

# Differences in flower and leaf bud responses to the environment drive shifts in spring phenological sequences of temperate woody plants

D.M. Buonaiuto<sup>1,2,a</sup>, E.M. Wolkovich<sup>3</sup>

## Abstract

The relative timing of growth and reproduction is an important driver of plant fitness. Deciduous woody species of temperate regions generally produce both leaves and flowers in the early spring but display significant variation in the order and duration of these phenological events among species, populations and individuals. These flower-leaf sequences (FLSs) appear to be shifting with climate change, which is likely to impact the reproduction, recruitment and survival of individuals and reshape forest communities. Predicting the effects FLS shifts require an improved understanding of how the environment dictates FLS patterns. We compared the phenological responses of flower and leaf buds to varying levels of temperature and light for a suite of temperate woody species to test two competing hypotheses regarding underlying physiology of FLS variation. We found that flower and leaf buds respond with differential sensitivity to environmental cues, with differences in their response to chilling being the dominant driver of FLS variation. Because climate change will amplify variability in chilling across time and space, these findings suggest that FLS shifts may be large, but are likely to vary substantially among populations and species. Our study indicates that wind-pollinated species that flower before leafing appear to be highly sensitive to negative fitness consequences of FLS shifts, and future FLS research should consider focusing on these taxa.

## Introduction

Among the most widely documented biological effects of anthropogenic climate change are shifts in phenology, the timing of life cycle events, in plants (Parmesan & Yohe, 2003; Menzel *et al.*, 2006; Cleland *et al.*, 2007). While phenology is generally advancing with climate change, the strength of these phenological shifts can vary substantially among specific phenological phases (Augspurger & Zaya, 2020). These differences alter the timing of phases relative to each other, changing the duration of inter-phase periods that make up phenological sequences (Ettinger *et al.*, 2018). As a major driver of plant fitness that impacts plant life history, resource allocation, demography and ecosystem processes (Post *et al.*, 2008), shifting phenological sequences with climate change will likely impact many of these processes. However the effects of these shifts depend both on their direction (whether distinct phases are shifting closer together or farther apart) and magnitude (how much they are shifting relative to each other).

Among deciduous woody plants, the relative timing of flower and leaf phenology, or flower-leaf sequences (FLSs), may be particularly consequential to fitness in temperate regions where flowering prior to leaf development is common (Rathcke & Lacey, 1985; Gougherty & Gougherty, 2018). Flowering before leafing may be a critical adaptation for pollination efficiency in wind-pollinated taxa by eliminating pollen interception by the forest canopy (Whitehead, 1969). In insect-pollinated taxa, flowering-first may increase the visibility of flowers to pollinators (Janzen, 1967; Savage, 2019) or alleviate hydraulic demand in dry conditions (Gougherty & Gougherty, 2018; Franklin, 2016).

Long-term phenological observations over the last several decades indicate that, like other phenological sequences, FLSs are shifting due to anthropogenic climate change (Buonaiuto *et al.*, 2020). For several species, the time between flowering and leafing appears to be increasing, but the strength of

this trend varies among species and the direction of FLS shifts are not consistent across populations (Buonaiuto *et al.*, 2020). These changes could affect the important functions of FLSs, potentially putting some species at greater risk for fitness declines while benefiting others.

Species with decreasing FLS interphases with climate change may see a reduction in pollination success as more pollen is intercepted by vegetative structures or flowers are obscured by developing leaves. Conversely, pollination efficiency could improve for species with lengthening FLS interphases (direction). A change in the FLS interphase of just a few days would likely have little impact on these processes, but if shifts were on the order of weeks, the impact on the pollination biology of a species could be highly significant (magnitude). For example, canopy structure influences the speed and direction of air flow (Niklas, 1985; Jackson & Lyford, 1999). For wind-pollinated taxa, decreasing FLS interphases could reduce pollen dispersal distances (Muller *et al.*, 2012), which would ultimately impact population structure and gene flow across a landscape.

Predicting the direction and magnitude of any FLS shifts requires identifying the underlying proximate mechanisms that drive responses to climate change among phenophases. Decades of research suggests that for woody plants in temperate regions, cool winter temperatures (chilling), warm spring temperatures (forcing) and day-length (photoperiod) are the primary drivers of both reproductive and vegetative phenology (Forrest & Miller-Rushing, 2010; Flynn & Wolkovich, 2018). However, observed FLS shifts indicate that there must be differences in how these cues influence phenological activity in floral and leaf buds (Buonaiuto *et al.*, 2020). Identifying these differences is a necessary step for predicting the direction, magnitude and—ultimately—fitness impacts of FLS shifts with climate change.

## Hypotheses for FLS variation

Studies that have attempted to identify the differences between reproductive and vegetative phenology in woody plants (mostly focused on crop species) have yielded two common explanations:

One hypothesis suggests that reproductive and vegetative buds utilize the same underlying environmental cues, but have different threshold responses to forcing, with whichever bud type bursts later—leaves or flowers—having a higher thermal requirement (duration of exposure to warming that triggers phenology) (Guo *et al.*, 2014; COSMULESCU & CALUSARU, 2020; Cosmulescu & Ionescu, 2018). Under this hypothesis, which we call the precocity hierarchy hypothesis (PHH), leaf and flower buds share the same suite of cues and develop similarly to non-forcing cues (i.e., chilling and photoperiod), but they differ in the thermal requirement for budburst.

In contrast, an alternative hypothesis suggests that flower and leaf buds differ in the strength of their phenological responses to the multiple environmental cues (Citadin *et al.*, 2001; Gariglio *et al.*, 2006; Aslamarz *et al.*, 2009; Mehlenbacher, 1991). Under this hypothesis, which we call the differential sensitivity hypothesis (DSH), each bud type relies more or less on certain cues. These differences generate FLS variability, even when leaf and flower buds are exposed to similar environmental conditions.

Under current field conditions, the PHH and DSH may produce similar phenological patterns but experiments that disentangle all three cues should differentiate between the two. A key signature of the PHH is that the sensitivity to forcing ( $\Delta$  day of phenological event/  $\Delta^\circ\text{C}$ ) of the second phase in the phenological sequence is 2x that of the first phase (Fig. 1 a.). By contrast, the signature of the DSH is that sensitivity estimates of flower and leaf buds to chilling and/or photoperiod will diverge,

but there is no characteristic pattern as to the strength or direction of this divergence. (Fig. 1 c.)

