Chilling outweighs photoperiod and forcing cues for temperate trees in experiments

A.K. Ettinger, C. Chamberlain, I. Morales-Castilla, D. Buonaiuto, D. Flynn, T. Savas, J. Samaha & E. Wolkovich

June 28, 2019

Abstract

Decades of fundamental research on woody plant species highlight three major cues that shape spring phenological events: chilling, forcing, and photoperiod (e.g., Campbell and Sugano, 1975; Heide, 2008; Flynn and Wolkovich, 2018). Increasing research on the phenological impacts of climate change has led to debate over how common all three cues are across species, and—if prevalent—whether chilling and/or photoperiod cues may be slowing phenological responses to warming in recent years (Heide, 2011; Koerner and Basler, 2010*b*; Fu et al., 2015; Zohner et al., 2016). Here we use a global meta-analysis of all published controlled environment studies to test for the relative effects of these three major cues across 203 species. We find almost all species show strong responses to all three cues, with chilling being the strongest cue (3.58 greater than forcing), and photoperiod the weakest (0.68 relative to forcing). Simple forecasts from our findings for a well-studied region (Central Europe), however, suggest that forcing cues dominate future phenological responses. Effects of chilling generally appear above 4°C warming for most locations, and thus are unlikely to underlie apparently slowing phenological responses. Our results unify both sides of the debate over phenological cues: while all species may respond to all cues strongly in experimental conditions, in current environmental conditions the dominant impact of climate change is—and may remain—from increased forcing.

Alternative: Decades of fundamental research on woody plant species highlight three major cues that shape spring phenological events: chilling, forcing, and photoperiod (e.g., Campbell and Sugano, 1975; Heide, 2008; Flynn and Wolkovich, 2018). Increasing research on the phenological impacts of climate change has led to debate over how common all three cues are across species, and—if prevalent—whether chilling and/or photoperiod cues may be slowing phenological responses to warming in recent years (Heide, 2011; Koerner and Basler, 2010*b*; Fu et al., 2015; Zohner et al., 2016). Here we use a global meta-analysis of all published controlled environment studies to test for the relative effects of these three major cues across 203 species. We find almost all species show strong responses to all three cues, with chilling being the strongest cue (3.58 greater than forcing), and photoperiod the weakest (0.68 relative to forcing). Simple forecasts from our findings for a well-studied region (Central Europe), however, suggest that forcing cues dominate future phenological responses. Effects of chilling generally appear above 4°C warming for most locations, and thus are unlikely to underlie apparently slowing phenological responses. Instead, we suggest statistical artifacts in calculations of phenological responses to warming may underlie the observed shifts. Our results thus show that all species may respond to all cues strongly in experimental conditions, but teasing out evidence of these cues in long-term observational is fraught with danger.

Original: Decades of fundamental research on woody plant species highlight three major cues that shape spring phenological events: chilling, forcing, and photoperiod (e.g., Campbell and Sugano, 1975; Heide, 2008; Flynn and Wolkovich, 2018). Increasing research on the phenological impacts of climate change has led to debate over whether forcing (associated with warm spring temperatures) cues may dominate for some species, while fewer species respond to chilling (associated with cool winter temperatures) and/or photoperiod (Heide,

2011; Koerner and Basler, 2010b; Zohner et al., 2016). The debate has wide-reaching consequences for the future of spring phenology, as the presence of strong chilling or photoperiod cues could slow, stall, or even reverse current trends towards ever-earlier spring phenology with warming (Fu et al., 2015; Koerner and Basler, 2010a). Here we use a global meta-analysis of all published controlled environment studies to test for the relative effects of these three major cues across 203 species. We find most species show strong responses to all three cues, with chilling being the strongest cue (3.58 greater than forcing), and photoperiod the weakest (0.68 relative to forcing). Simple forecasts from our findings for a well-studied region (Central Europe), however, suggest that forcing cues dominate future phenological responses. Impacts of chilling are more location-specific, and generally appear above 4°C warming. Our results unify both sides of the debate over phenological cues: while all species may respond to all cues strongly in experimental conditions, in current environmental conditions the dominant impact of climate change is—and may remain—from increased forcing.

