Forest Ecology and Management 409 (2018) 729–739

Contents lists available at ScienceDirect

Forest Ecology and Management

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

The timing of flowering in Douglas-fir is determined by cool-season temperatures and genetic variation

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ARTICLE INFO

Keywords:
Climate change
Pseudotsuga menziesii
Reproductive budburst
Seed orchards
Tree phenology

ABSTRACT

Trees have evolved to time flowering to maximize outcrossing, minimize exposure to damaging frosts, and synchronize development with soil moisture and nutrient availability. Understanding the environmental cues that influence the timing of reproductive budburst will be important for predicting how flowering phenology of trees will change with a changing climate, and aid in the time-sensitive management of seed orchards. We examined how temperature influenced the timing of female flowering of coastal Douglas-fir with over 4500 flowering observations of trees from 12 sites across western Oregon and Washington. We predicted flowering dates by modifying chilling and forcing effectiveness functions from a model of vegetative budburst of Douglas-fir. We also examined whether genetic variation in Douglas-fir influenced the relationships between chilling and forcing accumulations using flowering observations from two common-garden experiments with trees from 60 populations from a diverse range of climates. Our reproductive budburst model predicted the day of flowering within an average of five days of the observed flowering dates across all sites and years. Fewer hours of forcing temperatures were required for flowering on sites that had experienced high chilling. Warmer temperatures in the future will likely result in earlier flowering on sites which currently have high chilling; however, sites which currently experience low chilling may display no change or possibly even a delay in flowering. Douglas-fir genotypes from different geographic regions flowered in the same order from year to year in common gardens, indicating that both temperature and genetic variation influence flowering. Genetic variation in flowering dates was more strongly related to summer drought of seed source locations than to cold winters. Knowledge of the environmental cues and genetic variation in timing of flowering can help predict how future changes in temperature under various climate models could change flowering time across the range of coastal Douglas-fir.

1. Introduction

Phenology, the cyclic timing of biological events, is one of the primary ways that trees are adapted to their environment, and one of the most visible indicators of recent climate change (Parmesan and Yohe, 2003; Menzel et al., 2006). Phenology also strongly controls where trees can survive (Chuine, 2010; Hänninen and Tanino, 2011); thus, understanding the temperature cues that drive phenological changes will be important for predicting how both phenology and species ranges will change with a changing climate (Chuine et al., 2016; Carter et al., 2017). Trees that flower too early risk damage to vulnerable new tissues by frosts, and those that flower too late miss peak soil water and nutrients in spring before summer drought, or may not time flowering to be coincident with other trees, thus altering outcrossing rates and gene flow.

Previous research has found that the timing of vegetative budburst and initiation of height and diameter growth depend on the accumulation of both chilling and forcing temperatures over late fall, winter, and early spring - the dormant period for temperate tree species (Wommack, 1964; Harrington et al., 2010; Ford et al., 2016) and observations indicate that temperature cues influence the timing of flowering in many plant species (Cook et al., 2012). The vegetative and reproductive buds of most temperate and boreal tree species enter a dormant stage in late fall. In spring, budburst occurs after trees have experienced an accumulation of cold (chilling), and warm (forcing) temperatures (Cooke et al., 2012; Hänninen and Tanino, 2011). Longer periods of chilling should result in fewer forcing hours needed to induce reproductive budburst, whereas experience of fewer chilling hours should result in the requirement of more forcing hours prior to growth initiation (Murray et al., 1989). While the biochemical mechanisms directing bud dormancy and growth are only partially understood (Rinne et al., 2001; Cooke et al., 2012), multiple empirical studies have

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https://doi.org/10.1016/j.foreco.2017.11.062
Received 1 September 2017; Received in revised form 27 November 2017; Accepted 30 November 2017
0378-1127/ Published by Elsevier B.V.

shown that both chilling and forcing temperatures are important determinants of early season phenological events in temperate and boreal tree species (Harrington et al., 2010; Clark et al., 2014; Harrington and Gould, 2015).

A substantial body of research has examined the drivers of vegetative phenology in tree species (e.g. Hänninen, 1995; Hannerz, 1999; De Melo-Abreu et al., 2004; Nava et al., 2009); and some research has focused on the drivers of flowering phenology in temperate trees (e.g. Anderson et al., 1986; Chuine et al., 1998, 1999). Frequently, the same phenological models are used to predict both vegetative and flowering phenology of tree species (e.g. Linkosalo et al., 2008), but few studies have examined if the specific drivers may differ between the two phenological events in trees. Many phenology models simply use temperature sums above some threshold to calculate chilling and heating sums, although the exact biological basis for these distinctions is lacking, and the predictive capabilities of such models is often poor (Chuine et al., 1998).

To date, no predictive models of reproductive phenology have been developed for Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco), a commercially and ecologically important tree species. This is partially because flowering does not commonly occur in juvenile trees, so temperature-controlled growth chamber experiments are not feasible, unless grafted materials are used. Additionally, complete records of hourly temperatures are rarely available for the same locations where the flowering dates of conifers are being recorded. To address this gap in knowledge, we have assembled a unique database of flowering dates of Douglas-fir from 12 locations spanning over three decades, and matched the data with hourly temperature data from weather stations at or close to each location. We use this detailed dataset to fit a predictive model of timing of reproductive budburst for coastal Douglas-fir in the Pacific Northwest of the USA. **We hypothesized that both chilling and forcing temperatures would be important determinants of the timing of flowering**. Longer periods of chilling should result in less forcing needed to induce budburst, whereas shorter periods of chilling should require more forcing. We tested if the timing of flowering phenology could be predicted using phenology models similar to those developed for vegetative budburst and diameter growth initiation (e.g. Harrington et al., 2010; Ford et al., 2016), and, if so, could such models accurately predict the day of year of flowering of Douglas-fir across its range in the Pacific Northwest. In addition to predicting how the date of flowering could be impacted by future changes in climate, such models could also be used by seed orchard managers to help plan time-sensitive management decisions, such as timing of insecticide sprays, collecting pollen for long-term storage, or adding pollen to controlled-crosses.

Phenology can vary greatly among populations within a tree species as a result of local adaptation to different environmental conditions (Alberto et al., 2013; Campbell, 1979; Howe et al., 2003). Phenology of trees varies within species across climatic or geographical gradients (Gould et al., 2011; Vitasse et al., 2013). Douglas-fir has high genetic variability among individuals and populations, and past studies have found strong clines in traits across environmental gradients, so genetic differences in the timing of flowering may be particularly evident among populations of this species (Campbell, 1979; Rehfeldt, 1989; St. Clair et al., 2005; Gould et al., 2011; Ford et al., 2017). Previous research has found that chilling and forcing requirements for the timing of vegetative budburst differed among populations of Douglas-fir (Gould et al., 2011). Thus, we expect that the temperature cues for reproductive budburst will differ across populations as well. We recorded flowering dates of Douglas-fir from 60 populations in two common-garden trials, and considered the relationship between population variation in date of flowering and the climates of the seed sources. A relationship between population variation and the climates at the locations where the parent trees evolved suggests adaptation to local climates, particularly when the relationships make biological sense as suggested in the hypotheses below. Common-garden studies and provenance trials provide an excellent way to observe how differences in plant traits may differ among genotypes experiencing the same climatic conditions (Mátyás, 1996; Bansal et al., 2016). In this case, we utilized data from common gardens to test how forcing requirements may differ among trees experiencing the same chilling temperatures over the dormant season. We tested three alternative hypotheses: **Frost avoidance hypothesis**: Trees from colder environments grown in common-garden studies would have higher forcing requirements because they have evolved to have a conservative strategy that ensures they flower well after frost risk. **Growing season limitation hypothesis:** Trees from colder source populations would have lower forcing requirements than those from warmer locations. Trees adapted to long, cold winters and shorter periods of time between freezing temperatures should have evolved to expand buds quickly in spring after temperatures become favorable, to take advantage of the relatively short growing season. **Drought avoidance hypothesis**: Trees from environments that experience hot, dry summers should have lower forcing requirements, or increased frost tolerance, because they have evolved to time reproduction as soon as conditions are favorable, and before summer drought limits resources. Quantifying the influence of adaptive genetic variation in common-garden studies allows us to measure the relative influence of both temperature and a tree's seed source location on the timing of flowering in spring.