However, there are further complexities to differentiate these hypothesis. First, a species' buds could be both differentially sensitive to photoperiod or chilling and respond to forcing in a precocity hierarchy (Fig. 1 b.). Further, interactions between the chilling response and thermal requirements under the differential sensitivity framework can generate this signature of the PHH when secondary cues are at high levels (Fig. 1 c., chill x force interaction). It is therefore possible that the PHH is a special case of the DSH that occurs when the chilling and photoperiod requirements of both bud types have been met.

While the hypotheses may be indistinguishable under current field conditions, they have different implications regarding the potential for FLS shifts with climate change. The PHH suggests that FLS variation is largely a product of climate variation during the interphase. If spring temperatures increase with climate change, the second phenophase of the FLS will be accelerated relative to the first and the FLS interphases will decrease, but given the relative auto-correlation of spring temperatures (Di Cecco & Gouhier, 2018), these shifts should be relatively muted. If FLSs are structured by a precocity hierarchy, the direction of FLS shifts are relatively straight forward to predict, and it is less likely that shifts will be large enough in magnitude to significantly affect woody plant fitness.

The DSH suggests that with significant cue-use differences among bud types there will be strongly localized effects of climate change on FLSs. Shifts in FLS variation will depend on the direction and rate of change in cues at given locations and the species-specific differential sensitivity of reproductive and vegetative phenology to cue combinations. This hypothesis allows for larger magnitude shift in FLSs, on a scale that could impact pollen transport or the physiological functioning of woody plants. Further, the DSH also suggests that the magnitude of shifts may be highly divergent

both among species in a community, and among populations of the same species which could impact gene flow, population structure and demography.

In this study we tested PHH and DSH hypotheses via a fully factorial growth-chamber experiment manipulating chilling, forcing and photoperiod cues for flower and leaf buds of 10 temperate shrub and tree species. We then leveraged these data to project how FLSs may shift with climate change to identify species' characteristics associated with high magnitude FLS shifts.

## Methods

### Growth chamber study

We sampled all plant material from Harvard Forest in Petersham, MA (42.5314°N, 72.1900°W). On 25 October 2016, immediately after most plants in the area entered dormancy but before they could accumulate significant chilling in the field, we collected branch cuttings from 7-13 individuals of 12 woody plant species (4-12 cutting per individual for a total of 48-56 per species). The species consisted of a mix of deciduous shrubs, understory and canopy trees commonly found in mesic hardwood forests of the eastern United States (see tab.

We randomly assigned cuttings to a fully crossed set of eight experimental treatments; two levels of chilling (4 vs 8 weeks at 4°C), two levels of temperature (24°C:18°C (day/night) warm vs 18°C:12°C (day/night) cool) and two levels of photoperiod (12 vs 8 hours). We alternated day/night temperature periodicity on a 12 hour schedule to reduce co-variation with photo-periodicity. We re-cut all twig and changed the water every 7-10 days and rotated all treatments between growth chambers every two weeks to minimize chamber effects. We made phenological observations every 2-3 days

using a modified BBCH scale for woody plants (Finn *et al.*, 2007) for three months following release from chilling conditions. In this period we assess two phenological phases: budbreak (BBCH phase 07) and first flower open (BBCH 60). At the conclusion of this period we assessed all individuals that did not undergo budbreak and excluded 56 dead twigs from our analyses.

## Data analysis

To assess the sensitivity of each phase, we fit mixed-effect hierarchical models with chilling, forcing, photoperiod and all two-way interactions as the fixed effects and species as a grouping factor on both the slopes and the intercepts. We chose a Bayesian, hierarchical approach in order to identify systematic trends across species' responses while accounting for sample size, variance and the unique effect of each species. Two species *Betula allegheniensis* and *Acer saccharum* produced no flowers in our trial, so we excluded them from our analysis. In total, our analyses included 464 twigs from 10 species.

We modeled the effects of environmental parameters on flower opening and leaf budburst separately. We also fit a model with FLS interphase (day of budburst- day of flowering) as a response variable to compare these estimates with field observations.

The models we fit appear below:

$$y_{[i]} \sim N(\alpha_{sp[i]} + \beta_{forcing_{sp[i]}} + \beta_{chilling_{sp[i]}} + \beta_{photoperiod_{sp[i]}} + \beta_{forcing \times chilling_{sp[i]}} + \beta_{forcing \times photoperiod_{sp[i]}} + \beta_{chilling \times photoperiod_{sp[i]}})$$

Where  $y_{[i]}$  is either the day of the experiment leaf budburst, day of first flower opening or FLS

interphase length. We modeled the  $\alpha$  and each  $\beta$  parameter at the species level using the formula:

$$\alpha_{x_{sp}} \text{ or } \beta_{x_{sp}} \sim N(\mu_x, \sigma_x^2)$$

To test our hypothesis that the PHH is a special case of the DSH that occurs when all secondary cues requirements are met, we re-ran our models on a subset of our data which included both levels of forcing treatment but only the high photoperiod and chilling treatment levels. This model included forcing as the only main effect but, like our main models written above, included species as a grouping factor on the model slope and intercept.

We fit all models using the R package “brms” (Bürkner, 2018). We ran each model on four chains with 4000 iterations with a 3000 iteration warm up for a total of 4000 posterior draws for each parameter. In all models we used weakly informative priors and increasing the priors 5-fold did not affect the model results. We assessed model performance through ensuring  $\hat{R}$  were close to 1 and bulk and tail effective sample sizes were high (1800-2800 for most parameters, but as low as 800-900 for some.)

## Climate change predictions

To apply our model results to general climate change projections we chose our environmental treatments in this experiment to broadly reflect historic and future conditions at our sampling site. Our low forcing treatment approximated average spring temperature (March/April) at the site while our high temperature treatment reflects a 5 °C increase. Average field chilling (calculated from 15 Oct - 15 April, measured in Utah units) at Harvard Forest is 979.64, approximately 60% of the



185 difference between our low and high chilling treatment (Fig.

