/relative humidity data loggers (Hobos from Onset Computer Corp.). Temperature was recorded at least hourly.

2.3. Analysis

Budburst is considered to result from a combination of both chilling and forcing temperatures. We developed functions which assigned effectiveness values for specific temperatures for satisfying chilling and forcing (see details below); effectiveness values were between 0 (no effectiveness) and 1 (maximum effectiveness). We calculated chilling units (CU) and forcing units (FU) by multiplying the number of hours in each 1 $^{\circ}\text{C}$ temperature bin by the effectiveness value for each temperature and then summing the totals. We evaluated several alternative functions for effectiveness and also tried various dates for starting and stopping the summation. We defined a “possibility line” as the line that

represents the combinations of chilling units and forcing units where budburst can occur. The numbers of CU and FU that had accumulated up to the time of budburst were calculated for each seedling and an initial possibility line was fit to the average values of CU and FU for each treatment using the exponential model proposed by Cannell and Smith (1983). Our estimate of the possibility line is probably conservative since it was fit to the treatment means and budburst occurred in some individual seedlings with fewer CU and FU than the treatment means. In addition, we did not take into account potential differences among families when fitting the possibility line. While we argue here that different genotypes of Douglas-fir and different species sense chilling and forcing in essentially the same way (the effectiveness functions are the same), the possibility line (i.e., the combination of CU and FU needed for budburst to occur) may shift among species and genotypes within species.

The chilling and forcing functions that were used to calculate the CU and FU for each hour were initially constructed based on results reported in the literature as mentioned above, but they were refined using an iterative process of testing chilling and forcing functions and fitting possibility lines. Different time windows were tested during which seedlings accumulate chilling and forcing units (cf. Raulier and Bernier, 2000). Sequential models were also tested where forcing did not begin until after a critical chilling threshold had been reached (cf. Hänninen, 1995). One option for the chilling window was based on the time period when cell division should be lowest. Based on the information on mitotic index at various times during the year (Owens and Molder, 1973; Carlson et al., 1980; Cannell et al., 1990), we set this time to be December 15 to March 1. Another option evaluated was to assume that chilling was only sensed when cells were dividing as occurs for vernalization (Wellensiek, 1964; Burn et al., 1993); we set this time period as November 1–December 15 plus March 1–April 15. The final chilling and forcing functions were selected by finding those that agreed with other results from the literature, produced a low residual mean-square error to the fit of the possibility line, and were biologically reasonable.

2.4. Historical data

Hourly air temperature data was obtained for the period from fall 1947 to spring 2008 for Olympia, WA and Salem, OR (National Climate Data Center, 2009). Salem was the closest station to Corvallis to have decades of hourly data. Where the historical records for Salem and Corvallis overlapped, hourly temperatures were highly correlated during the period when chilling and forcing were thought to occur (November–June) ($r = 0.97$); however, the mean temperature was 0.52 $^{\circ}\text{C}$ lower in Salem. The hourly data were used to calculate accumulated chilling and forcing hours

based on our functions; we predicted date of budburst when the accumulated temperature units for chilling and forcing would first hit our possibility line of chilling versus forcing units for each year at each location ($n = 60 \text{ years} \times 2 \text{ locations} = 120$). We used these results to evaluate the predicted range of budburst dates over the past 60 years. A separate dataset of observed budburst date at Fall River, WA was used to compare the predicted budburst dates (based on temperature recorded hourly at the site) with the observed budburst date for 2001, 2002, and 2004 (Bailey and Harrington, 2006). This dataset was not used in fitting the chilling and forcing functions or the possibility line; therefore it provided an independent (although limited) test of the possibility-line approach for predicting budburst.

3. Results

3.1. Chilling and forcing functions

After evaluating several combinations of chilling functions, we settled on the one described below and presented in Fig. 1C.

$$CU = 3.13 \left(\frac{T + 4.66}{10.93} \right)^{2.10} e^{-(T + 4.66/10.93)^{3.10}} \quad (1)$$

where T is temperature and e is the base of natural logarithm. CU is set to zero when $T < -4.7^\circ\text{C}$, or $T > 16^\circ\text{C}$, and set to 1.0 when Eq. (1) yields a value >1.0 .

We evaluated several alternative forcing functions (Fig. 3). Neither a threshold value (e.g., $FU = 1$ when $T > 10^\circ\text{C}$; Fig. 3A) nor a simple linear function of forcing was as effective in producing a well-defined possibility line as a sigmoid function. We used the following to calculate forcing units:

$$FU = \frac{1}{1 + e^{-0.47T + 6.49}} \quad (2)$$

Under Eq. (2), temperatures of 11, 14, and 16°C have relative FU values per hour of 0.25, 0.50, and 0.75, respectively. Offsetting the function so that greater forcing was calculated at lower or higher temperatures than with the selected function produced possibility lines with higher residual mean-square error (Fig. 3B and C, respectively).