Alternative: Decades of fundamental research on woody plant species highlight three major cues that shape spring phenological events: forcing, photoperiod, and chilling (e.g., Campbell and Sugano, 1975; Heide, 2008; Flynn and Wolkovich, 2018). Increasing research on the phenological impacts of climate change has led to debate over how common all three cues are across species, and—if prevalent—whether chilling and/or photoperiod cues may be slowing phenological responses to warming in recent years (Heide, 2011; Koerner and Basler, 2010b; Fu et al., 2015; Zohner et al., 2016). Here we use a global meta-analysis of all published controlled environment studies to test for the relative effects of these three major cues across 203 species. We find almost all species show strong responses to all three cues, with chilling being the strongest cue (3.58 greater than forcing), and photoperiod the weakest (0.68 relative to forcing). Simple forecasts from our findings for a well-studied region (Central Europe), however, suggest that forcing cues dominate future phenological responses. Effects of chilling generally appear above 4°C warming for most locations, and thus are unlikely to underlie apparently slowing phenological responses. Instead, we suggest statistical artifacts in calculations of phenological responses to warming may underlie the observed shifts. Our results thus show that all species may respond to all cues strongly in experimental conditions, but teasing out evidence of these cues in long-term observational is fraught with danger.

Main text

For decades, plant phenology has been one of the most reported and consistent biological imprints of climate change (IPCC, 2014), with many temperate plants leafing and flowering days to weeks earlier with rising temperatures (Miller-Rushing and Primack, 2008; Menzel et al., 2006). Understanding such shifts is important as phenology shapes community assembly and a suite of ecosystem services, including pollination and carbon sequestration, and scales up to impact projections of climate change itself Cleland et al. (2007).

As research interest in phenology has progressed, critical discrepancies and uncertainties in our understanding have emerged. Though responses to warming are consistent on average, they show high unexplained variation across species and sites (Wolkovich et al., 2012). Furthermore, long-term observational studies provide increasing evidence that sensitivities of phenology to temperature are weakening in recent decades (Rutishauser et al., 2008; Yu et al., 2010), especially in Europe, where researchers suggest that responses to multiple environmental cues underlie declining temperature sensitivities (Fu et al., 2015).

Fundamental research in phenology outlines how three major environmental cues, chilling (cool temperatures, generally occurring in the fall and late winter), forcing (warm temperatures, generally occurring in the late winter and early spring), and photoperiod (daylength), provide multiple routes to budburst each spring, depending on the environment (Chuine, 2000). For example, in some species a cool winter will lower the amount of forcing required to trigger budburst, compared to a warmer winter (Harrington and Gould, 2015). Additionally, photoperiod may trigger budburst, given low chilling and/or forcing (Basler and Körner, 2014; Caffarra et al., 2011b; Zohner et al., 2016). Research suggests that all three cues may underlie spring phenology for many temperate woody species (Flynn and Wolkovich, 2018; Basler and Körner, 2014; Caffarra

et al., 2011a), which could have critical forecasting implications—predicting delays in spring phenology as increased warming reduces chilling in many areas (Fraga et al., 2019) or where earlier budburst shortens the experienced photoperiod. However, there is strong debate, with some research suggesting some cues—such as photoperiod—may be effectively absent in some species, but dominate in others (Zohner et al., 2016; Koerner and Basler, 2010a).

Resolving this debate requires overcoming major hurdles to estimate responses to each cue. Studies attempting to estimate cues using long-term observational data (e.g., Vitasse and Basler, 2013; Zohner et al., 2016) generally fail to overcome the fundamental challenge that all three cues are strongly correlated in nature (e.g., during the transition from winter to spring at temperate latitudes, forcing and photoperiod usually increase in step). In contrast to observational studies, controlled environment experiments can breakdown correlations between chilling and forcing. These experiments—most often conducted in growth chambers or similar systems to control temperature and light—have been conducted for decades. They have produced contrasting results, however, potential due to differences in focal species or study cites (Zohner et al., 2016; Laube et al., 2014; Basler and Körner, 2012; Caffarra et al., 2011b; Caffarra and Donnelly, 2011).