186 We used our flower and budburst models to project for each species in our study:

188 1. FLSs under average environmental conditions (treatments: low forcing, 6.5 weeks of chilling  
189 treatment)

190 2. FLS shifts with spring warming only (high forcing, 6.5 weeks of chilling treatment)

191 3. FLS shifts with warming and increased chilling (high forcing, 8 weeks of chilling treatment)

192 4. FLS shifts with warming and decreased chilling (high forcing, 4 weeks of chilling treatment)

193 To validate our predictions, we compared our FLS interphase model estimates of “average” condition  
194 FLS interphases to long term phenological records from Harvard Forest (O’Keefe, 2015) for five  
195 species common to both datasets (Fig.

196 To characterize FLS shifts for the three broad FLS groups (flowering first, concurrent, leafing-first)  
197 we extracted all sampling posterior estimates from our main models using the R package tidybayes  
198 (Kay, 2020), and grouped them by FLS pattern. We also include the species-specific estimates on  
199 which these grouped projects are base in Fig. ??.

## 201 Results

### 202 Growth chamber study

203 Both flower and leaf buds advanced with higher forcing and longer chilling duration (flowers– chill-  
204 ing effect: -21.23, forcing effect: -18.98, leaves– chilling effect: -30.41, forcing effect: -17.76), but

increases in both of these cues together offset these advances (flowers– force x chill effect: 7.01, leaves– force x chill effect: 12.37) (Fig. 2, Tab. 1). Leaf and flower buds diverged in their responses to increasing photoperiod, with flower phenology advancing and leaf phenology being delayed when the other two cues were at low levels (Fig. 2, Tab. 1). As seen in the interactions between photoperiod and chilling and photoperiod and forcing, increasing chilling or forcing with longer photoperiod advanced the phenology of both bud types. For both bud types, chilling and forcing were the dominant cues, while increasing photoperiod produced a more muted phenological response (Fig. 2, Tab. 1).

While leaf and flower bud phenological responses to environmental cues were qualitatively similar, the strength of their responses to each cue differed substantially. Leaf buds responded more strongly to chilling than flower buds (1.4x) , and had a stronger response to all cue interactions (forcing x chilling: 1.8x, photoperiod x chilling: 5.9x, photoperiod x forcing: 2.2x) (Fig. 2, Tab. 1). Across all species both bud types displayed a relatively proportionate advance with increased forcing. (Fig. 2, Tab. 1)

While there was significant variation among species in their strength of their response to forcing between bud types, no species displayed the characteristic sensitivity pattern of the PHH in which the sensitivity to forcing of the second phase twice as strong as the sensitivity of the first phase (Fig. 2), see Fig. 1,a.,b.). Rather, the differences in the strength of the responses of each bud type to each environmental cue combination reflects the DSH. However, when re-ran our models on the subset of data which included phenological observations at only high levels of chilling and photoperiod, we found the the sensitivity to forcing for most species followed with predicted pattern of the PHH, with the second phase of the FLS showing approximately double the sensitivity to forcing than the first phases (Fig. 3, Tab. 2).

## 231 Climate change predictions

232 Our model predict that both flower and leaf phenology will advance in most of our generalized  
 233 scenarios, but FLS shifts depended strongly on how forcing levels change relative to chilling ex-  
 234 posure (Fig. 4). Given the significant differences in sensitivity to chilling between flowering and  
 235 leafing phenology we found in our experiment, our projects showed that FLS interphases were more  
 236 strongly influenced by changes in chilling duration than increased forcing alone.

237

238 The direction and magnitude of shifts in FLS interphases depended on species and the specifics of  
 239 FLS phase order. Under some warming scenarios, our model predicted that FLS interphases for  
 240 some species may effectively disappear or the order of phenophases in the FLS may switch (Fig. 4,  
 241 Fig. ??). Several species, *Acer rubrum*, *Ilex verticillata*, *Prunus pensylvanica*, *Prunus virginiana*,  
 242 and *Viburnum acerifolium*, had FLSs that were relatively robust to changing environments. For  
 243 other species, *Acer pensylvanicum*, *Vaccinium corymbosum* and *Ilex mucronata*, which typically  
 244 begin to produce leaves shortly before flowers open, the magnitudes of projected FLS shifts were  
 245 moderate. The two species with the most significant FLS shifts in both direction and magnitude  
 246 across treatment combinations and climate change projections were *Comptonia peregrina* and *Cory-*  
 247 *lus cornuta* (Fig. ??). In all of our climate change scenarios, the FLS interphase was dramatically  
 248 reduced in these taxa.

249

## Discussion

Our experiments support the hypothesis that flower-leaf sequences are structured by differential sensitivity (DSH) to the environment between flower and leaf buds. Specifically, differences in the chilling response among buds types was the strongest driver of FLS variation. These result suggest that climate change has potential to significantly disrupt FLSs as global warming alters historic chilling patterns across the temperate zone. Yet, under the high chilling and photoperiod treatments, we found that FLSs for most species followed the predicted sensitivity pattern of the PHH, with the sensitivity to forcing of the second phase of the FLS approximately twice that of the first phase (Fig.3, Tab. 2). This may explain why the two FLS hypotheses have been difficult to distinguish under historic field conditions where in most locations chilling requirements were frequently met (Gauzere *et al.*, 2019). In conjunction with site-specific FLS shifts and species-specific FLS functions, this difficulty of assessing differential sensitivity in contemporary field conditions suggests there is a need for generalizing principles to anticipate the impacts of FLS shifts.

## Reconciling the differential sensitivity and the precocity hierarchy hypotheses

The strong differential sensitivity to chilling between flower and leaf buds we found in our study reveals a possible mechanistic link between the DSH and PHH, and offers insight into why these hypotheses have been difficult to differentiate in the past. Our data show that the PHH can be considered to be a special case of the DSH– when the chilling requirement for both flower and leaf buds is met, an individual appears to follow the predicted pattern of PHH, with temperature during the FLS interphase dictating the inter-annual variability in FLSs. Long term studies suggest that under historic climate conditions, chilling requirements were generally met (Gauzere *et al.*, 2019; Chuine *et al.*, 2016), which may explain why support for the PHH most often associated with

observational studies (e.g. COSMULESCU & CALUSARU, 2020; Guo *et al.*, 2014). This is consistent with findings in other phenological studies that suggest simple growing degree models (which underlie the PHH) accurately predict phenology under current climate, but under-perform under climate change scenarios when shifts in chilling accumulation become more pronounced (Linkosalo *et al.*, 2008; Chuine *et al.*, 2016).