Counting CU only during mid-winter when mitotic index should be close to zero (Fig. 4A) or counting CU only when cells should be dividing in the fall and early spring (Fig. 4C) resulted in fewer CU over the course of the experiments and possibility lines with higher residual mean-square errors. Extending the period of CU accumulation until budburst increased the totals for most treatments but also produced a possibility line with a higher residual mean-square error (Fig. 4B). Delaying the accumulation of FU until March 1 (when the optimum chilling requirement may have been met) reduced differences in forcing units among treatments, and resulted in a nearly horizontal possibility line (Fig. 4D). In our final model we started accumulating both chilling and forcing November 1, ceased the accumulation of CU on March 21, and continued to count FU until budburst occurred (Fig. 5).

3.2. Experiment 1

The treatments in Experiment 1 from both locations represented a wide range in chilling and forcing units (Fig. 5). All treatments from that experiment fit well with the proposed possibility line which indicates the amount of forcing needed for each level of chilling (i.e., it describes the range of conditions possible for budburst). The possibility line was fit within the range of our data and should not be extrapolated beyond those extremes. Douglas-fir has an obligate chilling requirement (Irgen-Moller,

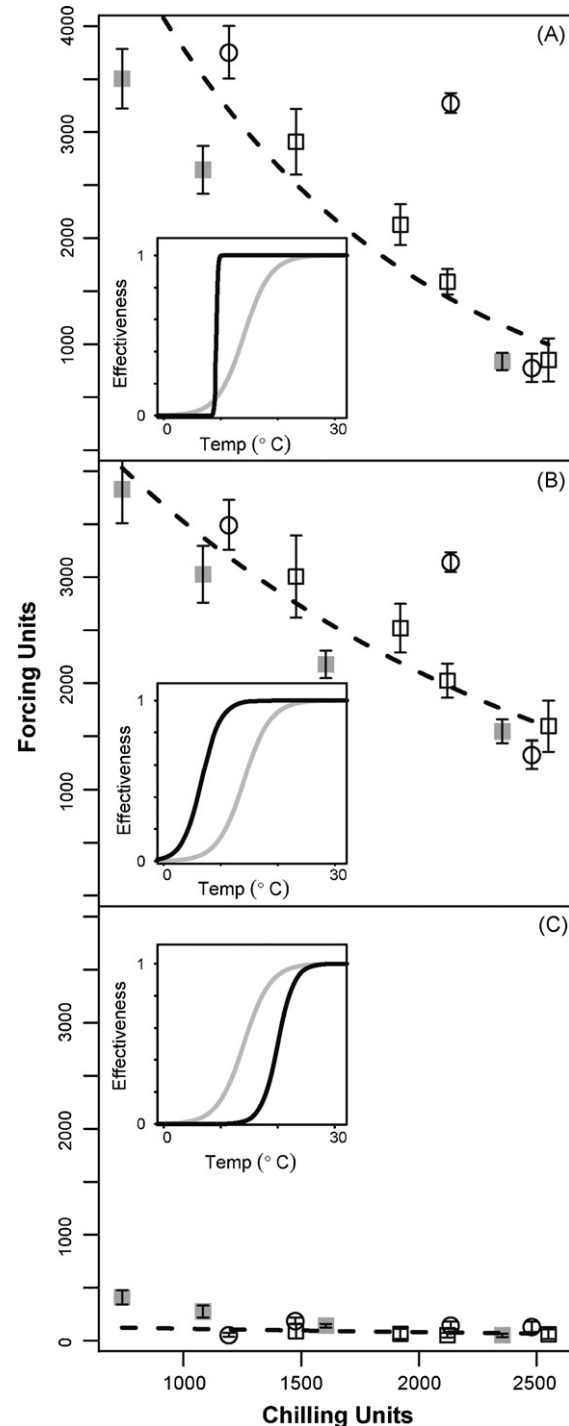


Fig. 3. Relationships between forcing units and chilling units as calculated for selected forcing functions. Shown are the means and standard deviations by treatment and the best fit line through the points. Shaded squares are from Experiment 1 in Corvallis, open squares are from Experiment 1 in Olympia, open circles are from Experiment 2 in Olympia. In each inset panel the grey line indicates the final function chosen (sigmoid function beginning at 0°C) and the black line indicates the alternative being considered. The alternative were: (A) threshold or step function with no effectiveness at temperatures $\leq 10^\circ\text{C}$ and maximum effectiveness at temperatures $\leq 10^\circ\text{C}$; (B) sigmoid function shifted for greater forcing at colder temperatures; (C) sigmoid function shifted for greater forcing at higher temperatures.