Here, we leverage nearly 40 years of controlled environment studies to understand how chilling, forcing, and photoperiod determine budburst timing in woody species. Using a meta-analytic approach we can synthesize experimental results across many taxa to find generalizaeable patterns. We reviewed 193 papers from controlled environment studies, then extracted data from all experiments that reported budburst responses, yielding data from 49 studies across 39 years and 203 species (Fig. 1S). The resulting OSPREE database includes only studies for which we could identify forcing, photoperiod, and chilling treatments quantitatively and includes a mix of studies where tissue was grown in greenhouses or brought in from the field and exposed to experimental conditions. As chilling was rarely reported, we calculated chilling when possible, using a common but imperfect method (cite utah paper), in which chilling does not accumulate below 1.4 °C or at high temperatures (see Supplemental Methods). We estimated the effects of chilling, forcing, and photoperiod using a Bayesian hierarchical model. Our model averages over interactions between predictors to estimate both species-level responses (generally yielding more accurate estimates for well-studied species, such as *Fagus sylvatica* and *Betula pendula*), and the distribution from which they are drawn, yielding an estimate of the overall response across species (see Supplemental Materials).

Across studies, all cues—chilling, forcing, and photoperiod—advance budburst phenology (Fig. 1). Chilling was the strongest cue (-2.84 days/standard unit or -8.89 days per chill unit, Fig. 2), followed by forcing (-0.79 days/standard unit or -4.36 days per °C of warming, Fig. 2), and photoperiod (-0.54 days/standard unit or -3.15 days per hour of daylength; see Supplemental Materials for comparison of models using standardized versus unstandardized predictors). While photoperiod had the smallest effect among the three cues, our results contrast with the extensive literature suggesting photoperiod is an unimportant existent cue for many species (Zohner et al., 2016; Koerner and Basler, 2010a)—instead we found it was surprisingly large, even in a model accounting for its interaction with latitude (Fig. ??, see also Supplemental Materials for details, especially Fig. 1S). It was also generally consistent across species, onle deviating in *Fagus sylvatica*, a species well-known for having a large response to photoperiod (which we also found, see Fig. 1). Species also showed fairly consistent responses to chilling (variance = 2.07 days per chill unit, Fig. 1). Responses to forcing, in contrast, were the most variable across species (variance = 0.91 days per °C of warming).

As temperature is fundamentally altered by climate change, our finding that different ends of the temperature spectrum—chilling and forcing—have the strongest effects on budburst suggests that understanding these cues will be critical for forecasting phenology with climate change. Many previous studies attribute advances in budburst to increased forcing (Basler and Körner, 2014; Bradley et al., 1999; Menzel et al., 2006; Harrington and Gould, 2015). Our results, however, suggest chilling has a greater effect on budburst than forcing (Fig. 1). This has not been widely suggested previously, perhaps because little work has directly manipulated chilling, and the few studies that have were designed to compare chilling versus photoperiod effects (e.g., Basler and Körner, 2014; Caffarra et al., 2011a; Laube et al., 2014; Zohner et al., 2016), not forcing versus chilling effects.

A simple interpretation of our results supports the hypotheses that chilling and photoperiod cues may underlie declining sensitivities to warming in long-term Central European data (Rutishauser et al., 2008; Yu et al., 2010; Fu et al., 2015). Under these hypotheses, warming increases forcing and thus advances budburst, but such advances become muted if warming also causes declines in chilling and shorter photoperiods experienced near the timing of budburst (Koerner and Basler, 2010a). This basic agreement between our results and long-term observational trends, however, integrates across experimental conditions—a more robust comparison requires examining predictions under conditions closer to those found in nature.

Reinterpreting our model using climate and phenology data that have led to observations of declining temperature sensitivities in Central Europe suggests that chilling and photoperiod are unlikely to cause the observed declines in sensitivity. Our model predicts such declines occur only at extreme warming for most sites (Supplemental Materials for details). In contrast to the common hypothesis that chilling declines with warming we found that—for many sites—total estimated chilling increased with warming (Fig. 3 A,D), though this varied with local climate prior to warming (Fig. 2S). Portions of Central Europe have experienced more dramatic warming in winter versus summer (Balling Jr et al., 1998), but even if warming only occurs in the winter, our results suggest that delays due to decreased chilling occur at warming above 4°C for most sites (Fig. 3). At high warming, predicted declines in sensitivity were due to declines in chilling—photoperiod had comparitively little effect on budburst day of year, even for the photosensitive species *F. sylvatica* (Fig. **??**).