By contrast, experimental studies which manipulate chilling levels beyond that which was historically observed in the field tend to support the DSH (e.g. Aslamarz *et al.*, 2009; Gariglio *et al.*, 2006). The results of our study in wild species are consistent with experimental manipulations of tree-crop phenology which also found a higher sensitivity to chilling for leaf buds (Gariglio *et al.*, 2006; Citadin *et al.*, 2001). Our findings suggest that as climate continues to change, differential sensitivity to the environment between flower and leaf phenology should become more apparent in field observations, and that individual FLS variation is likely to extend beyond historically observed reaction norms.

## Population-level implications of the DSH with climate change

The strong differential sensitivity to chilling between flower and leaf buds we found in our study suggests complex FLS dynamics with climate change. Predicted shifts in chilling are highly variable across both time and space—because chilling only accumulates at intermediately low temperatures, warming may increase chilling at some locations while decreasing it in others (Man *et al.*, 2017; Zhang *et al.*, 2007). This suggests that the direction and magnitude of FLS shifts is likely to vary substantially among populations based on the specific cue combinations at a given locality (Chmielewski *et al.*, 2012). Long-term phenology records show there was already substantial intra-specific variation in FLSs at the population level (Buonaiuto *et al.*, 2020) and our findings suggest

that these populations level differences may be further amplified by climate change. In this way, all the three generic FLS climate change scenarios depicted in Fig. 4 should not be considered alternatives to each other, but rather, could occur contemporaneously across a species' range.

Population-level heterogeneity has potential to influence patterns of pollen dispersal across the landscape (Borycka *et al.*, 2017; Pace *et al.*, 2018). For example, advancing canopy closure relative to flowering can impede long-distance pollen transport (Milleron *et al.*, 2012), which may in turn alter patterns of gene flow across a landscape. Our findings regarding FLS shifts should be integrated with projections of pollinator movement or prevailing wind directions (Kling & Ackerly, 2020) to better understand how FLS variation may contribute to population structure in the long term.

The implications of our study's observed differential sensitivity to photoperiod to FLS shifts with climate change are more difficult to characterize. Climate change does not directly impact photoperiod, but warming does shift the time of year when plants become phenologically active, changing the photoperiod they experience. However, depending on the latitude, phenology would have to shift by at minimum several weeks before the experienced photoperiod would change substantially (Us, in review). For this reason we modeled climate change scenarios with a constant photoperiod in our FLS projections with climate change, but at high latitudes where photoperiod changes more rapidly over the season, the experienced photoperiod may mute or amplify the FLS shifts captured in our projections. This may be particularly important as species shift their distribution poleward with climate change and begin to encounter novel photoperiod regimes (WAY & MONTGOMERY, 2015).

## Species-level implications of the DSH with climate change

Our study highlights that the direction and magnitude of FLS shifts with climate change are species-specific. Not only is it likely that the function of FLS variation differs among species (Buonaiuto *et al.*, 2020), but we found that FLSs of some species were very sensitive to changing climate conditions while other remain fairly resilient (Fig. 4, Fig. ??).

These differences suggest that some FLS shifts will impact some species more than others, and researchers should focus their efforts towards species or populations that are likely to be most vulnerable. However, identify vulnerable species is challenging. At present, observational studies cannot capture the magnitude of FLS shifts with climate, and using artificial environments to manipulate FLSs for all species of interest is unfeasible. Therefore, there is a strong need for generalizing principles to identify species with potential for consequential FLS shifts with climate change. While one study cannot begin to represent the taxonomic diversity of a temperate forest, we identified several patterns in the FLS responses of our multi-species experiment that may serve as starting point for further inquiry.

It is likely that the different FLS response patterns we observed correlate to broader anatomical, physiological and phenological differences among species. The species that maintained FLS structure across climate change scenarios generally shared a strongly leafing-first FLS, with a fairly long FLS interphase (Fig 4). These species tended to have mixed buds so there may be strong physical constraints on their FLSs. By contrast, the species that were most sensitive to FLS shifts were monoecious, flowering-first, wind-pollinated shrubs (Fig 4, Fig ??). This result may reflect other evidence that the reproductive phenology of wind-pollinated species appears to be more sensitive to climate change than for biotically pollinated taxa (Ziello *et al.*, 2012). Given the hypothesized

function of FLS in wind-pollinated species, the significant reductions in FLS interphases we observed could suggest that these species, and flowering-first, wind-pollinated taxa in general, may face particular risk for reproductive performance reductions.

While much of the conversation around phenology and pollination in the context of global change has centered around trophic mismatches between pollinator and floral phenology (Memmott *et al.*, 2007), which is of little relevance to abiotically pollinated taxa, our study identified the possibility that the effect of FLS shifts with climate change may be particularly important for wind-pollinated woody plants. The direction and magnitude of FLS shifts we observed in these taxa, coupled with the hypothesized function of a flowering-first FLS in wind-pollinated species, suggests that FLS variation in this functional group should be explored in greater detail in the future. More research is needed to identify species' traits that may correlate with the potential for FLS shifts, but flowering-first, wind-pollinated species may be particularly sensitive to FLS shifts, and species in this functional group should be considered a research priority for the study of spring phenological sequences in deciduous, woody plants.

## References

- Aslamarz, A.A., Vahdati, K., Rahemi, M. & Hassani, D. (2009) Estimation of chilling and heat requirements of some persian walnut cultivars and genotypes. *HortScience* **44**, 697–701.
- Augspurger, C.K. & Zaya, D.N. (2020) Concordance of long-term shifts with climate warming varies among phenological events and herbaceous species. *Ecological Monographs* **n/a**, e01421.
- Borycka, K., Ortyl, B. & Kasprzyk, I. (2017) Temporal variations and spatial differentiation in the



black alder and silver birch pollination pattern-the impact of local climate or something more?

*AGRICULTURAL AND FOREST METEOROLOGY* **247**, 65–78.

Buonaiuto, D.M., Morales-Castilla, I. & Wolkovich, E.M. (2020) Reconciling competing hypotheses regarding flower–leaf sequences in temperate forests for fundamental and global change biology. *New Phytologist* **n/a**.

Bürkner, P.C. (2018) Advanced bayesian multilevel modeling with the r package brms. *R Journal* **10**, 395–411.