1958; Wommack, 1960), thus, the possibility line should not intercept the Y axis and instead should approach infinity at the critical minimum number of CU required for budburst (CU_{crit}). Similarly, the function should level off at the optimum CU value for

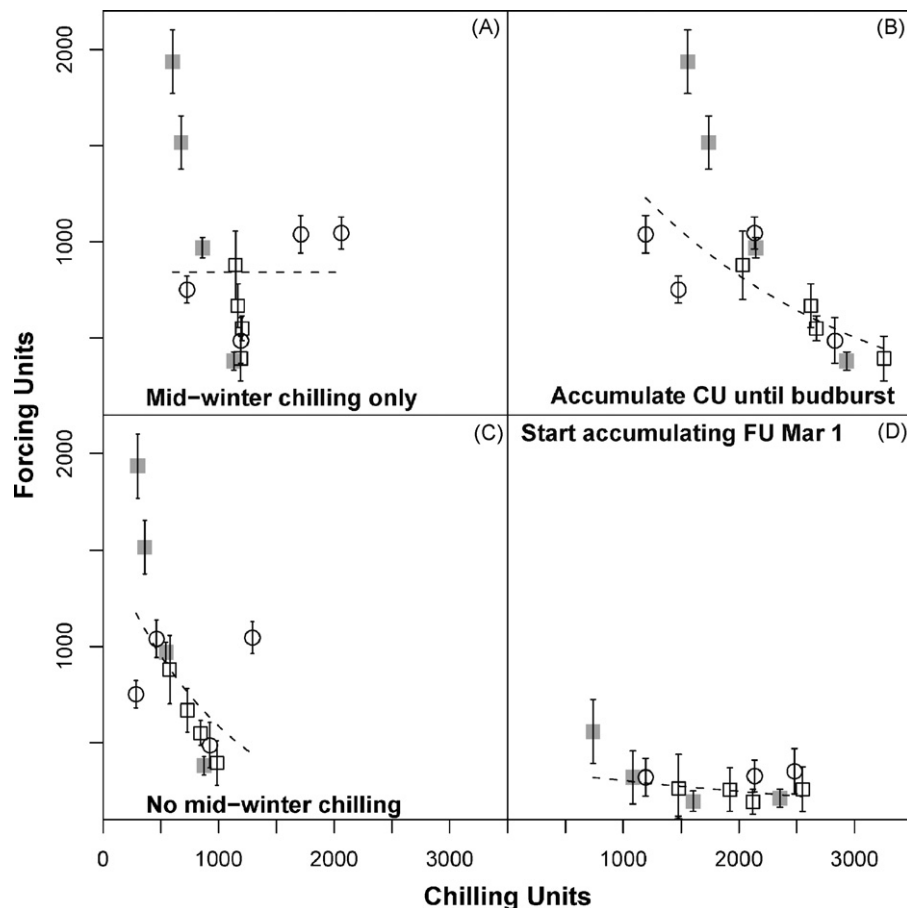


Fig. 4. The relationship between forcing units and chilling units as calculated with different start and stop dates for accumulating chilling and forcing. Shown are the means and standard deviations for 12 treatments of Douglas-fir and the best fit line. Symbols as defined in Fig. 3. The alternatives were: (A) chilling units accumulated mid-winter only (December 15–March 1), forcing accumulated from November 1 to budburst; (B) chilling and forcing units start accumulating November 1 and continue until budburst; (C) chilling units accumulated November 1–December 15 and March 1–April 15 (no accumulation of chilling units mid-winter), forcing accumulated from November 1 until budburst; and (D) chilling units accumulated from November 1 until budburst, forcing units accumulated starting March 1.

the genotypes being evaluated (CU_{Opt}). The values of CU_{Crit} and CU_{Opt} were not determined although it appears we were at or very close to the CU_{Opt} value for these genotypes.

Under ambient conditions, mean date of budburst was slightly later in Olympia (day 125.4) than in Corvallis (day 118.2). Both locations showed faster budbreak under treatment Ambient-400 (2.5 days faster in Corvallis and 11.4 days faster in Olympia) than under the Ambient treatment. At Corvallis, the warmest two treatments (Ambient-800 and Ambient-1200) resulted in much longer times to budburst (budburst at day 126.1 and 147.3, respectively) than in the cooler treatments. At Olympia, however, days to budburst did not change much in the warmest two treatments (Ambient-800 and Ambient-1200 had budburst at 126.0 and 118.2 days). At both locations the coldest treatment had the narrowest range in days to budburst and the warmest treatment had the greatest range in days to budburst.

The trees from Experiment 1 in Olympia had greater height growth in the two better chilled treatments (Ambient, Ambient-400) than in the treatments receiving less chilling (data not shown). This result was not evident in the trees from Experiment 2 in Olympia or those from Experiment 1 in Corvallis.