2. Methods

2.1. Modelling the timing of female reproductive budburst of Douglas-fir

We assembled a database of flowering of Douglas-fir from ten seed orchards and two common-garden studies across the Pacific Northwest (Fig. 1, Table 1). Here, we use the term 'flowering' to refer to the first observed date when the first female strobili per tree became receptive. Flowering dates spanned the time-period from 1980 to 2016, with between one to nine years of data collected at each site (Fig. 1, Table 1). Observers at seed orchards gathered detailed data on the timing of female strobili receptivity in order to correctly time the application of pollen to make controlled crosses for tree breeding. Seed orchards for economically important tree species, such as Douglas-fir, have been established to provide reliable yields of genetically-improved seeds for forestry and restoration efforts (Fashler and El-Kassaby, 1987). It is possible that exact dates of flowering were biased by different observers at different orchards and over different years, however, all flowering observations were collected by experienced personnel. Additionally, the large quantity of data from many sites and years should lead to robust model parameterization. To parameterize the reproductive phenology model, we only included flowering dates of relatively local populations from the common gardens (seed-source populations had to be within ~150 km of the common garden where the trees were grown) since populations for the common gardens were selected from widely different climatic regions. Additionally, we also only included flowering observations from common gardens that flowered in every year to parameterize the reproductive model. However, all data from all genotypes were used in analyses of genetic variation (below). For all locations, we compared the dates of flowering of female strobili to hourly temperature data collected on-site or from nearby weather stations to develop models of reproductive phenology. If on-site weather data were not available, we obtained weather station data from stations within 70 km of sites, at similar elevations, that had the most complete hourly temperature records (Table 1). For short periods of missing temperature values (4 or less hours), we replaced missing values with the value directly prior to the missing one. For longer periods (5–10 h) we used linear interpolation between the hourly temperature values directly before and after the missing values to close the gaps. No weather station records had > 0.4% data missing over the periods of phenological observations.

We used equations from a previous model of vegetative budburst

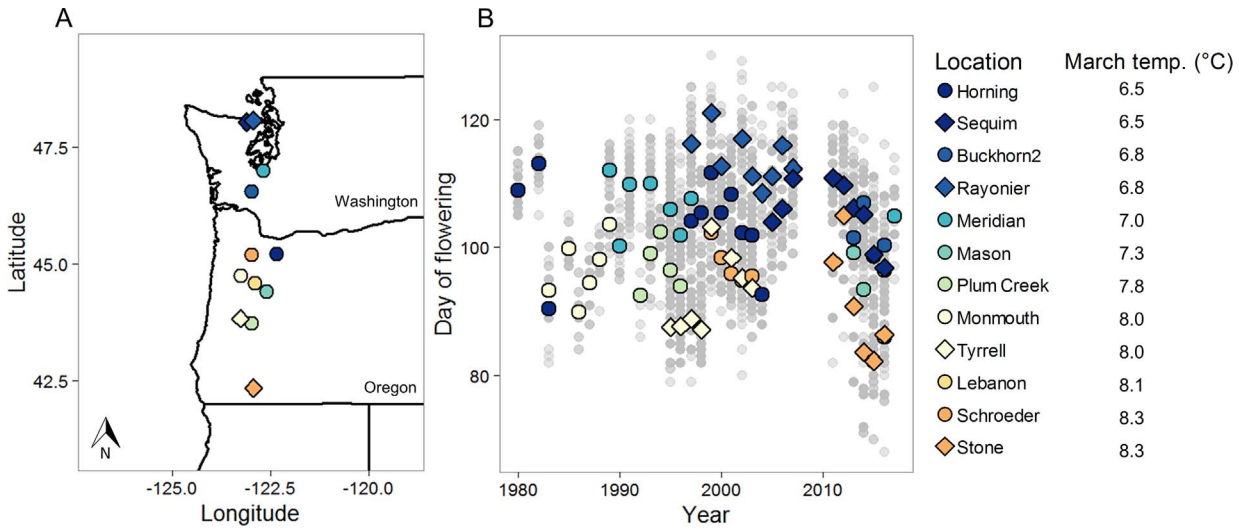


Fig. 1. (A) Locations of the 12 sites across Oregon and Washington that contributed reproductive phenology data for Douglas-fir, colored by mean March temperature (°C). (B) Colored circles denote the average flowering dates of Douglas-fir per site, per year (n = 77 mean site by year observations), and grey circles show the flowering dates for each tree (n = 4518 individual observations). Different shapes distinguish between sites with the same mean March temperature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

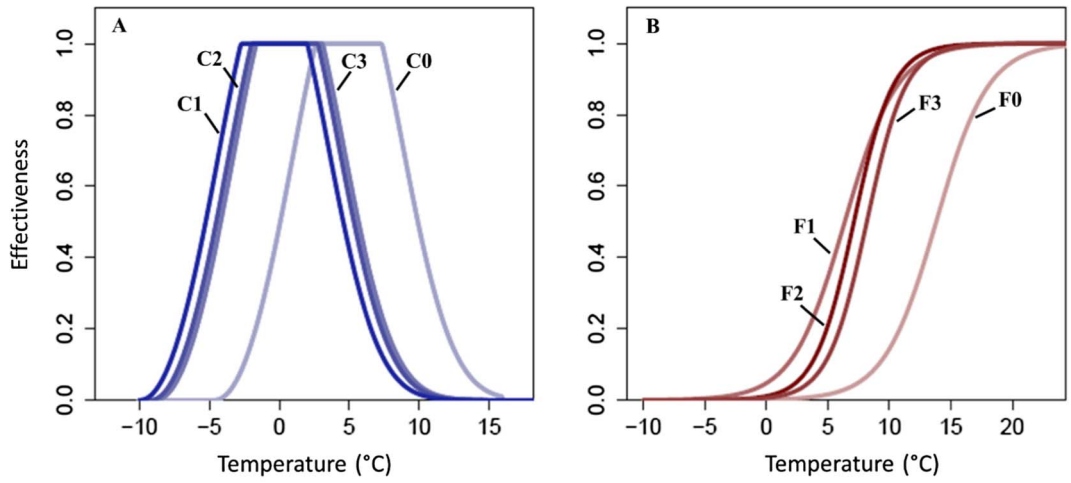
presented in Harrington et al. (2010) as a starting point for developing the chilling and forcing functions for reproductive budburst. Based on the previous literature and growth chamber and greenhouse experiments, Harrington et al. (2010) developed functions that gave effectiveness units to hourly temperatures over fall, winter and spring that vary from 0 to 1 for satisfying chilling and forcing requirements before vegetative budburst (Fig. 2). Then, both chilling and forcing units for hourly temperatures were summed from Nov. 1 through the day of vegetative budburst and a possibility line was fit to the data, with the possibility line indicating all combinations of chilling and forcing unit accumulation that could result in budburst.