Chmielewski, F.M., Blümel, K. & Pálesová, I. (2012) Climate change and shifts in dormancy release for deciduous fruit crops in germany. *Climate Research* **54**, 209–219.

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

Citadin, I., Raseira, M.C.B., Herter, F.G. & da Silva, J.B. (2001) Heat requirement for blooming and leafing in peach. *HortScience HortSci* **36**, 305–307.

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

COSMULESCU, S. & CALUSARU, F.G. (2020) Influence of temperature on blackthorn (*prunus spinosa* l.) phenophases in spring season. **advpub**, –.

Cosmulescu, S. & Ionescu, M. (2018) Phenological calendar in some walnut genotypes grown in romania and its correlations with air temperature. *International Journal of Biometeorology* **62**.

Di Cecco, G.J. & Gouhier, T.C. (2018) Increased spatial and temporal autocorrelation of temperature under climate change. *Scientific Reports* **8**, 14850.

- Ettinger, A., Gee, S. & Wolkovich, E. (2018) Phenological sequences: how early season events define those that follow. *American Journal of Botany* **105**.
- Finn, G.A., Straszewski, A.E. & Peterson, V. (2007) A general growth stage key for describing trees and woody plants. *Annals of Applied Biology* **151**, 127–131.
- Flynn, D.F.B. & Wolkovich, E.M. (2018) Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytologist* **219**, 1353–1362.
- Forrest, J. & Miller-Rushing, A.J. (2010) Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 3101–3112.
- Franklin, D.C. (2016) Flowering while leafless in the seasonal tropics need not be cued by leaf drop: evidence from the woody genus brachychiton (malvaceae). *Plant Ecology and Evolution* **149**, 272–279.
- Gariglio, N., González Rossia, D.E., Mendow, M., Reig, C. & Agustí, M. (2006) Effect of artificial chilling on the depth of endodormancy and vegetative and flower budbreak of peach and nectarine cultivars using excised shoots. *Scientia Horticulturae* **108**, 371–377.
- Gauzere, J., Lucas, C., Ronce, O., Davi, H. & Chuine, I. (2019) Sensitivity analysis of tree phenology models reveals increasing sensitivity of their predictions to winter chilling temperature and photoperiod with warming climate. *Ecological Modelling* **411**, 108805.
- Gougherty, A.V. & Gougherty, S.W. (2018) Sequence of flower and leaf emergence in deciduous trees is linked to ecological traits, phylogenetics, and climate. *New Phytologist* **220**, 121–131.
- Guo, L., Luedeling, E., Dai, J. & Xu, J. (2014) Differences in heat requirements of flower and leaf buds make hysteranthous trees bloom before leaf unfolding. *Plant Diversity and Resources* **36**, 245–253.

- 411 Jackson, S.T. & Lyford, M.E. (1999) Pollen dispersal models in quaternary plant ecology: Assump-  
412 tions, parameters, and prescriptions **65**, 39–75.
- 413 Janzen, D.H. (1967) Synchronization of sexual reproduction of trees within the dry season in central  
414 america. *Evolution* **21**, 620–637.
- 415 Kay, M. (2020) *tidybayes: Tidy Data and Geoms for Bayesian Models*. R package version 2.1.1.
- 416 Kling, M.M. & Ackerly, D.D. (2020) Global wind patterns and the vulnerability of wind-dispersed  
417 species to climate change. *Nature Climate Change* **10**, 868–875.
- 418 Linkosalo, T., Lappalainen, H.K. & Hari, P. (2008) A comparison of phenological models of leaf  
419 bud burst and flowering of boreal trees using independent observations. *Tree Physiology* **28**,  
420 1873–1882.
- 421 Man, R., Lu, P. & Dang, Q.L. (2017) Insufficient chilling effects vary among boreal tree species and  
422 chilling duration. *Frontiers in Plant Science* **8**, 1354.
- 423 Mehlenbacher, S.A. (1991) Chilling requirements of hazelnut cultivars. *Scientia Horticulturae* **47**,  
424 271–282.
- 425 Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007) Global warming and the disruption  
426 of plant–pollinator interactions. *Ecology Letters* **10**, 710–717.
- 427 Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kuebler, K., Bissolli, P.,  
428 Braslavska, O., Briede, A., Chmielewski, F.M., Crepinsek, Z., Curnel, Y., Dahl, A., Defila, C.,  
429 Donnelly, A., Filella, Y., Jatcza, K., Mage, F., Mestre, A., Nordli, O., Penuelas, J., Pirinen, P.,  
430 Remisova, V., Scheifinger, H., Striz, M., Susnik, A., Van Vliet, A.J.H., Wielgolaski, F.E., Zach,  
431 S. & Züst, A. (2006) European phenological response to climate change matches the warming  
432 pattern. *Global Change Biology* **12**, 1969–1976.

- 433 Milleron, M., Lopez de Heredia, U., Lorenzo, Z., Perea, R., Dounavi, A., Alonso, J., Gil, L. &  
 434 Nanos, N. (2012) Effect of canopy closure on pollen dispersal in a wind-pollinated species (*Fagus*  
 435 *sylvatica* L.). *Plant Ecology* **213**, 1715–1728.
- 436 Niklas, K.J. (1985) The aerodynamics of wind pollination. *The Botanical Review* **51**, 328–386.
- 437 O’Keefe, J. (2015) *Phenology of Woody Species at Harvard Forest since 1990*. Harvard Forest Data  
 438 Archive: HF003., Petersham, MA, USA.
- 439 Pace, L., Boccacci, L., Casilli, M., Di Carlo, P. & Fattorini, S. (2018) Correlations between weather  
 440 conditions and airborne pollen concentration and diversity in a mediterranean high-altitude site  
 441 disclose unexpected temporal patterns. *Aerobiologia* **34**, 75–87.
- 442 Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across  
 443 natural systems. *Nature* **421**, 37 EP –.
- 444 Post, E.S., Pedersen, C., Wilmers, C.C. & Forchhammer, M.C. (2008) Phenological sequences reveal  
 445 aggregate life history response to climatic warming **89**, 363–370.
- 446 Rathcke, B. & Lacey, E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of*  
 447 *Ecology and Systematics* **16**, 179–214.
- 448 Savage, J.A. (2019) A temporal shift in resource allocation facilitates flowering before leaf out and  
 449 spring vessel maturation in precocious species. *American Journal of Botany* **106**, 113–122.
- 450 WAY, D.A. & MONTGOMERY, R.A. (2015) Photoperiod constraints on tree phenology, perfor-  
 451 mance and migration in a warming world. *Plant, Cell & Environment* **38**, 1725–1736.
- 452 Whitehead, D.R. (1969) Wind pollination in the angiosperms: Evolutionary and environmental  
 453 considerations. *Evolution* **23**, 28–35.
- 454 Zhang, X., Tarpley, D. & Sullivan, J.T. (2007) Diverse responses of vegetation phenology to a  
 455 warming climate. *Geophysical Research Letters* **34**.