Our predictions leave open the question of what underlies declining sensitivities across Europe, but simple analyses suggest it could be a statistical artifact of how temperature sensitivities are calculated. Physiologically, budburst is triggered by the accumulation of forcing temperatures during the spring (Hänninen, 1995; Chuine et al., 2016). Yet, researchers today often estimate temperature sensitivities from long-term observational data using a linear regression of annual budburst date versus mean or other aggregated metrics of spring temperature (e.g., Wolkovich et al., 2012). This approach has the benefit of yielding an easily interpretable metric—days change per °C—but will systematically estimate lower sensitivities given warmer daily temperatures, even with no change in the underlying cues (Fig. 3S). We found the declining sensitivities observed in European data are of the same magnitude as those predicted from a statistical artifact (sensitivity declines of 0.8±0.3 days/°C in European data versus 0.9±0.5 days/°C in simulations), and the data also show a related decline in leafout variance that would not be immediately predicted from shifting cues (see *Understanding declines in temperature sensitivity in European long-term data* in the Supplemental Materials and Güsewell et al., 2017, for further details). This statistical artifact is likely not confined to phenological studies; it should apply to any research using a similar days/°C metric to estimate an underlying thermal accumulation model where the thermal sum per day is non-stationary, as is the case with climate change.

A consistent result of our findings—across both the experimental and in situ environmental conditions—is the importance of chilling. Yet chilling and its related physiological stage, endodormancy, are not well understood (Chuine et al., 2016). Models of how species accumulate chilling are poorly developed for forest trees, with few relevant tests evaluating the particular temperatures at which species do or do not accumulate chilling. Instead, researchers generally rely on models developed for perennial fruit trees (i.e., Utah (Richardson, 1974) and chill portions (Fishman et al., 1987), both of which were developed for peach species). These models are themselves *hypotheses* for how chilling may accumulate and produce dormancy release, but are likely to be inaccurate for many species (Dennis, 2003).

Progress on developing chilling models for wild species is especially slow, as only a few studies (5 out of the total 66 studies) manipulate chilling directly. Instead many studies (13 out of 66; the remaining studies did not not appear to manipulate chilling) estimate chilling effects through sequential removal of tissue from the field followed by exposure to 'forcing' conditions (Weinberger et al., 1950), with the assumption that tissues collected later experience more chilling. This method allows more natural chilling conditions but introduces other challenges: first, chilling duration may not always co-vary with the magnitude of total accumulated chilling (Dennis, 2003), and, second, photoperiod and other factors also change over time. Indeed, we found that sequential-removal studies tended to result in later budburst, weaker effects of forcing and stronger effects of chilling compared to estimates from studies that directly manipulated chilling (Fig. 4S Weinberger

et al., 1950; Polgar et al., 2013), suggesting a study's design of chilling impacts both forcing and chilling estimates. An improved understanding of chilling could in turn alter our understanding of forcing. Although researchers often define 'chilling' and 'forcing' treatments based on temperatures, physiologically plants appear to accumulate forcing mainly after chilling requirements have been met, thus identifying processes plants undergo when accumulating chilling versus forcing will be critical for the most accurate forecasts (Chuine et al., 2016).

Our results unify decades of experimental studies, which have shown the importance of chilling, forcing, and daylength to determining budburst timing, with long-term observational data, where forcing appears to dominate responses to recent warming. We do not find strong evidence for delaying budburst in the near future, and suggest recent observed declines in temperature sensitivity may be related to statistical artifacts from estimating complex cues from observational data. Instead, our predictions suggest budburst will continue to advance in many well-studied European regions in the future with the most dramatic changes coming from regions were winter warming causes dramatic decreases in chilling, with implications for ecosystem services related to phenology.

References

Balling Jr, R. C., P. J. Michaels, and P. C. Knappenberger. 1998. Analysis of winter and summer warming rates in gridded temperature time series. Climate Research 9:175–181.

Basler, D., and C. Körner. 2012. Photoperiod sensitivity of bud burst in 14 temperate forest tree species. Agricultural and Forest Meteorology 165:73–81.

———. 2014. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. Tree physiology 34:377–388.

Bradley, N. L., A. C. Leopold, J. Ross, and W. Huffaker. 1999. Phenological changes reflect climate change in Wisconsin. Proceedings of the National Academy of Sciences 96:9701–9704.

Caffarra, A., and A. Donnelly. 2011. The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. International journal of Biometeorology 55:711–721.

Caffarra, A., A. Donnelly, and I. Chuine. 2011a. Modelling the timing of *Betula pubescens* budburst. II. Integrating complex effects of photoperiod into process-based models. Climate Research 46:159–170.

Caffarra, A., A. Donnelly, I. Chuine, and M. B. Jones. 2011b. Modelling the timing of *Betula pubescens* bud-burst. I. Temperature and photoperiod: A conceptual model. Climate Research 46:147.