Since flowering occurs earlier than vegetative budburst in Douglas-fir, and may be responding to different environmental cues, we decided to test a wider range of chilling and forcing effectiveness values for hourly temperatures than those used in the vegetative model. We iteratively tested different combinations of chilling and forcing effectiveness functions (adjusting peak effectiveness of both functions by ± 0.5 °C per iteration) using the entire reproductive phenology database. We also altered the shapes of both functions, and tested the fit of the model using different dates for the start of chilling and forcing unit accumulation (between Sept. 1 – and Dec 31st) and stop (Feb. 15th – date of budburst). We then selected the three candidate models from the iterations that resulted in the best fits (highest R^2 values and lowest residual mean-square errors) for the relationship between chilling and forcing accumulations, i.e. the possibility lines, for reproductive budburst. The shape of the functions for the original model and the three candidate models are shown in Fig. 2.

To select the final model, we fit possibility lines to each candidate model using half of the reproductive phenology dataset (training data) to calculate coefficients for possibility lines for each model. We divided the data into training and test datasets by assigning flowering observations from every other year per site to one dataset or the other. For each set of candidate equations, we fit both a natural log and a linear possibility line. We then tested those model predictions for chilling and forcing accumulations by the date of flowering against the observed chilling and forcing received by the flowering dates from the other half of the database (test data), so that we were testing the validity of the model predictions against data that was not also used to fit the models. We chose the model that resulted in the lowest residual mean-square error between observed and predicted forcing accumulations and observed and predicted day of flowering as the final model. To test our hypothesis that both chilling and forcing temperatures would be important for correctly predicting the date of reproductive budburst, we compared the fit statistics of our final model to models using only forcing accumulations through reproductive budburst. The forcing-only models utilized the same forcing equation as the best-fit model and started accumulating forcing units either on November 1st or January 1st. Finally, we compared predictions from our best-fit model to a

Table 1
Location information for the sites that contributed Douglas-fir flowering data and the weather stations for which we received temperature data. All temperature data was downloaded from the NOAA CDO website (www.ncdc.noaa.gov/cdo-web), except for on-site weather stations. Under 'Location', SO indicates a seed orchard, and CG indicates a common-garden experiment.

Location	Ownership	Latitude	Longitude	Years of data	State	Weather station	Station ID (WBAN)	Dist. from site (km)
Buckhorn2 CG	Pt. Blakely	46.55	−122.99	6	WA	On-site weather station	N/A	0
Horning SO	BLM	45.22	−122.34	6	OR	Portland Intl. Airport	24229	47
Lebanon SO	Roseburg Forest Products	44.60	−122.90	1	OR	Corvallis	24020	32
Mason SO	Cascade Timber	44.42	−122.61	4	OR	Eugene Mahlon Sweet Airport	24221	59
Meridian SO	WA Dept. of Natural Res.	46.99	−122.69	8	WA	Olympia Airport	24227	16
Monmouth SO	US FS	44.75	−123.26	7	OR	Salem McNary Field	24232	27
Plum Creek SO	Plum Creek Timberlands	43.74	−122.99	5	OR	Eugene Mahlon Sweet Airport	24221	47
Rayonier SO	Rayonier	48.07	−122.95	9	WA	Whidbey Island NAS	24255	38
Schroeder SO	Oregon Dept. of Forestry	45.20	−122.99	6	OR	Salem McNary Field	24232	11
Sequim SO	Weyerhaeuser	48.03	−123.11	9	WA	Port Angeles Intl. Airport	94266	31
Stone Nursery CG	USFS	42.34	−122.94	6	OR	On-site weather station	N/A	0
Tyrrell SO	BLM	43.83	−123.27	8	OR	Roseburg	24231	66



(C0) $CU = 3.13\left(\frac{T+4.66}{10.93}\right)^{2.10} e^{-\left(\frac{T+4.66}{10.93}\right)^{3.10}}$

(C1) $CU = 3.13\left(\frac{T+10}{10.93}\right)^{2.10} e^{-\left(\frac{T+10}{10.93}\right)^{3.10}}$

(C2) $CU = 3.13\left(\frac{T+9.24}{10.93}\right)^{2.10} e^{-\left(\frac{T+9.24}{10.93}\right)^{3.10}}$

(C3) $CU = 3.13\left(\frac{T+8.91}{10.93}\right)^{2.10} e^{-\left(\frac{T+8.91}{10.93}\right)^{3.10}}$

(F0) $FU = \frac{1}{1+e^{-0.47T+6.49}}$

(F1) $FU = \frac{1}{1+e^{-0.47T+3}}$

(F2) $FU = \frac{1}{1+e^{-0.63T+4.49}}$

(F3) $FU = \frac{1}{1+e^{-0.63T+5.15}}$

Fig. 2. Shapes and equations of the candidate (A) chilling and (B) forcing functions for the reproductive budburst model. For all equations: *CU* represents chilling units and *FU* represents forcing units, *T* is hourly temperature, and *e* is the base of the natural logarithm. 'C0' and 'F0' are the shapes and equations for chilling and forcing effectiveness functions parameterized for vegetative budburst from Harrington et al. (2010), where *CU* is set to zero when $T < -4.7$ °C, or $T > 16$ °C. 'C1-C3' and 'F1-F3' represent the three candidate chilling and forcing curves used for the candidate reproductive budburst models in Table 3.

prediction based on the average day of flowering of the training data across all sites and years.

Once the final model was identified, we fit the possibility line for reproductive budburst of Douglas-fir using a hierarchical linear model that included all observations in the dataset to predict forcing accumulations by flowering based on the fixed effect of chilling accumulation by flowering, with random intercepts for each site and individual trees within each site (St. Clair et al., 2005). We used a hierarchical model to account for variation in flowering dates resulting from effects of site location, and repeated measures on individual trees. Model parameters were calculated using the lmerTest package in the statistical program R (Kuznetsova et al., 2017).

While most research on temperate tree phenology indicates that temperatures over the dormant season are the determining factors of the timing of budburst (Chuine et al., 1999; Hänninen and Kramer, 2007; Laube et al., 2014), it is possible that other environmental factors, such as precipitation, solar radiation, photoperiod, and nutrient availability, influence phenology as well, although probably to a lesser degree (Cannell and Smith, 1986; Körner and Basler, 2010; Basler and Körner, 2014; Fu et al., 2014). However, it is beyond the scope of this model to incorporate all possible influences on phenological development. Temperature has been shown to be the primary driver of vegetative budburst of Douglas-fir (Ford et al., 2016, *supplementary material*), and we hypothesize that temperature should be important for reproductive budburst as well. Here, we focus only on the effectiveness of chilling and forcing temperatures to determine dates of reproductive budburst. Temperature cues have been shown to strongly influence phenology (Cannell and Smith, 1986; Murray et al., 1989; Hänninen and Kramer, 2007) and temperature data are readily available for many locations for use in simple predictive models.

