

Chilling outweighs photoperiod and forcing cues for temperate trees in experiments

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Abstract

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. 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.

Original: 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 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, 2010*b*; 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, 2010*a*). 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.

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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 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 environmental cues beyond forcing (e.g., chilling and photoperiod) underlie declining temperature sensitivities Fu et al. (2015).

Fundamental research in phenology outlines how three major 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., 2011*b*; Zohner et al., 2016). Research suggests that all three cues may underlie spring phe-

nology 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, but have produced contrasting results (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 reviewed 193 papers from controlled environment studies, then extracted data from any papers 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. As field chilling was rarely reported, we estimated chilling using local climate data (see Supplemental Materials). 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 a higher-level 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 240 Utah units, which is typically about 10 days of chilling, 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 a weak or non-existent cue for many species (Zohner et al., 2016; Koerner and Basler, 2010a)—instead we found it was surprisingly large and consistent across species, even in a model accounting for its interaction with latitude (Fig. ??, see also Supplemental Materials for details, especially Fig. 1S). Only *Fagus sylvatica*, a species well-known for having a large response to photoperiod deviated far from the overall estimate (Fig. 1). Species also showed fairly consistent responses to chilling (variance = 2.07 days per 240 Utah units, 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 comparatively 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 (Vitasse et al., 2018; Güsewell et al., 2017). 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 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 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, daylength, and forcing to determining budburst timing in temperate woody species, with long-term climate and observational data, where forcing appears to dominate responses to recent warming. By combining experimental and long-term data we do not find strong evidence for delaying budburst in the near future, and suggest recent observed declines in forcing may be more 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 potential extensions to other temperate regions where winter warming does not lead to dramatic decreases in chilling. [Something about how these findings have widespread consequences for carbon sequestration.]