3.3. Experiment 2

The five treatments in this experiment represented a wide range in timing of chilling as well as in amount of chilling. Terminal buds in the Fall chilling treatment in Experiment 2

never completed budburst during the experiment; in fact by the following fall, 10% of the seedlings had still not burst the terminal bud and many of the seedlings which did burst bud had little or almost no elongation of the terminal shoot (Fig. 6). In contrast, the lateral buds burst promptly. Somewhat surprisingly, the None treatment also burst terminal and lateral buds normally; this indicated that our assumption that temperatures $\geq 10^\circ\text{C}$ would not provide chilling was incorrect (and provided additional evidence that a wide range in temperature was effective in satisfying the chilling requirement). The None, Ambient, and Winter treatments fit the possibility function well (Fig. 5). The Spring chilling treatment appeared to require more forcing than would be expected based on the number of chilling hours it had accumulated; however, the None and Spring treatments were in the same environment up until the Spring treatment went in the chilling chamber and the None treatment started budburst a few days after the Spring treatment was put into the chilling chamber. Thus, we believe that the Spring treatment would have burst bud sooner if it had not been put into a cold environment just prior to budburst. For that reason, it was excluded from fitting the final possibility line. The possibility line indicates when a combination of chilling and forcing conditions could result in budburst; however, the conditions present at the time the line is reached will determine the specific timing of when the plants will actually burst bud (i.e., the possibility line should theoretically be the boundary below all observations of budburst).

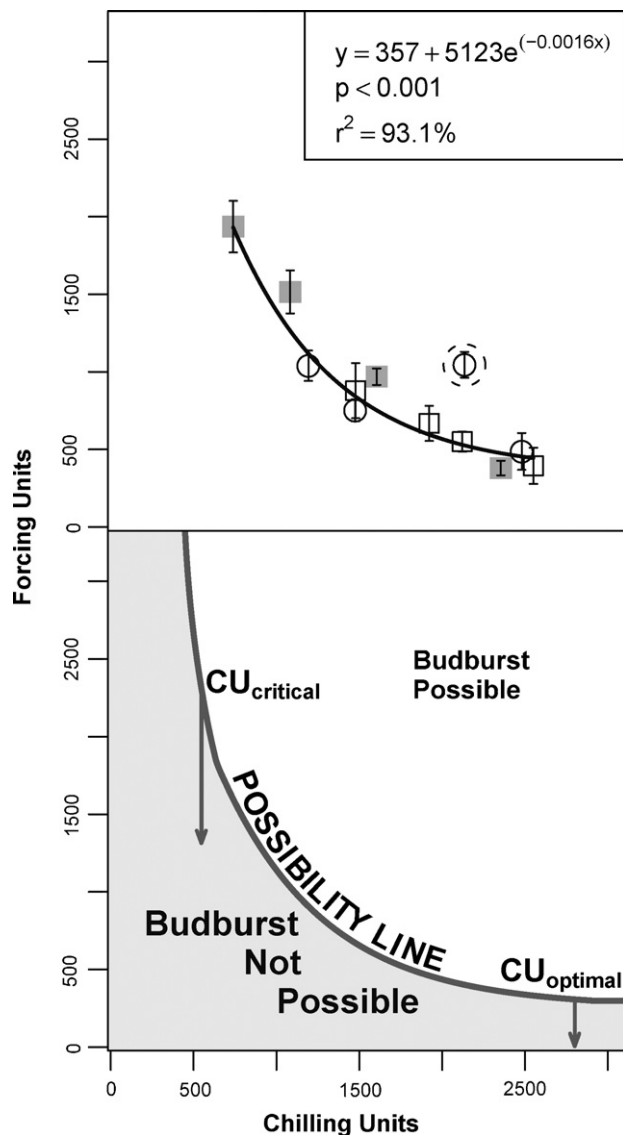


Fig. 5. The best relationship between chilling and forcing units for Douglas-fir seedlings in 12 treatments. Chilling units were accumulated from November 1 to March 21; forcing units were accumulated from November 1 until budburst. The points represent the treatment combinations that resulted in budburst (shown are the means and standard deviations). The equation for the line was fit without including the circled point (the Spring treatment in Experiment 2) for reasons described in the text.



Fig. 6. Stunted terminal shoot and normal lateral shoot resulting from the Fall treatment in Experiment 2. See text for details.

3.4. Historical data

The hourly data from two long-term weather stations near the study sites provided an indication of the historical range in mean temperature from November 1 through April 30 (Fig. 7). The relationship between mean winter temperature and predicted date of budburst was negative for the historical data, indicating that we would predict earlier dates of budburst as mean winter temperature increases. Considerable variability in the predicted date of budburst can be found, however, for any mean winter temperature. The variation in temperature patterns within winters with the same mean temperature can result in very different amounts of chilling and forcing units and thus lead to differences in predicted date of budburst.