2.2. Influence of genetic variation on forcing requirements for reproductive budburst of Douglas-fir

To examine how genetic variation affects the timing of reproductive budburst of Douglas-fir, we analyzed how climatic variables from seed-source environments related to the timing of flowering using flowering observations from two common-garden studies that were part of a reciprocal transplant experiment called the Douglas-fir Seed-Source Movement Trial (Bansal et al., 2015; Gould et al., 2012).

Seeds for this experiment were collected from 120 open-pollinated parent trees from 60 populations across elevational and climatic gradients in western Washington, western Oregon, and northern California. Tree seedlings from all seed-source locations were grown for 16 months in styro-blocks in Corvallis, OR, and Olympia, WA, then transplanted to Deepots® for four months prior to planting at nine different common gardens during the winter of 2008–2009. Trees that became reproductive were monitored for the timing of male and female reproductive budburst at two common-garden locations on a weekly basis during spring, from 2011 through 2016. One common garden, Stone Nursery, was located in a warm climate in southern Oregon, and the other, Buckhorn2, was located in a cool climate in western Washington. More detail on the experimental design of the Douglas-fir Seed-Source Movement Trial can be found in Bansal et al. (2015). For this analysis, we limited the dataset to female reproductive budburst to match the data used to fit the reproductive phenology model.

To test which climatic factors were most strongly related to flowering phenology of different Douglas-fir genotypes, we obtained

Table 2
Descriptions of environmental variables selected to test for support of the three hypotheses for flowering dates in common-garden studies. Variables were obtained for each seed-source location from ClimateWNA (http://www.climatewna.com/).

Variable type	Variable	Description
Frost avoidance	DD_0_sp	Spring degree-days below 0 °C
	Tave_sp	Spring mean temperature (°C)
	Tmin_sp	Spring mean minimum temperature (°C)
	NFFD	Annual number of frost-free days
	NFFD_sp	Spring number of frost-free days
	FFP	Frost-free period
	bFFP	The day of the year on which FFP begins
Growing season limitation	Tmin_at	Autumn mean minimum temperature (°C)
	FFP	Frost-free period
	NFFD	Annual number of frost-free days
	DD_0_at	Autumn degree-days below 0 °C
	Tmin_at	Autumn mean minimum temperature (°C)
	eFFP	The day of the year on which FFP ends
Drought limitation	MAP	Mean annual precipitation (mm)
	MSP	Mean annual May to Sept. precipitation (mm)
	MAT	Mean annual temperature (°C)
	MWMT	Mean warmest month temperature (°C)
	CMD_sm	Summer Hargreaves climatic moisture deficit (mm)
	AHM	Annual heat-moisture index (MAT + 10)/(MAP/1000))
	Eref	Hargreaves reference evaporation (mm)
	CMD	Hargreaves climatic moisture deficit (mm)
	RH	Mean annual relative humidity (%)
	MAR	Mean annual solar radiation (MJ m^{-2} d^{-1})

climatic variables for each seed-source location from ClimateWNA (Wang et al., 2011, Table 2) based on biological relevance. Several variables were selected that would potentially support either the 'frost-avoidance', 'growing season limitation' or 'drought-avoidance' hypotheses. To test which hypotheses were supported, we first identified the single variable that explained the most variation in flowering dates within each hypothesis group (Table 2). All predictor variables were scaled by subtracting the mean of the variable and then dividing the result by the variable's standard deviation, so standardized regression coefficients and R^2 values could be directly compared between models. The 'frost-avoidance' hypothesis would be supported if the frost-avoidance variables were highly significant and had positive coefficients (i.e., increased frost would be associated with higher forcing requirements), whereas the 'growing-season limitation hypothesis' would be supported by highly significant variables with negative coefficients (shorter growing seasons would be associated with lower forcing requirements). The 'drought-avoidance hypothesis' would be supported by a majority of significant variables belonging to the drought-avoidance group (the sign of the coefficients would vary based on whether they were precipitation or temperature variables).

We were also interested in examining whether multiple hypotheses might explain variation in timing of flowering and forcing accumulations for different genotypes, which could show that genotypes were adapted to both frost-avoidance and drought, for example. For that test, we first selected the climatic variable for each hypothesis that explained the greatest amount of variation in forcing accumulations after the influence of chilling accumulations was accounted for. We then fit candidate models that included as predictor variables all combinations of the top climatic variables for each hypothesis, along with chilling accumulations and common-garden site, and compared fit statistics to select the most parsimonious model. We chose not to analyze models which included all variables, since many climatic variables were highly collinear. Candidate models were fit as hierarchical linear models that predicted forcing unit accumulation based on the fixed effects of chilling unit accumulation and all combinations of the top climatic variables for each hypothesis with random intercepts for site, and population within site, to account for variation between sites, populations, and populations measured over multiple years. Model parameters were calculated using the lmerTest package in the statistical program R (Kuznetsova et al., 2017).

3. Results

3.1. Modelling the timing of female reproductive budburst of Douglas-fir

Flowering dates of Douglas-fir ranged over a 2-month period in western Washington and Oregon, with substantial variation across sites and years (Fig. 1B). The best-fit reproductive model predicted the mean average flowering date per site and year of the test dataset to within an average of 5.4 ± 0.5 days (RMSE = 6.72, R^2 = 0.50, Table 3, Fig. 4). The final model functions shifted optimal chilling and forcing temperatures c.a. −5 °C compared with the original model in Harrington et al. (2010), retained the same start date of Nov. 1st, and accumulated chilling and forcing from Nov. 1st through the date of flowering (Fig. 3). The chilling and forcing functions for the final model were:

$$Chilling\ units = 3.13\left(\frac{T+10}{10.93}\right)^{2.10} e^{-\left(\frac{T+10}{10.93}\right)^{3.10}} \quad (1)$$

$$Forcing\ units = \frac{1}{1+e^{-0.63T+4.49}} \quad (2)$$

where T is hourly temperature and e is the base of a natural logarithm. Chilling units are set to zero when $T < -10$ °C or $T > 16$ °C, and set to

Table 3
Fit statistics for predictive capabilities of the original vegetative budburst model from Harrington et al. (2010), four candidate reproductive phenology models, a model based on the average day of year of flowering of the test dataset, and a model using only a forcing requirement. All data were trained on the average DOY of flowering per site and year in the test dataset (half of the reproductive data) and then the fit was compared to the average DOY of flowering in the other half of the dataset. Function numbers correspond to equation numbers in Fig. 2. R^2 values, MAE (mean absolute error) and RSME (root mean absolute error) are calculated for error between predicted and actual dates of flowering. The best-fit model is in bold.