456 Ziello, C., Böck, A., Estrella, N., Ankerst, D. & Menzel, A. (2012) First flowering of wind-pollinated  
 457 species with the greatest phenological advances in europe. *Ecography* **35**, 1017–1023.

458 **Tables**

|             | Estimate | Est.Error | Q25    | Q75    |
|-------------|----------|-----------|--------|--------|
| Intercept   | 70.81    | 9.18      | 64.94  | 76.88  |
| Chill       | -30.41   | 5.40      | -33.89 | -27.15 |
| Light       | 5.87     | 5.13      | 2.42   | 9.16   |
| Force       | -17.76   | 5.21      | -21.10 | -14.29 |
| Chill:Light | -5.17    | 4.35      | -8.03  | -2.31  |
| Chill:Force | 12.37    | 4.84      | 9.26   | 15.51  |
| Light:Force | -12.62   | 4.10      | -15.37 | -9.87  |

|             | Estimate | Est.Error | Q25    | Q75    |
|-------------|----------|-----------|--------|--------|
| Intercept   | 77.53    | 9.92      | 71.05  | 83.88  |
| Chill       | -21.23   | 7.42      | -26.14 | -16.32 |
| Light       | -5.72    | 5.70      | -9.01  | -2.03  |
| Force       | -18.98   | 6.51      | -23.02 | -14.93 |
| Chill:Light | -0.88    | 6.11      | -4.72  | 3.21   |
| Chill:Force | 7.01     | 6.62      | 2.98   | 11.11  |
| Light:Force | -5.61    | 6.42      | -9.51  | -1.46  |

Table 1: Phenological sensitivity of leaf (top) and flower (bottom) buds to environmental cues.  
 Caption needs work.

459 **Figures**

|    | Species | Estimate | error | Q25    | Q75    | phase        | sequence |
|----|---------|----------|-------|--------|--------|--------------|----------|
| 1  | ACE.PEN | -10.71   | 3.92  | -13.48 | -8.19  | vegetative   | first    |
| 2  | ACE.PEN | -17.43   | 6.15  | -20.68 | -14.00 | reproductive | second   |
| 3  | ACE.RUB | -16.76   | 7.25  | -20.21 | -13.09 | reproductive | first    |
| 4  | ACE.RUB | -28.39   | 6.22  | -32.69 | -24.08 | vegetative   | second   |
| 5  | COM.PER | -13.28   | 3.33  | -15.50 | -11.17 | reproductive | first    |
| 6  | COM.PER | -15.47   | 3.69  | -17.82 | -13.01 | vegetative   | second   |
| 7  | COR.COR | -15.55   | 4.50  | -18.13 | -12.87 | reproductive | first    |
| 8  | COR.COR | -19.82   | 4.04  | -22.41 | -17.10 | vegetative   | second   |
| 9  | ILE.MUC | -10.44   | 3.81  | -13.09 | -8.05  | vegetative   | first    |
| 10 | ILE.MUC | -16.05   | 4.06  | -18.58 | -13.47 | reproductive | second   |
| 11 | ILE.VER | -8.66    | 3.73  | -11.19 | -6.19  | vegetative   | first    |
| 12 | ILE.VER | -20.43   | 10.72 | -25.92 | -14.18 | reproductive | second   |
| 13 | PRU.PEN | -10.24   | 4.14  | -12.99 | -7.50  | vegetative   | first    |
| 14 | PRU.PEN | -13.85   | 4.02  | -16.46 | -11.40 | reproductive | second   |
| 15 | PRU.VIR | -26.68   | 5.11  | -30.02 | -23.09 | vegetative   | first    |
| 16 | PRU.VIR | -23.69   | 7.67  | -28.74 | -17.84 | reproductive | second   |
| 17 | VAC.COR | -7.06    | 3.85  | -9.62  | -4.56  | vegetative   | first    |
| 18 | VAC.COR | -13.10   | 3.60  | -15.49 | -10.79 | reproductive | second   |
| 19 | VIB.ACE | -12.68   | 3.78  | -15.14 | -10.29 | vegetative   | first    |
| 20 | VIB.ACE | -21.60   | 8.52  | -26.63 | -16.00 | reproductive | second   |

Table 2: Phenological sensitivity of leaf and flower buds to forcing at high levels of chilling and forcing. Caption needs work.