The Ambient-400 treatments at both locations and the Ambient-800 treatment at Olympia are within the range of winter temperature variability experienced in recent decades while the more extreme treatments (A-800 at Corvallis and Ambient-1200 at both locations) are close to or outside the historical range of variability in mean winter temperature. Thus, data from our warmest treatments (and our model) would suggest the negative linear relationship between mean winter temperature and budburst evident on the left side of the graph will flatten (no change in date of budburst with temperature) with small increases in temperature from the historical values and then reverse as temperatures warm further. For Ambient-1200 at Olympia, the date of budburst was *earlier* than predicted with data for most past years but still within the range of date of budburst predicted from the historical data. However, the date of budburst for the Ambient-1200 treatment in Corvallis is 9 days *later* than the latest date predicted from the historical data (and this is in agreement with our model predictions). Thus, our model predicts that the date of budburst would occur later than has occurred historically if winter temperatures rise substantially beyond their range exhibited in recent decades.

The dates of budburst in 2001, 2002, and 2004 at Fall River, WA were predicted fairly accurately by determining when cumulative CU and FU reached the possibility line (Fig. 8). Predicted dates

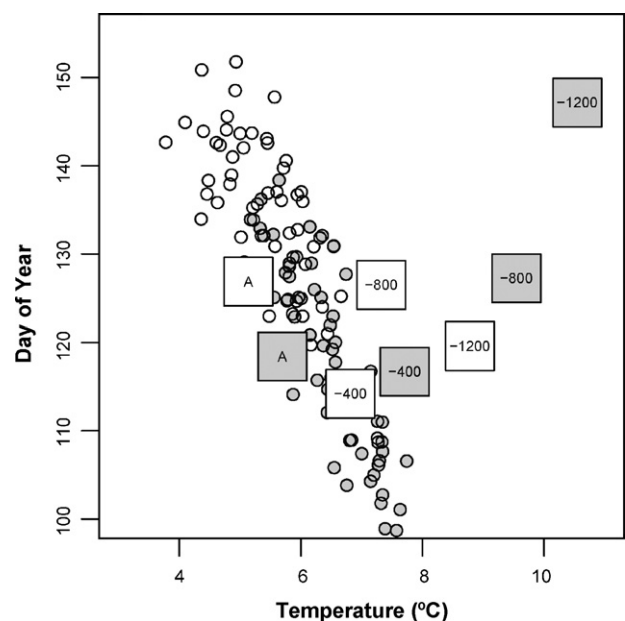


Fig. 7. The relationship between mean winter temperature in historical climate data from Olympia, WA (open circles) or Salem, OR (shaded circles) and predicted date of spring budburst. Also shown are the values for the 8 treatments in Experiment 1 (A is Ambient, open boxes are treatments from Olympia, shaded boxes are treatments from Corvallis, see text for description of the other treatments).

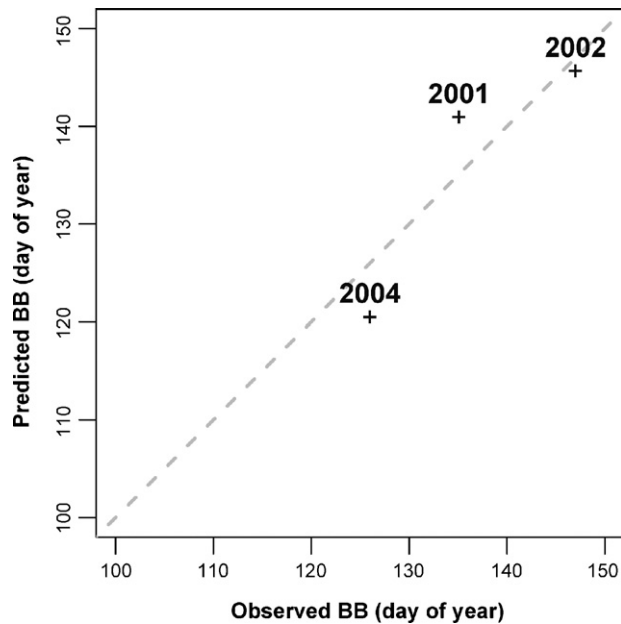


Fig. 8. Application of the possibility-line approach to prediction of date of budburst for 2001, 2002, and 2004 at Fall River, WA. The dotted line shows the 1:1 relationship between observed and predicted dates.

differed from observed dates by 2–5 days, but the average difference was <1 day. Although the sample was small, the range in budburst dates was fairly large (21 days) and the possibility-line approach was successful at predicting whether budburst would occur relatively early (2004) or late (2002).