Model	Chilling function	Forcing function	R^2	MAE	RSME
Vegetative model (linear)	C0	F0	0.20	14.53	17.31
Vegetative model (log)	C0	F0	0.22	14.88	17.57
Reproductive model 1 (linear)	C1	F1	0.46	5.58	7.00
Reproductive model 1 (log)	C1	F1	0.39	6.09	7.45
Reproductive model 2 (linear)	**C1**	**F2**	**0.50**	**5.37**	**6.72**
Reproductive model 3 (log)	C2	F2	0.31	6.52	7.91
Reproductive model 3 (linear)	C2	F2	0.39	5.89	7.43
Reproductive model 4 (log)	C3	F3	0.42	6.26	7.38
Reproductive model 4 (linear)	C3	F3	0.46	5.75	7.09
Forcing only model (Nov. 1)	N/A	F2	0.07	21.35	25.68
Forcing only model (Jan. 1)	N/A	F2	0.19	15.89	18.74
Average DOY model	N/A	N/A	N/A	7.60	9.53

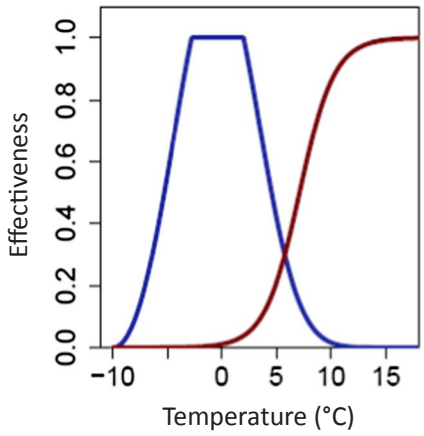


Fig. 3. Final curves for temperature effectiveness for accumulation of chilling and forcing units for the final reproductive phenology model calculated with Eqs. (1) and (2).

1.0 when Eq. (1) gives a value > 1.0 (c.a. −4 °C to 2 °C).

This model provided a better fit than using the average date of flowering across the dataset, which predicted the average site DOY of flowering to within 7.6 ± 0.74 days, or a model that included only forcing accumulations from January 1st, which predicted the average site DOY of flowering to within 15.9 ± 1.6 days.

The final model equation for the possibility line predicting forcing unit accumulation based on chilling unit accumulation was: $FU = 2734.34–0.76\ (CU)$ ($DF = 4211$, $t = -107.98$, $p < .000001$, Fig. 4).

3.2. Genetic influences on forcing requirements for reproductive budburst of Douglas-fir

Flowering dates varied by as much as 40 days among populations growing at the same site in the same year; thus, the forcing requirements for trees from different seed-source populations that experienced the same winter conditions differed greatly (Fig. 5). Models which tested how well environmental variables, along with chilling unit accumulation, explained variation in forcing required for flowering showed that variables associated with summer heat or drought explained more variability in forcing requirements than variables related to winter minimum temperatures or frost risk (all $t \geq 3.5$, $p \leq .001$, Table 4), however, no single variable explained more variation in forcing than chilling unit accumulation (Table 4).

None of the single variable models showed support for the 'growing-season limitation' hypothesis (i.e. there were no significant frost-risk variables with positive coefficients, Table 4), thus only the top variable for the 'drought-avoidance' and 'frost-avoidance' hypotheses were used in the final model selection (Hargreaves climatic moisture deficit (CMD), and the beginning of the frost free period (bFFP), respectively, Table 5, Figs. 5 and 6). The best-fit model for the common-garden flowering data was one that predicted forcing accumulations needed for flowering including both the effects of CMD and bFFP, as well as chilling unit accumulations (Table 6). However, the influence of CMD on forcing accumulations was much stronger than that of bFFP (Table 5).

4. Discussion

Flowering dates of Douglas-fir can vary over 60 days across low elevation sites and years in western Washington and Oregon. Our reproductive budburst model predicted the date of flowering to within 5 days of observed dates across eleven different sites from southern Oregon to northern Washington, indicating that this model can be a useful tool for predicting flowering of coastal Douglas-fir. Detailed models such as this, which utilize budburst data from sites across a large geographic range, incorporate cumulative effects of hourly temperatures over the entire period of dormancy, and which allow for the simultaneous accumulations of both cold and warm temperature units for satisfying chilling and forcing requirements, can assist in accurately predicting the date of flowering for tree species under current and future climates. Phenology models that only consider effects of growing degree days or forcing temperatures, or sequential models that require chilling requirements to be filled before forcing units begin accumulating, may give less accurate predictions, especially in very warm years (Carter et al., 2017). Seed orchard managers can use our reproductive phenology model to track how close seed orchard blocks are to

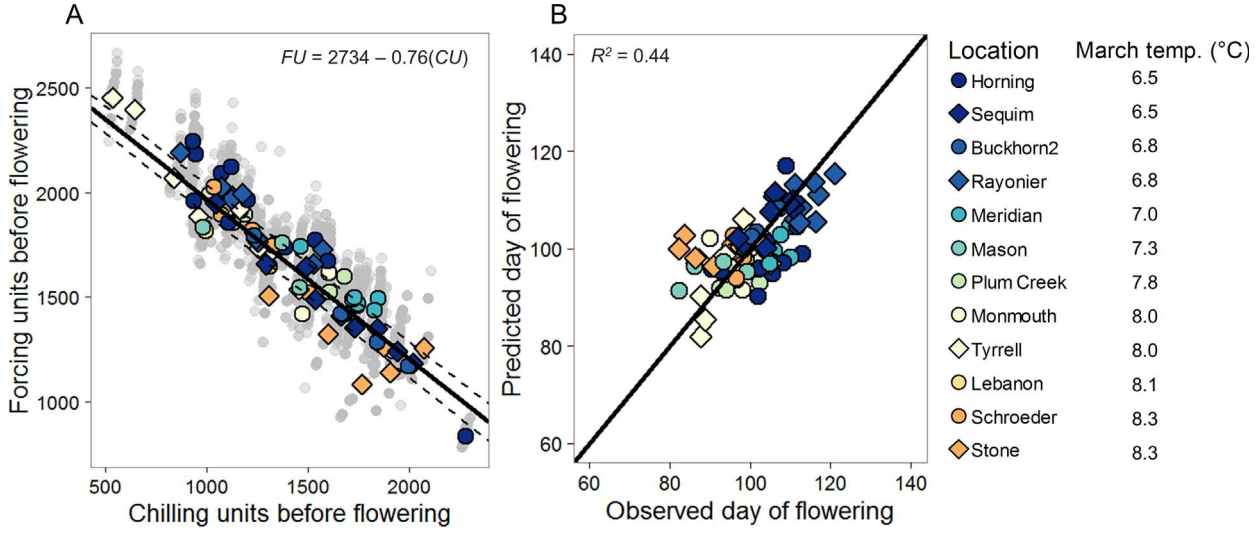


Fig. 4. (A) Chilling and forcing unit accumulations calculated for all observations using the final reproductive phenology model. Colored points represents the average accumulated chilling and forcing units reached prior to reproductive budburst of Douglas-fir for each site and year ($n = 77$). Grey points represent the average chilling and forcing hours before reproductive budburst of individual trees at sites ($n = 4518$). Chilling and forcing units began accumulating on November 1st of the year prior to flowering and stopped accumulating on midnight of the day prior to flowering. The solid line shows the linear relationship between chilling and forcing accumulations, and the dashed lines show the 95% confidence intervals of the slope. (B) Observed versus predicted dates of flowering of Douglas-fir calculated with the final reproductive model. Colored circles denote the average flowering dates of Douglas-fir per site, per year. The solid line is the 1:1 line, however, the R^2 value is for the linear relationship between the average observed and predicted values per site and year for the entire dataset. Statistics shown here are for the entire dataset, not just the test data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