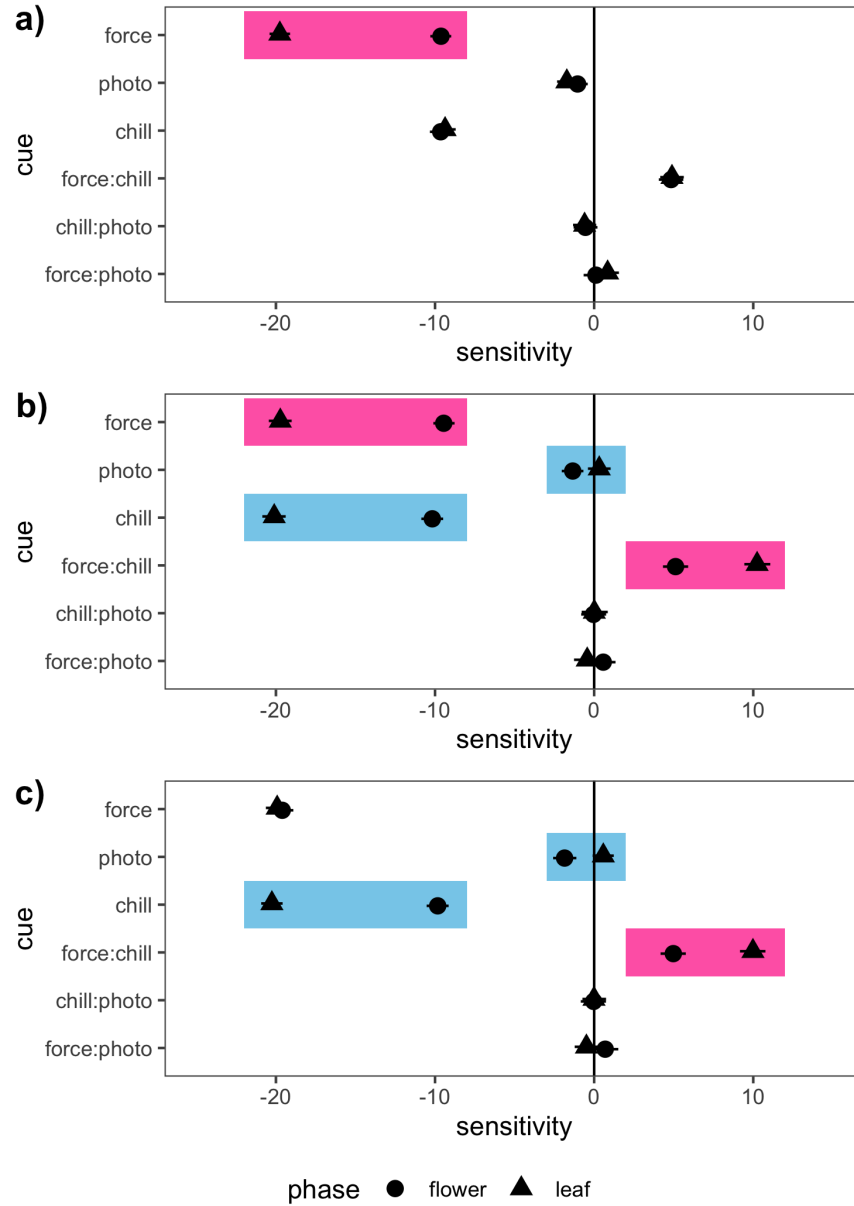


Figure 1: **Characteristic sensitivity ( $\Delta$  day of phenological event/  $\Delta$  environmental cue) patterns of the phenological response to changing cues level for the two major flower-leaf sequence hypotheses. a)** displays a signature pattern of the precocity hierarchy hypothesis (PHH) (pink boxes) — with the second phenophases in the sequence (in this case leafing) having twice the sensitivity to forcing than the first. **b)** depicts a scenario where both the PHH and the differential sensitivity hypothesis (DSH) contribute to flower-leaf sequence variation. Here the characteristic forcing sensitivity of the PHH is still apparent but the differential sensitivity to chilling and photoperiod is observable as well (blue boxes). **c)** highlights a typical sensitivity pattern produced by the DSH without the PHH. However the forcing x chill interaction suggests that at high levels of chilling, the response to forcing would follow the characteristic PHH response, demonstrating that the two hypotheses are difficult to disentangle. All plots above are based on simulations. We simulated the PHH by assigning flowering a lower thermal requirement ( $F^*$ ) than leafing but assigned similar responses to chilling and photoperiod variation. In **b)** we maintain the differences in  $F^*$  values between flowering and leafing but also assigned them different responses to forcing and photoperiod. For simulation of the DSH only scenario, we assigned identical  $F^*$  values to both phenophases but maintained differences in their chilling and photoperiod responses. We produced the plots using Bayesian hierarchical models to evaluate the phenological sensitivity of flower and leaf buds under each of these scenarios. Points are the mean estimates and lines represent the 95% credible intervals.

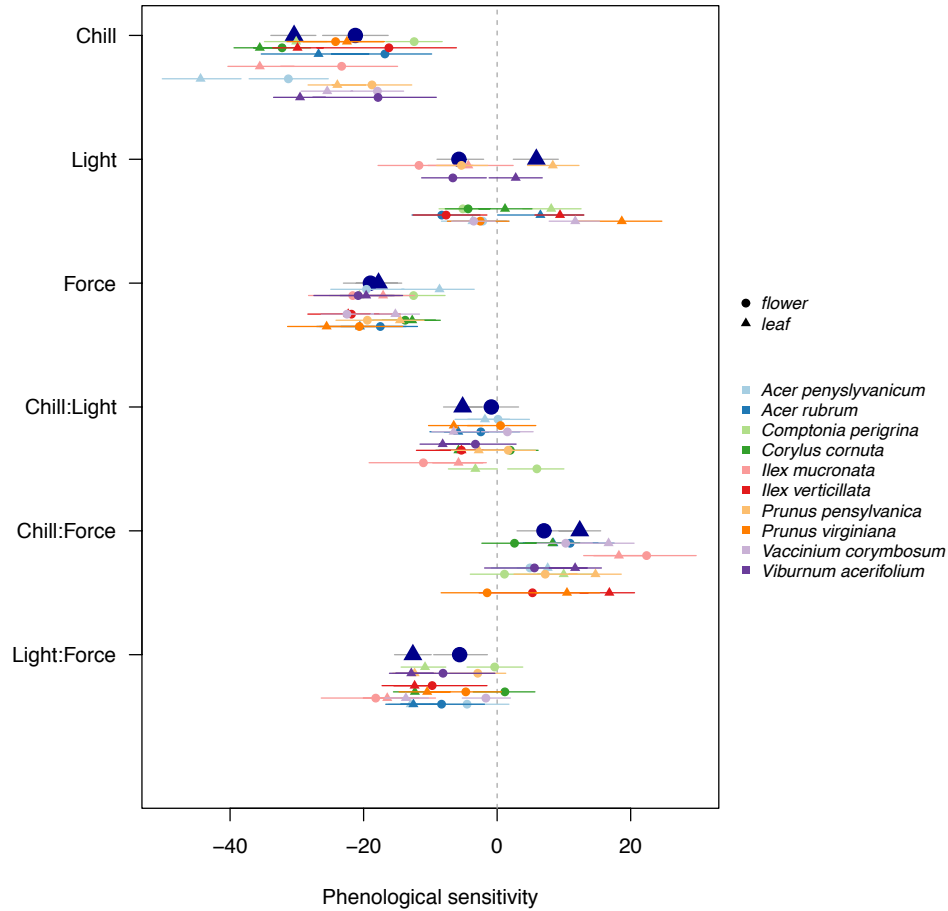


Figure 2: **Experimental results suggest differential sensitivity to environmental cues between flower and leaf buds.** We used a growth chamber manipulation and Bayesian hierarchical models to evaluate the phenological sensitivity ( $\Delta$  day of phenological event/  $\Delta$  environmental cue) of flower and leaf buds to varying forcing temperatures, photoperiods, and duration of chilling. Vegetative buds (circles) were more sensitive to chilling and cue interactions. Flower buds (triangles) advanced with photoperiod increases under all treatment combinations but leaf phenology was delayed with increasing photoperiod when chilling and forcing levels were low. Points indicate mean estimates and lines represent the 50% credible intervals. These differential sensitivities dictate how FLS patterns vary with changing environmental conditions.