Campbell, R. K., and A. I. Sugano. 1975. Phenology of bud burst in douglas-fir related to provenance, photoperiod, chilling, and flushing temperature. Botanical Gazette pages 290–298.

Chuine, I. 2000. A unified model for budburst of trees. Journal of Theoretical Biology 207:337–347.

Chuine, I., M. Bonhomme, J.-M. Legave, I. García de Cortázar-Atauri, G. Charrier, A. Lacointe, and T. Améglio. 2016. Can phenological models predict tree phenology accurately in the future? the unrevealed hurdle of endodormancy break. Global change biology 22:3444–3460.

Cleland, E. E., I. Chuine, A. Menzel, H. A. Mooney, and M. D. Schwartz. 2007. Shifting plant phenology in response to global change. Trends in Ecology & Evolution 22:357–365.

Dennis, F. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. HortScience 38:347–350.

Fishman, S., A. Erez, and G. Couvillon. 1987. The temperature dependence of dormancy breaking in plants: mathematical analysis of a two-step model involving a cooperative transition. Journal of Theoretical Biology 124:473–483.

Flynn, D. F. B., and E. M. Wolkovich. 2018. Temperature and photoperiod drive spring phenology across all species in a temperate forest community. New Phytologist 0.

Fraga, H., J. G. Pinto, and J. A. Santos. 2019. Climate change projections for chilling and heat forcing conditions in european vineyards and olive orchards: a multi-model assessment. Climatic Change pages 1–15.

Fu, Y. S. H., H. F. Zhao, S. L. Piao, M. Peaucelle, S. S. Peng, G. Y. Zhou, P. Ciais, M. T. Huang, A. Menzel, J. P. Uelas, Y. Song, Y. Vitasse, Z. Z. Zeng, and I. A. Janssens. 2015. Declining global warming effects on the phenology of spring leaf unfolding. Nature 526:104–107.

Güsewell, S., R. Furrer, R. Gehrig, and B. Pietragalla. 2017. Changes in temperature sensitivity of spring phenology with recent climate warming in switzerland are related to shifts of the preseason. Global change biology 23:5189–5202.

Hänninen, H. 1995. Effects of climatic change on trees from cool and temperate regions: an ecophysiological approach to modelling of bud burst phenology. Canadian Journal of Botany 73:183–199.

Harrington, C. A., and P. J. Gould. 2015. Tradeoffs between chilling and forcing in satisfying dormancy requirements for pacific northwest tree species. Frontiers in Plant Science 6:120.

Heide, O. M. 2008. Interaction of photoperiod and temperature in the control of growth and dormancy of *Prunus* species. Scientia Horticulturae 115:309–314.

———. 2011. Temperature rather than photoperiod controls growth cessation and dormancy in *Sorbus* species. Journal of experimental botany page err213.

IPCC. 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Koerner, C., and D. Basler. 2010a. Phenology under global warming. Science 327:1461–1462.

———. 2010b. Warming, photoperiods, and tree phenology response. Science 329:278–278.

Laube, J., T. H. Sparks, N. Estrella, J. Höfler, D. P. Ankerst, and A. Menzel. 2014. Chilling outweighs photoperiod in preventing precocious spring development. Global Change Biology 20:170–182.

Menzel, A., T. H. Sparks, N. Estrella, E. Koch, A. Aasa, R. Ahas, K. Alm-Kuebler, et al. 2006. European phenological response to climate change matches the warming pattern. Global Change Biology 12:1969–1976.

Miller-Rushing, A. J., and R. B. Primack. 2008. Global warming and flowering times in Thoreau's Concord: A community perspective. Ecology 89:332—341.

Polgar, C. A., R. B. Primack, E. H. Williams, S. Stichter, and C. Hitchcock. 2013. Climate effects on the flight period of lycaenid butterflies in Massachusetts. Biological conservation 160:25–31.

Richardson, E. 1974. A model for estimating the completion of rest for'redhaven'and'elberta'peach trees. HortScience 9:331–332.

Rutishauser, T., J. Luterbacher, C. Defila, D. Frank, and H. Wanner. 2008. Swiss spring plant phenology 2007: Extremes, a multi-century perspective, and changes in temperature sensitivity. Geophysical Research Letters 35:L05703. Rutishauser, This Luterbacher, Juerg Defila, Claudio Frank, David Wanner, Heinz.