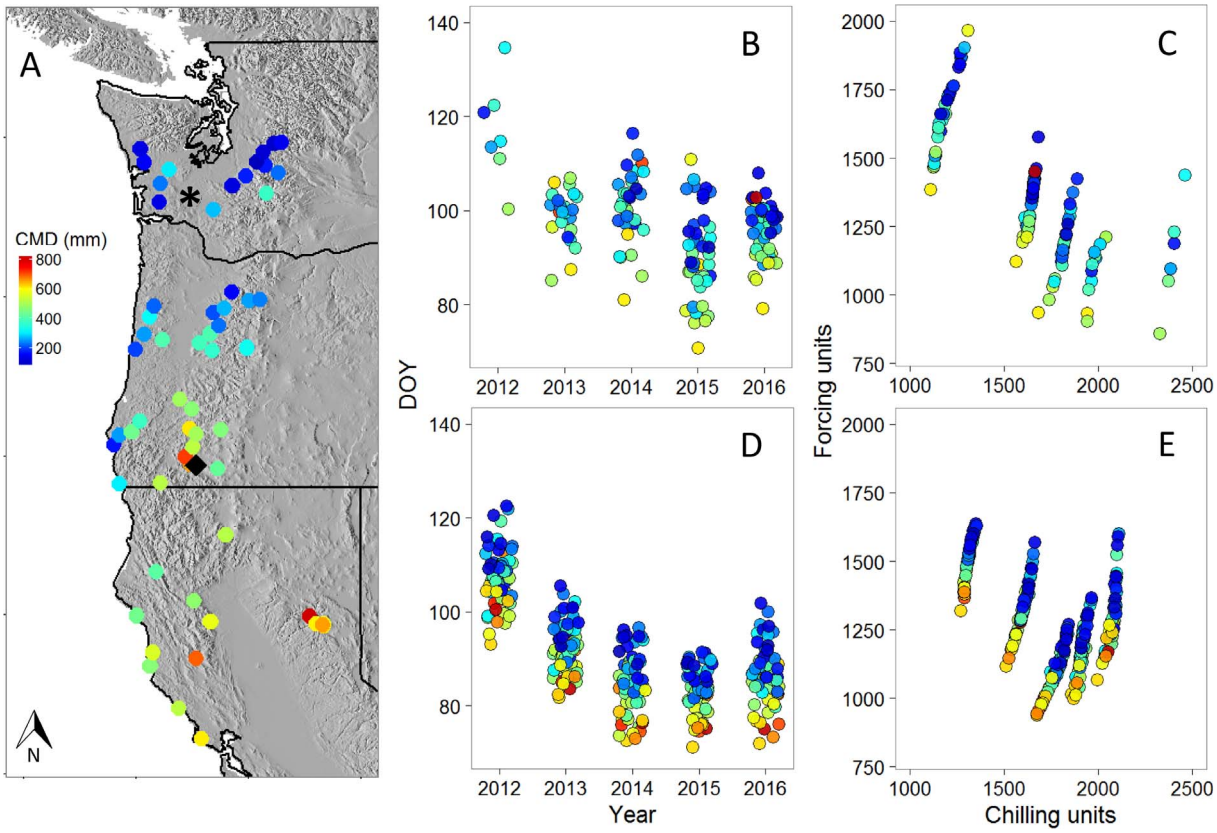


Fig. 5. (A) Seed-source locations of the 60 populations included in the common-garden studies. All points are colored by annual Hargreaves climate moisture deficit (the sum of monthly differences between reference evaporation and precipitation in mm). Higher values and warmer colors indicate drier seed-source locations. The two common-garden studies are identified by a black asterisk (Buckhorn2) and a black diamond (Stone Nursery). The average dates of flowering per population per year are shown for Buckhorn2 (B) and Stone Nursery (D). Points are jittered along the x-axis to better display population means. The average chilling and forcing accumulations per population per year are shown for Buckhorn2 (C) and Stone Nursery (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Scaled regression coefficients and statistics for the amount of variation in forcing requirements explained by different scaled environmental variables in hierarchical linear models. All models included a fixed effect of chilling unit accumulation. Variables explaining the most variation for each chosen hypothesis, with slopes in the hypothesized direction, are in bold. See Table 2 for descriptions of environmental variable acronyms.

Hypothesis	Variable	Coef.	R^2	*p* value	Δ AIC
Base model	Chilling units	−121.29	0.31	< .0001	0
Frost avoidance	DD_0_sp	10.21	0.31	.239	−5.55
	Tave_sp	−25.22	0.32	.004	−12.33
	Tmin_sp	−15.72	0.31	.09	−7.14
	NFFD	−11.85	0.31	.22	−5.9
	NFFD_sp	−8.67	0.31	.35	−5.16
	FFP	−25.36	0.32	.008	−11.42
	bFFP	**27.18**	**0.32**	**.004**	**−12.58**
	Tmin_wt	−14.05	0.31	.14	−6.51
Growing season limitation	Tmin_at	−19.31	0.32	.04	−8.46
	FFP	−25.36	0.32	.008	−11.42
	NFFD	−11.85	0.31	.22	−5.9
	DD_0_at	15.42	0.31	.08	−7.32
	Tmin_at	−19.31	0.32	.04	−8.46
	eFFP	−22.26	0.32	.02	−9.69
Drought limitation	MAP	48.61	0.35	< .0001	−7.32
	MSP	67.52	0.40	< .0001	−70.99
	MAT	−33.26	0.33	.0001	−18.69
	MWMT	−30.67	0.33	.0004	−16.42
	CMD_sm	−66.87	0.40	< .0001	−70.78
	AHM	−46.95	0.36	< .0001	−36.57
	Eref	−54.48	0.37	< .0001	−45.97
	CMD	**68.88**	**0.40**	**< .0001**	**−72.78**
	RH	39.95	0.34	< .0001	−22.18

Table 5
Comparisons between candidate models to explain forcing accumulations needed for flowering at common-garden sites based on chilling accumulations and climatic moisture deficit (CMD) and the beginning of the frost-free period (bFFP) from seed-source populations. Marginal R^2 are calculated for mixed-effects models without including random effects, and conditional R^2 values include random effects. The delta AIC is the difference in fit between the listed model and the model with the lowest AIC. Lower AICs indicate models that better explain variation in the data.

Rank	Model	Marginal R^2	Conditional R^2	Δ AIC
1	Chilling units + CMD + bFFP	0.40	0.49	0.00
2	Chilling units + CMD	0.40	0.49	0.75
3	Chilling units + bFFP	0.33	0.51	53.98
4	Chilling units	0.31	0.51	59.70
5	Null model (random effects only)	0.00	0.22	1685.08

flowering by plotting accumulated chilling and forcing units from temperatures at on-site or nearby weather stations against the curve of the reproductive phenology model. If accumulated chilling and forcing sums are far from the possibility line, then trees are unlikely to flower. Conversely, if the line of accumulated chilling and forcing approaches the possibility line, then it should be biologically possible for trees to flower.