4. Discussion

Many types of models have been suggested for predicting budburst from temperature records. These models have used a wide range of functions describing forcing and chilling (cf. Hänninen, 1995 for a summary of model types) and different windows when temperatures affect budburst. Some researchers have used specific (often arbitrary) dates for starting the accumulation of chilling or forcing (e.g., November 1, January 1), or suggested dates corresponding to the winter solstice, spring equinox or dates associated with predicted cessation or resumption of bud activity. Our evaluation which excluded chilling temperatures mid-winter, or only accumulated chilling mid-winter (as suggested by the dates when cell division is or is not occurring), indicated these shorter time periods were not as effective as using a wider time period. Many modelers working with historical data have found that it makes very little difference which date is used or which temperature thresholds are used (Hänninen, 1995; Raulier and Bernier, 2000). The fit for our possibility line was insensitive to small changes (~1 month) in the times forcing and chilling started and stopped but was quite sensitive to larger changes. Our fits were also sensitive (reduced mean-square error) to functional form and to varying the effectiveness of different temperatures in satisfying chilling or promoting budburst. The lack of sensitivity in other models (to temperature functions or starting and stopping dates) could be because some modelers did not have access to hourly data which would allow more sensitive analyses or because the temperature ranges within a particular year or treatment environment are often highly correlated (e.g., the number of hours at 2–4 °C may be highly correlated with the number of hours at 4–6 °C). We suggest that a more detailed examination of relationships between temperature and effectiveness of chilling and forcing will require data from

controlled treatments which create a wide range of temperature environments.

It is often assumed that forcing does not begin until after the chilling requirement has been met and this undoubtedly is what occurs naturally in boreal regions where extended periods of cold temperatures are never or rarely interrupted by warmer (forcing temperatures) until spring. We believe, however, that chilling and forcing can occur at the same time (in the temperature range where they overlap) and both systems are accumulating “time” over the dormant period. The systems involved in such accumulations (named the “memory of winter” by Amasino, 2004) are not understood. Phytochrome systems have been implicated in inducing dormancy (e.g., dormancy increases as day length decreases). Phytochrome-R and Phytochrome-FR both sense light over a range of wavelengths but their peak sensitivities differ (Buchanan et al., 2000, p. 948). Thus, it could be speculated that there are parallel sensing systems operating during dormancy and that chilling and forcing have different modes of control (e.g., different genes or transcription factors). At the molecular level we would want to find systems that can overlap temporally (perceive and integrate both chilling and forcing) and also interact to result in resumption of cell division in the buds. As a part of the dormancy or cold acclimation process, biochemical changes occur in the buds (cf. Rinne et al., 2001; Jordy, 2004); dormancy release is complete when those changes are reversed, cell-to-cell communication is re-established and cell division resumes. The increased range in days to budburst in our warmest treatments could imply that process was not complete in all buds, and thus, it took longer for cell division to resume in some plants.

Our proposed functions of chilling and forcing effectiveness suggest that a wide range in temperature can be effective and that the range of temperatures where chilling effectiveness is optimum may be fairly broad. The early step function proposed by Richardson et al. (1974) also suggested that the optimum temperature (for two varieties of peach tree) was quite broad. In contrast to those who have suggested different ranges of temperature effectiveness for different species, however, we suggest that general curves of effectiveness versus temperature for chilling and forcing may be adequate for multiple species. This assumption can be tested in new dormancy release studies or by reanalysis of existing data. Future work should elucidate the basic biochemical mechanisms of dormancy induction and release. At that time, experiments can be designed to evaluate the temperature rate functions for the biological subsystems specifically involved in sensing and translating cues involved in chilling and forcing. Determination of the temperatures which have maximum effectiveness in satisfying chilling requirements have been developed for various species and have worked well in predicting practical aspects such as the equivalency of natural or artificial chilling, chilling needed for storage, or the number of chilling units to budburst (cf. Richardson et al., 1974; Anderson et al., 1986; van den Driessche, 1975; Garber, 1983; Carlson, 1985). Some of those operational functions have ignored the effectiveness of temperatures higher or lower than the optimum value or have assumed that a very broad range of temperatures are equivalent in their effectiveness. We suspect that past models which assume only a narrow temperature range is effective or that all temperatures within a wide range have equal effectiveness are not likely to be very robust in predicting the effects of temperature regimes outside those used in developing the original relationships. For example, if we assume only temperatures ≥ 0 °C and ≤ 5 °C were effective in satisfying the chilling requirement for Douglas-fir, we would predict for many sites that any winter warming would result in later budburst. Based on our experimental data and our model of budburst, however, we believe that budburst can occur from any combination of chilling and forcing units which meet or

exceed the possibility line and many of these combinations will result in dates of budburst within the range of historic variability.