Vitasse, Y., and D. Basler. 2013. What role for photoperiod in the bud burst phenology of european beech. European Journal of Forest Research 132:1–8.

Weinberger, J. H., et al. 1950. Chilling requirements of peach varieties. Pages 122–28 *in* Proceedings. American Society for Horticultural Science. Vol. 56.

Wolkovich, E. M., B. I. Cook, J. M. Allen, T. M. Crimmins, J. L. Betancourt, S. E. Travers, S. Pau, J. Regetz, T. J. Davies, N. J. B. Kraft, T. R. Ault, K. Bolmgren, S. J. Mazer, G. J. McCabe, B. J. McGill, C. Parmesan, N. Salamin, M. D. Schwartz, and E. E. Cleland. 2012. Warming experiments underpredict plant phenological responses to climate change. Nature 485:494–497.

Yu, H. Y., E. Luedeling, and J. C. Xu. 2010. Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. Proceedings of the National Academy of Sciences of the United States of America 107:22151–22156.

Zohner, C. M., B. M. Benito, J. C. Svenning, and S. S. Renner. 2016. Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. Nature Climate Change .
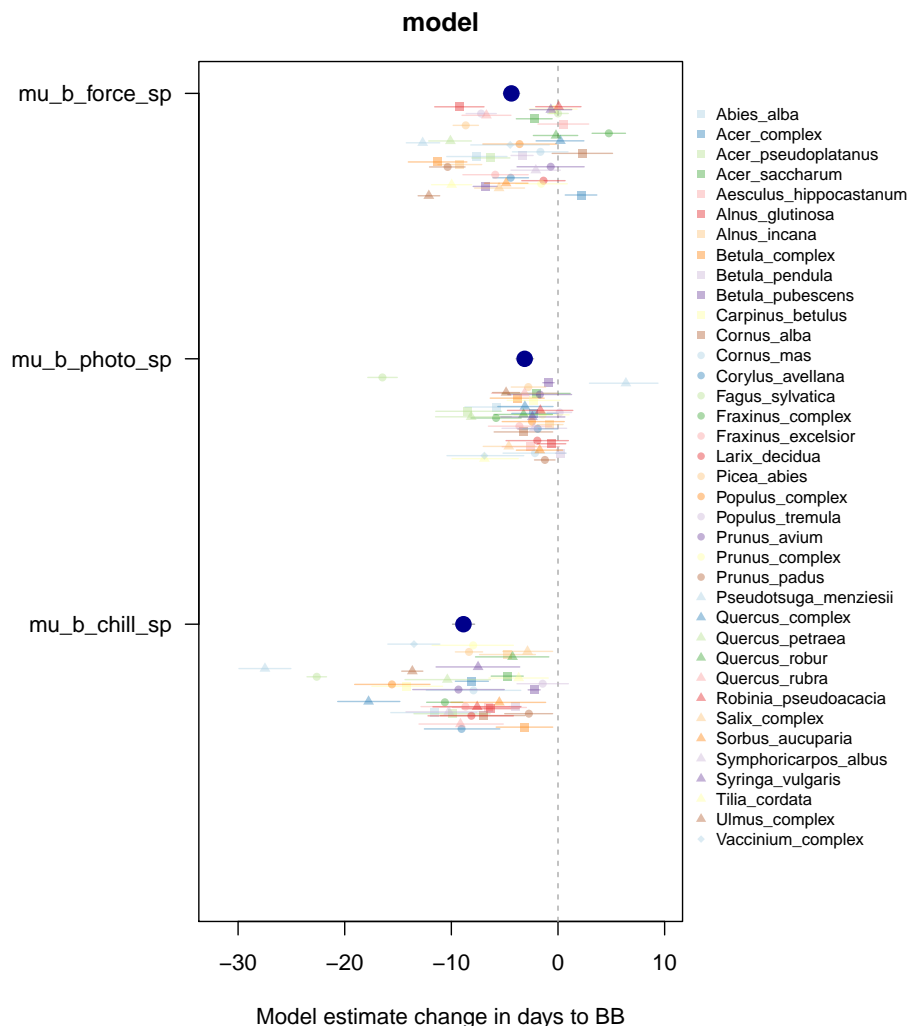
Figures