This model agrees with previous research that both cold and warm temperatures are important determinants of the timing of spring phenological development of temperate tree species (Cannell and Smith, 1986; Andersen, 1991; Cesaraccio et al., 2004; Harrington et al., 2010; Cook et al., 2012). Fewer forcing units were required for flowering at sites in colder locations, and over years with colder winters. Warmer temperatures in the future will likely result in earlier flowering on sites

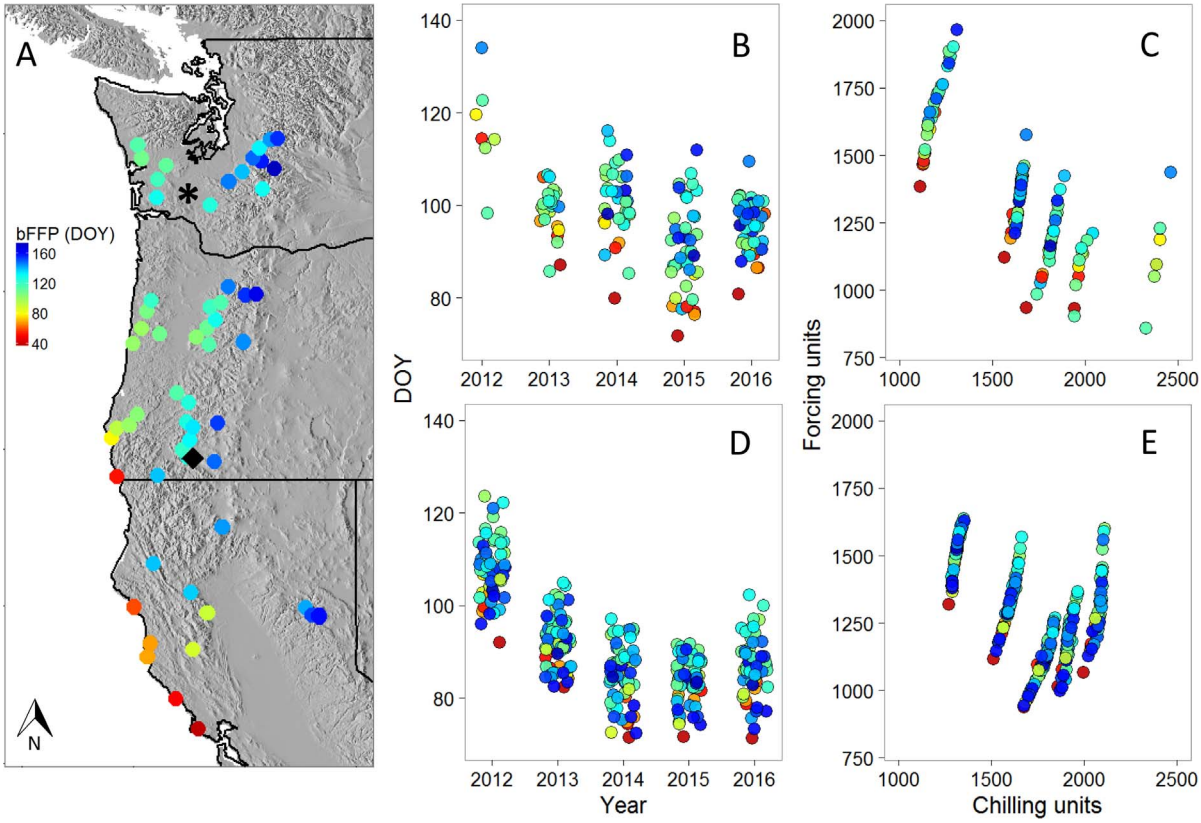


Fig. 6. (A) Seed-source locations of the 60 populations included in the common-garden studies. All points are colored by day of year marking the beginning of the frost free period (i.e., the average day of year after which there are no more average daily temperatures below 0° C). Higher values and cooler colors indicate seed-source locations with later days of the last frost in spring. The two common-garden studies are identified by a black asterisk (Buckhorn2) and a black diamond (Stone Nursery). The average dates of flowering per population per year are shown for Buckhorn2 (B) and Stone Nursery (D). Points are jittered along the x-axis to better display population means. The average chilling and forcing accumulations per population per year are shown for Buckhorn2 (C) and Stone Nursery (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Coefficients and statistics for best-fit model of forcing accumulations needed for flowering based on chilling unit accumulations, Hargreaves climate-moisture index (CMD), and the beginning of the frost free period (bFFP) for seed-source locations.

Variable	Coef.	Std. error	df	*t* value	*p* value
Intercept	2137.70	39.62	23.69		
Chilling units	−0.45	0.01	3408.21	−45.57	< .0001
CMD	−0.36	0.04	104.70	−9.40	< .0001
bFFP	0.45	0.22	99.28	1.99	.05

which currently have high chilling; however, sites which currently experience low chilling may demonstrate no change or possibly even a delay in flowering. Previous studies across temperate regions have shown a delay in spring greening (Yu et al., 2010) and first flowering dates (Cook et al., 2012) with warmer winter temperatures. The reproductive model shown here differs from the previous vegetative budburst model developed for Douglas-fir in that the best-fit model for the reproductive data was a linear relationship between chilling and forcing accumulations, not an exponential relationship (Harrington et al., 2010). However, based on the previous findings for vegetative budburst, we hypothesize that the relationship between chilling and forcing temperatures would likely be exponential if a larger range of winter temperatures were included. This is based on the assumption that a certain minimum amount of forcing or chilling would be required to initiate reproductive budburst (Harrington et al., 2010). The model in Harrington et al. (2010) resulted from both an extensive literature review and experiments that subjected trees to very warm and very cold winters. The results showed that with very warm winter temperatures, Douglas-fir was delayed in breaking vegetative bud. Since we did not have data for extremely warm or extremely cold years in the reproductive phenology dataset, it is possible that the linear relationship between chilling and forcing would need to be modified to be effective under very warm or very cold fall and winter conditions. Alternatively, photoperiod may play a role as a secondary cue that can help induce flowering past a certain day length (Gyllenstrand et al., 2007). Studies have found photoperiod to influence the timing of vegetative budburst of some tree species (Basler and Körner, 2014), and the timing of flowering of herbaceous species (Falusi and Calamassi, 1996; Keller and Körner, 2003; Jackson, 2009), however, few studies have examined how photoperiod influences reproductive budburst of trees. In general, research indicates that photoperiod is a less important cue for spring phenological events than temperatures over the dormant season (Laube et al., 2014). Additional controlled experiments that manipulate both temperature and photoperiod are needed to accurately parameterize the possibility line for reproductive budburst, and test for any influence of photoperiod. If climate change results in much warmer temperatures over winter, chilling requirements may not be met, and it is possible that Douglas-fir may flower later, especially at the more southern edges of its range. This will be especially relevant for seed orchards, as many are currently located in the warmer regions of the coastal Douglas-fir range. Although most observations to date have shown an advancement of spring phenology with climate change (e.g. Parmesan and Yohe, 2003); it will be important to continue to consider the contrasting effects that warmer winters may exert on the current trend of advancing phenology (Cannell and Smith, 1986; Cook et al., 2012; Gould et al., 2012; Ford et al., 2016).