Our model does not provide any particular weight to alternating day/night temperatures or to cold temperatures followed by warm temperatures or vice versa. Our proposed relationships of temperature to chilling or forcing may be helpful in interpreting why some researchers have suggested alternating temperatures can have positive, negative or no effect on days to budburst (van den Driessche, 1975; Campbell and Sugano, 1975; Erez et al., 1979; Myking, 1997; Partanen et al., 1998). Under our proposed relationships, an alternating day/night temperature regime could have many different effects (in comparison to a constant temperature regime) depending on the relative values of forcing and chilling associated with the alternating temperatures. It has been suggested that warm temperatures following cold ones negate chilling effects or that cold temperatures followed by warm temperatures negate forcing effects (Bailey and Harrington, 2006). Major temperature changes can undoubtedly influence the timing of budburst but we suggest small changes in daily or weekly temperature do not eliminate the effects of previous environments. If temperature fluctuations negate previous summations, it is hard to understand how our model of chilling versus forcing would fit well across diverse treatments as since many of our treatments included ambient conditions with alternating temperatures over long periods of time. High temperatures have been known, however, to induce secondary dormancy in seeds (Bewley and Black, 1994, p. 230; McLemore and Barnett, 1966) and in buds (Vegis, 1964). Negation of chilling at temperatures $\geq 21^\circ\text{C}$ has been reported for peach (Erez et al., 1979) and pear (Sugiura et al., 2002) flowers. Our study trees did not experience enough hours above 20°C to test if similar temperatures would cancel previous chilling for Douglas-fir. Cold temperatures can also delay seed germination and this factor accounts for the rapid germination of seed after cold stratification. That is, low temperatures in stratification inhibit germination of non-dormant seed until temperatures are favorable; by that time additional seeds have become non-dormant (Bonner, 2008, p. 27). Extended cold temperatures could account for the delayed budburst in the Spring treatment in Experiment 2; it probably would have started budburst much sooner if it had not been placed in the chilling chamber. That does not mean that chilling negated the previously experienced warm temperatures but only that the plants were not in an environment which was conducive to budburst when they crossed the possibility line. Heide (2003) tested fall temperatures (during dormancy induction) of 9, 15 and 21°C on alder and birch saplings and reported that the two warmer temperatures delayed subsequent budburst. This effect was attributed in part to the negation of chilling at warm temperatures; it is not known when the trees became dormant. If they were dormant for part of the fall treatment period, our model would predict the same effect would occur by “counting” additional chilling occurring at 9°C and might suggest some effectiveness at 15°C . Future studies are needed to determine the relative effectiveness of a wide range of temperatures. Such experiments need to be carefully designed and monitored to ensure that observed temperature effects are directly related to the physiological mechanisms associated with dormancy induction and release and not on other factors, for example, tissue damage, increased respiration (reducing vigor) or hardening of bud scales. Our model does not account for all observed phenomena in the plethora of published studies on dormancy but it does help explain some of the effects that were previously perceived as inconsistencies.

Our study used five open-pollinated families selected to differ little in days to budburst (based on previous results from the common-garden experiment by St. Clair et al., 2005). We suspect that the relationship of relative effectiveness by temperature is not

likely to vary by species or genotype; however, the possibility line which describes the combinations of chilling and forcing units which will result in budburst may shift for individual genotypes or species. The implied intercept on the chilling unit axis is related to the minimum or critical chilling requirement for the plant material under consideration. Thus, for all plants with an obligate chilling requirement, there is a minimum number of CU (CU_{crit}) that are required for a particular population before budburst will occur even with very high FU. In addition, the right-hand asymptote of the curve suggests that after an optimum chilling requirement is met additional chilling will not accelerate budburst (CU_{opt}). Several researchers have previously documented population or varietal differences in the optimal chilling level for several species (cf. Douglas-fir: Wommack, 1964; van den Driessche, 1975; Campbell and Sugano, 1979; loblolly pine: Carlson, 1985; birch: Myking and Heide, 1995; fruit trees: Weinberger, 1950). We suspect the possibility line will shift for genotypes from widely different environmental conditions.

The basic shape of the curves of temperature versus dormancy release (chilling effectiveness) or promotion of budburst (forcing effectiveness) seems reasonable based on previous reports in the literature and our data. There is, however, very little information to determine the correct shape of the curves at the extremes. For some climates, the shape of the curve at the extremes could seem unimportant as under current climate regimes there are few hours in those temperature ranges. However, if we are to rigorously evaluate the curve shapes and test if the curve shapes are the same for multiple species, it will be important to have data from the extremes as well as the middle of the curves. Many researchers have attempted to predict the effects of future climate regimes using correlations from past years. We believe modeling the effects of different climate scenarios will be most robust if the curve shapes are accurate because the distribution of temperatures in future climates could differ from those observed in the past.