Figure 1: **Estimates for effects of chilling exceeded forcing and photoperiod estimates** in the budburst models fit to data from the OSPREE database. Here we show estimates from the model fit to centered data, enabling comparisons of effects sizes across predictors, and using Utah units to quantify chilling. Estimates to models fit to uncentered data and using Chill Portions were qualitatively similar and can be found in the Supplemental Materials.
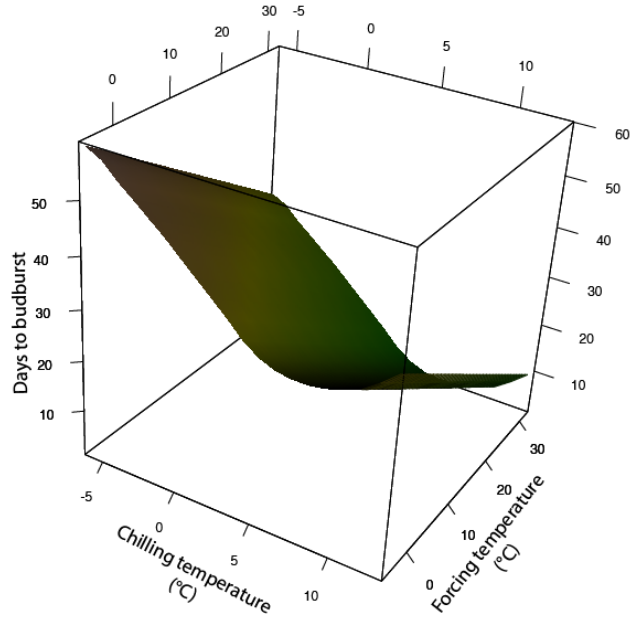
Figure 2: **Based on the OSPREE model, days to budburst decrease linearly with forcing temperature and vary nonlinearly with chilling temperature** due to the way that chilling is estimated (in this case, the Utah model; the model with Chill Portions is shown in the Supplemental Materials). Forcing treatment temperatures in growth chamber experiments ranged from 0-32 °C and chilling temperatures ranged from -10-16 °C(see Table 2S for details). Budburst responses predicted by the main budburst model are shown across the full range of experimental conditions in the OSPREE database with chilling calculated varying temperatures and durations, using field conditions across multiple sites within the distribution of *Betula pendula*, a European species that is one of the most common in OSPREE. See supplemental materials for details.
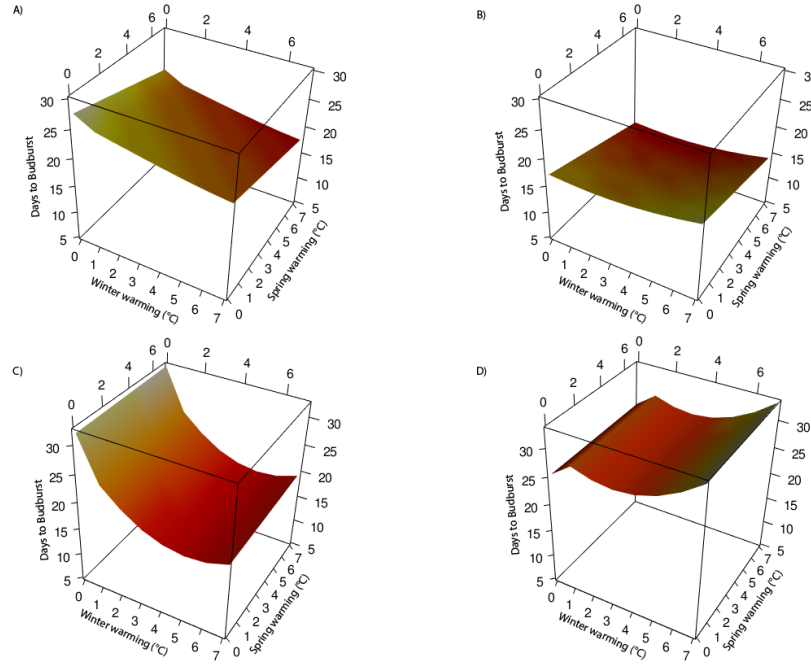
Figure 3: **Implications of global warming on budburst varies by species and site**, depending on pre-warming climate for the two most common species in the OSPREE database: *Betula pendula* (A,B) and *Fagus sylvatica* (C,D), as predicted by the OSPREE model. For sites in A and C, chilling increases with warming, whereas chilling decreases with warming for the sites in B and D. See Supplemental Materials, especially Fig. XX, for details.