Our final reproductive phenology model utilized equations that

implied greater effectiveness at lower temperatures for both chilling and forcing than the phenology model parameterized for other aspects of Douglas-fir growth (Harrington et al., 2010; Gould et al., 2011; Ford et al., 2016). These results are in line with several previous studies that have tested temperature effectiveness for satisfying chilling and forcing requirements for flowering of woody species. Sunley et al. (2006) found that chilling models which included all temperatures below a threshold of 7.2 °C predicted dates of flowering of shrubs better than models that did not include sub-zero temperatures or allowed temperatures higher than 7.2 °C to be effective for chilling. Nikkanen (2001) found that giving forcing effectiveness to all temperatures above 5 °C accurately predicted dates of flowering of Norway spruce. It is important to note that the air temperatures used to parameterize these models may differ greatly from the actual temperatures experienced inside reproductive buds, since bud temperature is also influenced by solar radiation, wind speed, and vapor pressure deficit (Savvides et al., 2013). Thus, air temperature effectiveness values that most accurately predict flowering dates may not accurately represent the temperatures experienced by organs of the tree. Additionally, temperatures may differ between sensor heights at different locations or between seed orchards and the nearest weather stations, so caution should be used when attempting to translate temperature thresholds used for this predictive model to precise temperature thresholds that govern biological processes in trees. Future research should focus on experimentally altering temperatures, as well as photoperiod, to refine estimates of phenological cues for reproductive budburst of Douglas-fir.

Results from the two common garden experiments strongly supported our drought-avoidance hypothesis, and also provided a small amount of support for the frost avoidance hypothesis. However, the influence of chilling unit accumulation on forcing requirements for flowering was almost twice as large as that of any climate variable, indicating that temperature during the dormant season is the single strongest predictor of flowering dates in spring. Genotypes from warmer, drier locations required fewer forcing units to flower after receiving the same amount of chilling over winter. Trees from hot, dry regions in the Pacific Northwest and California may be under selective pressure to time reproduction to occur as soon as conditions are favorable, and before summer drought limits resources (St. Clair et al., 2005; Gould et al., 2011). Thus, trees growing in drought-prone regions may already be adapted to grow and flower earlier as the growing season also shifts earlier. Additionally, the average day of year when the frost-free period begins at seed-source locations was positively correlated with forcing needed for flowering, indicating that trees from colder source populations may have adapted to flower later to avoid frost risk. In contrast, populations from warmer, more southern locations that do not experience frost risk late into spring may not have adapted to delay flowering. These intraspecific results suggest that, although Douglas-fir along the southern portions of its range may receive less chilling in the future, the natural populations in warmer locations may also be more resilient to warmer winter temperatures. Populations from warmer and drier locations may flower with fewer forcing hours in spring than populations from cooler areas, as long as minimum chilling requirements are met. Selection of genotypes from provenances at the southern range limits of Douglas-fir for tree breeding programs, or selection of early flowering genotypes from within current tree breeding programs, may reduce the possibility of delayed flowering with warmer winters in the future.

These results on the timing of flowering also have important implications for gene flow between Douglas-fir populations in the future. In our common-garden studies, trees from different climatic regions flowered up to 35 days apart in the same year. Changes in winter and spring temperatures may differentially affect the timing of flowering of trees along climatic gradients in wild populations, and from different provinces within seed orchards. Differences in the timing of flower receptivity and pollen shedding of different clones can influence seed quantity, quality, and reproductive success of trees in seed orchards (Erickson and Adams, 1989; El-Kassaby et al., 1984, 1988; Copes and Sniezko, 1991; Slavov et al., 2005). Depending on how dormant season temperatures change with climate change, these differences could result in a compression or extension of the flowering season across populations or seed orchards (Copes and Sniezko, 1991; Alizoti et al., 2010) and this can influence the outcrossing efficiency of early-flowering versus late-flowering trees (Copes and Sniezko, 1991). Previous studies have found that increased chilling over winter, or cold spring temperature treatments, resulted in more synchronous flowering in Douglas-fir and berry orchards (El-Kassaby et al., 1984; Sunley et al., 2006). Similarly, Alizoti et al. (2010) found that abnormally warm winter temperatures resulted in highly asynchronous flowering in a *Pinus nigra* orchard. The increase or decrease of flowering synchrony with climate change could drastically affect gene flow between populations, as well as the rate of successful pollination within populations or seed orchards (Copes and Sniezko, 1991; Alizoti et al., 2010). Future research should address how temperature change and changes in timing of reproductive budburst influence outcrossing rates and reproductive success of Douglas-fir to aid managers in selection of genotypes for seed orchards, and inform predictions of gene flow in forests across climatic gradients in a warmer world.

The relative importance of summer drought as a selective pressure for timing of reproductive budburst in the common-garden experiments mirrors results found for the timing of vegetative budburst of Douglas-fir (St. Clair et al., 2005, Gould et al., 2011). Climatic variables involving winter temperatures, minimum temperatures, or frost-free periods did not explain as much variation in flowering timing as those related to summer temperatures and drought. These results differ from those of fall cold-hardiness traits from trees in the same common gardens (Bansal et al., 2015). Seed-source environmental variables related to winter minimum temperatures and fall frost dates best predicted population-level differences in fall cold hardiness traits of Douglas-fir grown in common-garden experiments (Bansal et al., 2015). Together, results of these studies show that plants have adapted in different ways to a complex suite of environmental factors (Bansal et al., 2016). This may limit the ability of models to predict future suitable habitat and survival of trees based on changes in simple climate variables, such as changes in mean annual temperature or precipitation (Benito Garzón et al., 2011). The importance of both cold hardiness and drought avoidance traits also highlights the utility of using seeds from climatically delineated seed zones for reforestation (Bower et al., 2014). Additionally, progeny tests should be designed to look at performance over a broad range of climate conditions to insure that trees selected for production orchards are well adapted to the present and future climatic conditions at that location.

Our model of reproductive budburst predicted the day of flowering of coastal Douglas-fir across a range of sites and yearly temperatures based on chilling and forcing accumulations over the dormant season. Flowering time was also strongly influenced by genetic variation, with trees from warmer, drier climates requiring fewer forcing units to flower than those adapted to colder, wetter locations. Our results provide detailed information on the temperature and genetic cues that drive the timing of flowering, and can be used to help predict how changes in temperature under various climate models may change the timing of reproductive phenology. These models may also indicate the geographic areas where future climate could advance or delay flowering of Douglas-fir in the future.

Acknowledgements

We thank Sam Brooks, Erin Hooten and Mike Crawford, Bureau of Land Management; Brian Baltunis, Weyerhaeuser; Sara Lipow, Roseburg Forest Products; Bill Marshall, Cascade Timber Consulting; Norah Young and Jeff DeBell, Washington Dept. of Natural Resources; Jim Smith, Plum Creek Timberlands; Candace Cahill, Rayonier; and Rick Quam, Oregon Department of Forestry, for breeding data for

Douglas-fir. We thank all our coworkers for data collection at the Buckhorn2 and J. Herbert Stone Nursery common-garden sites, especially Teresa Vail, Yianna Bekris, Leslie Brodie, and Chris Poklemba. We thank Port Blakely Tree Farms and the USDA Forest Service J. Herbert Stone Nursery for providing access to those sites. We thank Peter Gould, Kevin Ford, and David Thornton for assistance with data handling and analysis, and Kevin Ford, Brian Baltunis, Sam Brooks and two anonymous reviewers for editorial suggestions on earlier versions of this manuscript. Finally, we thank the USDI Bureau of Land Management and USDA Forest Service Pacific Northwest Research Station for providing funding. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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