Based on our data as well as previous reports (cf. Wommack, 1964; Sugano, 1971; van den Driessche, 1975), it is clear that Douglas-fir can burst bud under a wide range of chilling and forcing conditions. Although it has long been known that chilling is ineffective if the bud has not entered true dormancy, we were surprised that the fall treatment (beginning October 15) was so ineffective in promoting budburst as operational models use October 15 as the starting time to begin accumulating chilling. Sugano (1971) also reported October chilling (beginning October 22) to be less effective than later chilling beginning December 5 but shoots were normal after his October chilling and only slightly shorter in length than those which developed after December chilling. We suspect that a November 1 start date for accumulating chilling for Douglas-fir would be more dependable than October 15 in the lowlands of western WA and OR but other dates may be more appropriate for other species or other environments. Seeley (1996) suggested that the date at which chilling would start accumulating depended on past temperatures and so would vary from year to year (p. 366, the plot of chilling unit accumulation starts to become positive at different dates; his model assigned negative values to temperatures above 16°C). Undoubtedly photoperiod is a strong trigger in inducing budset (and presumably also in inducing dormancy) and is likely to interact with both temperature and water or nutrient stress.

The lack of budburst or the poor shoot elongation in the Fall treatment in Experiment 2 indicates that those buds were not physiologically ready to perceive chilling. Romberger (1963, pp. 161–162) commented that when dwarf shoots arise in this type of situation it may indicate that cell division is blocked at the cellular level (as no difference in pith cell length was recorded for dwarfed and non-dwarfed plants in Holmsen, 1960 as cited in Romberger, 1963). Our seedlings were in their first season after germination

and such seedlings can have delayed budset and development of dormancy. It would be valuable if a simple rapid test (e.g., a biochemical analysis or anatomical stage) could be developed to indicate if buds were ready to perceive chilling. The normal elongation of shoots developing from upper lateral buds in the Fall treatment could indicate a lack of apical dominance, a lower chilling requirement of lateral buds, a later stage of bud development, or a change in the transport of auxin or sensitivity of the lateral buds to auxin. It has been previously noted that apical dominance which is present during the year lateral buds are formed is erased over winter; thus, most buds which were previously inhibited will grow out the following spring (Zimmerman and Brown, 1971).

It will not be possible to predict with certainty the effects of different temperature regimes on budburst until the mechanisms inducing and releasing dormancy are clearly understood. The recent and current work on changes in cell cycle (Horvath et al., 2003), gene expression and molecular markers (Jermstad et al., 2001; Schrader et al., 2004), hormone levels (Druart et al., 2007; Ruonala et al., 2006; Ormenese et al., 2006), plant metabolites (Espinosa-Ruiz et al., 2004), and factors blocking cell to cell communication (Rinnie et al., 1999) in dormant plants suggest we are getting closer to understanding some of the mechanisms by which plants transition into and out of dormancy. It will be very helpful to modelers when the physiological studies are complete enough to allow them to be used for prediction models for budburst.

5. Implications related to climate change

Our functions predicting the accumulation of chilling and forcing units worked well in defining the lower boundary line of minimum conditions required for budburst in our experimental data. Use of historical weather data resulted in predicted dates of budburst within previously reported ranges for Douglas-fir and budburst from an independent site was also well predicted with our functions. Thus, we believe our chilling and forcing functions could be used to predict time of budburst for Douglas-fir under various climate scenarios (the location of the possibility line may need to be shifted for specific genotypes). The effectiveness functions for temperature we developed for chilling and forcing may work well for multiple species but they need to be tested with species-specific data. Additional information may be needed to determine the appropriate starting and stopping times for accumulating chilling and forcing for each species or for specific conditions. For both Douglas-fir and other species, additional work is needed to determine the exact shape of the effectiveness functions across the full range of possible temperatures as well as the minimum and optimum values for chilling for genotypes of interest.

Our historical climate data predicted earlier mean date of budburst as winter temperature increased (i.e., there was a linear negative trend). This is in agreement with direct observations of phenology (cf. Linkosalo et al., 2008). Future temperature in temperate and boreal regions is predicted to rise under various assumptions of emissions (IPCC, 2007). Our experimental data suggests that if winter temperatures rise much beyond the historical range in variability, the relationship will first flatten and then reverse. Thus, substantial winter warming would cause later budburst. Based on our observations (data not shown), we would also predict greater range around the mean value for days to budburst as winter temperature increases. This could imply greater range in within stand growth or damage as trees with early budburst could sustain frost damage and trees with late budburst would have a shorter growing season.

Acknowledgements

The work was supported in part with a grant from the USDA Forest Service, Western Regional Agenda 2020 Program. We thank our coworkers for patience with the budburst assessments and our reviewers for their comments which were very helpful in revising the paper.

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