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Figures

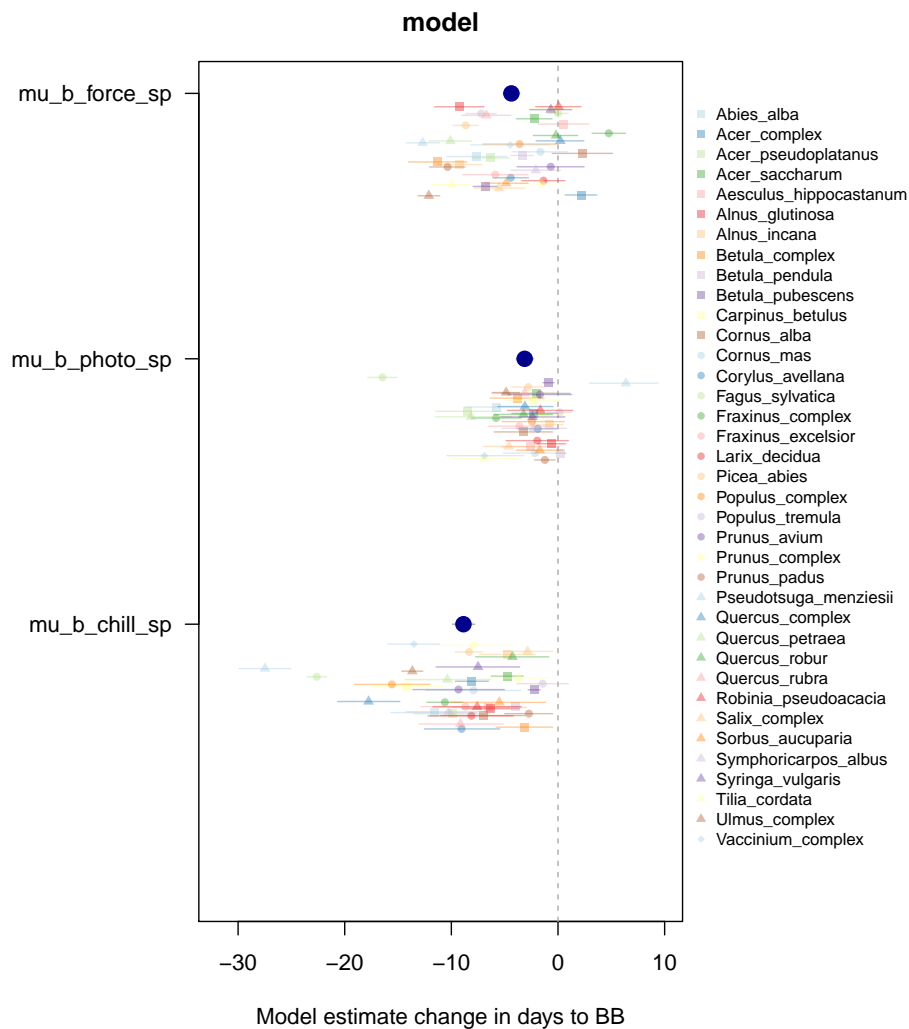


Figure 1: **Estimates for effects of chilling exceeded forcing and photoperiod estimates** in the budburst models fit to data from the OSPREE database. Here we show estimates from the model fit to centered data, enabling comparisons of effects sizes across predictors, and using Utah units to quantify chilling. Estimates to models fit to uncentered data and using Chill Portions were qualitatively similar and can be found in the Supplemental Materials.

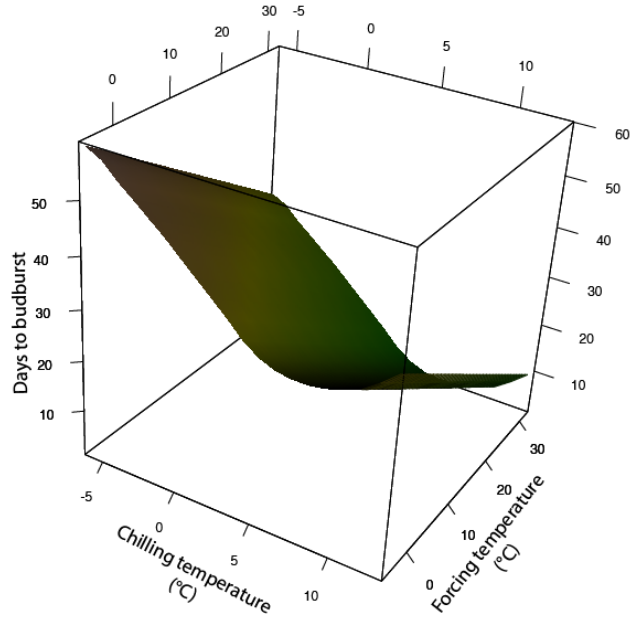


Figure 2: **Based on the OSPREE model, days to budburst decrease linearly with forcing temperature and vary nonlinearly with chilling temperature** due to the way that chilling is estimated (in this case, the Utah model; the model with Chill Portions is shown in the Supplemental Materials). Forcing treatment 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.

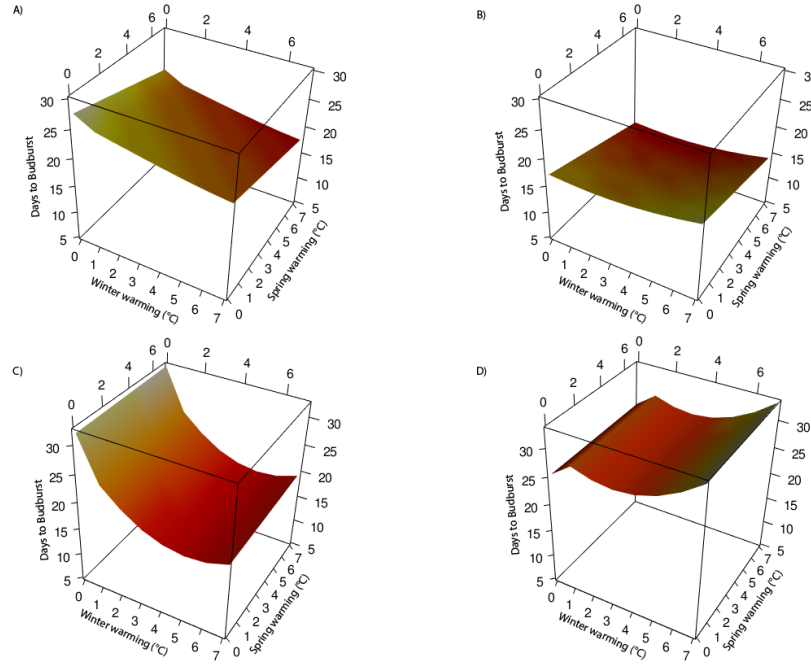


Figure 3: **Implications of global warming on budburst varies by species and site**, depending on pre-warming climate for the two most common species in the OSPREE database: *Betula pendula* (A,B) and *Fagus sylvatica* (C,D), as predicted by the OSPREE model. For sites in A and C, chilling increases with warming, whereas chilling decreases with warming for the sites in B and D. See Supplemental Materials, especially Fig. XX, for details.