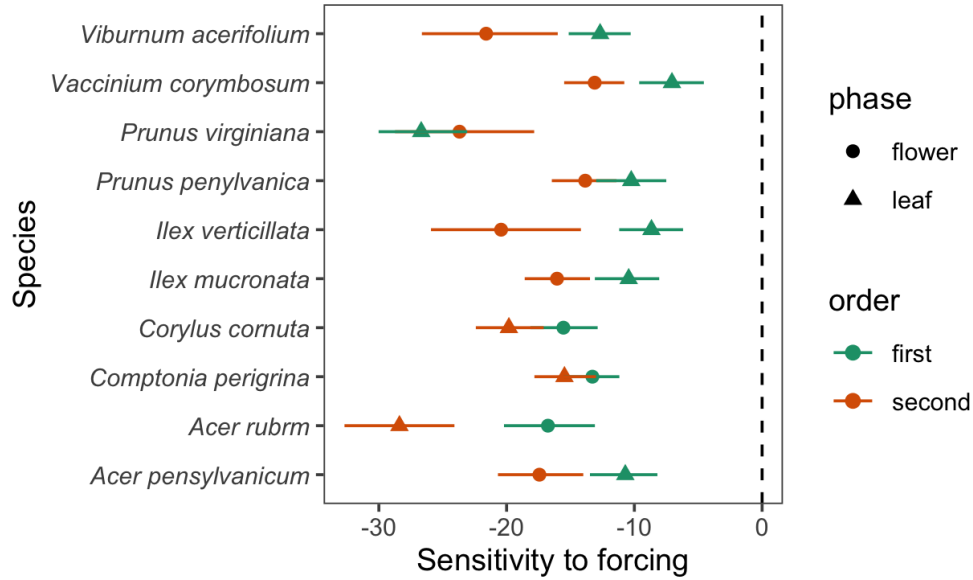


Figure 3: Under adequately long chilling duration and photoperiods, the phenological sensitivity ( $\Delta$  phenological event/  $\Delta$  C°) follow the predicted pattern of the precocity hierarchy hypothesis (PHH), with the second phenophase of the sequence being approximately twice as sensitive to forcing as the first. After performing a growth chamber manipulation evaluate the phenological sensitivity of flower and leaf buds to varying level forcing temperatures, photoperiods, and duration of chilling, we subset out data to include only observation at high chilling and photoperiod levels. Using Bayesian hierarchical models, we quantified the differences in sensitivity to forcing for all species in our study. Points indicate mean estimates and lines depict 50% credible intervals. Our finding indications that the PHH should be considered a special case of the differential sensitivity hypothesis (DSH) that occurs when the chilling and photoperiod requirements are well met for both bud types.

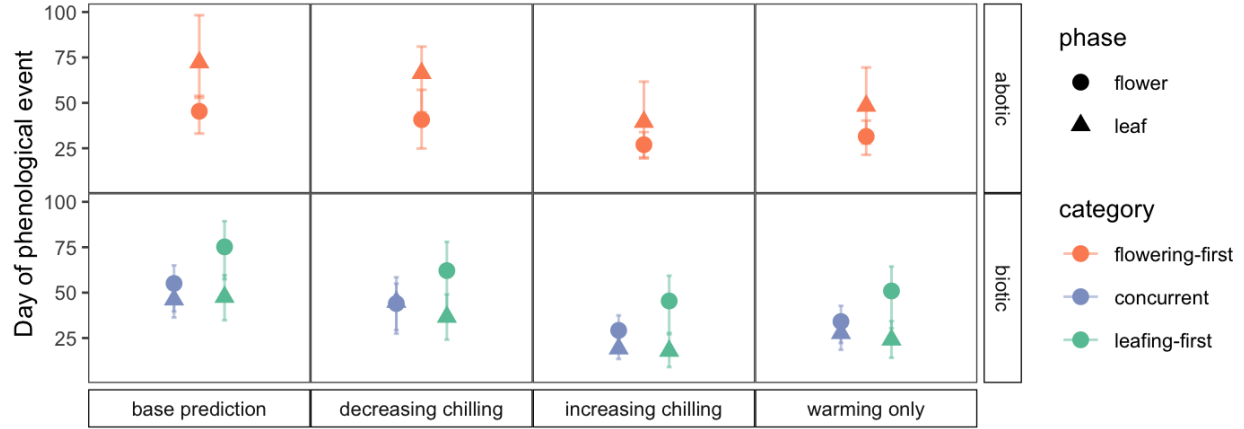


Figure 4: **Flower-leaf sequences (FLSs) of temperate, woody species will shift with climate change, but the magnitudes of these shifts vary by among FLS categories and depend on the specific dynamics of temperature at a given location.** We used Bayesian, hierarchical models comparing flower and leaf bud responses to variable temperature combinations to predict FLSs patterns under current climate conditions and three climate change scenarios; an increase in spring warming alone (warm 5), increase in spring warming and increase in winter chilling (warm 5 +chill) and an increase in spring warming and decrease in winter chill (warm 5 -chill). We grouped the species-level posterior estimates by FLS category (flowering-first, concurrent, leafing-first). The points represent the mean estimates and the lines represent the 50% credible intervals. In our study, all flowering-first species are wind-pollinated, and projected FLS shifts are most pronounced in some of these wind-pollinated, flowering-first shrubs. However, FLS shifts for all species depend on the relationship between forcing and chilling changes which is likely to vary by location with climate change.