

# Differences between flower and leaf phenological responses to environmental variation drive shifts in spring phenological sequences of temperate woody plants

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

*Author affiliations:*

<sup>1</sup>Arnold Arboretum of Harvard University, Boston, Massachusetts, USA. ORCID: 0000-0003-4022-2591

<sup>2</sup>Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA

<sup>3</sup>Forest & Conservation Sciences, Faculty of Forestry, University of British Columbia, Vancouver, British Columbia, Canada

<sup>a</sup>Corresponding author: 617.823.0687; dbuonaiuto@g.harvard.edu

# Abstract

The relative timing of growth and reproduction is an important driver of plant fitness. For deciduous woody species in temperate regions leaves and flowers both appear in the early spring, but the order and duration of these phenological events vary among species, populations, and individuals. Researchers have long hypothesized that this variation in flower-leaf sequences (FLSs) may be important—affecting the reproduction, recruitment and survival of individuals. Further, FLSs appear to be shifting with climate change; thus anticipating the extent of these shifts may influence projections of how climate change may impact species' performance and reshape forest communities. Predicting FLS shifts requires an improved understanding of how environmental variation dictates FLS patterns. To address this, we compared the phenological responses of flowers and developing leaves for 10 temperate woody species to varying levels of temperature and photoperiod in a lab experiment. Our experimental design allowed us to test competing hypotheses for how environmental cues determine FLS variation—specifically whether forcing (warm temperatures) alone drives variation or differential sensitivity to chilling (cool temperatures generally in the fall and winter) and/or photoperiod matter. Within species, we found that flower and leaf phenology responded with differential sensitivity to environmental cues, with differences in their response to chilling being the dominant driver of FLS variation. These differences between flowering and leaf responses were consistent across species, but because species differ the order of phenological events in their FLSs (flowering-first vs. leafing-first), differences between flower and leaf phenology will have contrasting impacts on FLS variation across species. Because climate change will amplify variability in chilling across time and space, our findings suggest that FLS shifts may be large, but are likely to vary substantially among populations and species. Simple projections of FLS shifts with climate change, based on our results, showed large shifts in wind-pollinated species that flower before leafing, with flower-leaf interphases substantially shortened. This shorter interphase would reduce the time period for efficient pollen

transfer, and thus raises the possibility that wind-pollinated taxa especially may experience reproductive declines due to FLS shifts in the decades to come.

*Keywords:* chilling, climate change, deciduous forests, flower-leaf sequences, forcing, hysteresis, phenology, wind-pollination

## Introduction

Among the most widely documented biological effects of anthropogenic climate change are shifts in plant phenology, the timing of life cycle events (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 between events that make up phenological sequences (Ettinger *et al.*, 2018). Phenological sequences are a major driver of plant fitness that impact plant life history, resource allocation, demography and ecosystem processes (Post *et al.*, 2008). Thus, shifting sequences with climate change will likely impact many of these processes. The effects of these shifts, however, 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.

For 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). There are several hypotheses regarding the function of FLS variation (see Gougherty & Gougherty, 2018), and it is likely that the adaptive significance of FLSs vary among species, and may co-vary with

other plant traits.

The flowering-first FLS is strongly correlated with wind-pollination (Buonaiuto *et al.*, 2021; Friedman & Barrett, 2009) and models of pollen movement show that for wind-pollinated species, flowering-first increases pollen dispersal distances and significantly reduces the amount of pollen intercepted by non-reproductive structures (Di-Giovanni *et al.*, 1989; Tauber, 1967; Whitehead, 1969). Flowering-first is also prevalent in some biotically-pollinated taxa, but its function is less clear. Some authors suggest that flowering-first impacts floral visibility to pollinators (Janzen, 1967; Bukovac *et al.*, 2017; Forrest & Thomson, 2009) or modifies hydraulic demand (Gougherty & Gougherty, 2018; Franklin, 2016), while others suggest that in biotically-pollinated taxa there is no unique function to the sequence and flowering-first a by-product of selection for early flowering in general (Primack, 1987).

Phenological observations over the last several decades indicate that, like other phenological sequences, FLSs are shifting with climate change (Ma *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.*, 2021; Ma *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.

The impact on FLS shifts with climate change on the fitness of woody plants depends on 1) the function of FLSs for that species and 2) the direction and magnitude of the shift. For example, in wind-pollinated species that rely on a substantial flower-leaf interphase for effective pollen transport, decreasing FLS interphases with climate change may drive a reduction in pollination success as more pollen is intercepted by vegetation. Conversely, pollination

efficiency could improve for species with lengthening FLS interphases. However, a proportionate FLS shift in biotically-pollinated taxa may have different fitness implications because of the contrasting function of FLS variation in these species.

While several recent analyses have examined the function of FLS variation (e.g. Buonaiuto *et al.*, 2021; Gougherty & Gougherty, 2018), the factors that influence the magnitude and direction of FLS shifts are less well studied (but see Ma *et al.*, 2020). Predicting FLS shifts requires identifying the proximate mechanisms that drive and constrain FLS variation, and how these mechanisms differ among species.

Decades of research suggest that cool winter temperatures (chilling), warm spring temperatures (forcing), and day-length (photoperiod) are the primary drivers of both reproductive and vegetative phenology for woody plants in temperate regions (Körner & Basler, 2010; Flynn & Wolkovich, 2018). However, observed FLS shifts indicate that there must be differences in how these cues influence the phenology of flowers and leaves (Buonaiuto *et al.*, 2021).

It is also likely that FLS variation is mediated by other internal mechanisms like developmental construction (Diggle, 1995), or other physical constraints like inflorescence architecture or bud type (Pope *et al.*, 2013). For example, FLS variation in species with separate buds (buds containing either embryonic leaves or flowers) may be less constrained than species with mixed buds (buds containing both embryonic leaves and flowers together). Other factors like growth form (tree vs. shrub) or colonization-competition tradeoffs that have been shown to influence the phenological sensitivity of specific phenophases (Basler & Körner, 2012; Donnelly & Yu, 2021) may also influence the sensitivity of phenological sequences to climate.

While FLS variation in woody plants is no doubt the product of interactions between species-specific biology and complex environmental inputs, identifying the differences in how flower and leaf phenology responds to environmental change is a necessary step for predicting the direction, magnitude and—ultimately—fitness impacts of FLS shifts with climate change. 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 which we present below.

## Hypotheses for FLS variation

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 (that is, they need a greater sum of warm temperature to trigger the phenological event, Guo *et al.*, 2014; Cosmulescu & Calusaru, 2020; Cosmulescu & Ionescu, 2018). Under this hypothesis, which we call the forcing hierarchy hypothesis (FHH), leaf and flower buds share the same suite of cues and develop similarly to non-forcing cues (i.e., chilling and photoperiod), but 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 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, generating FLS variability.

While observational studies of phenology in the field are commonly used to evaluate the

relationship between environmental conditions and phenology (Cleland *et al.*, 2007), under current and recent climatic conditions, the FHH and DSH may produce similar phenological patterns, making it difficult to evaluate these hypotheses with such methods. However, experiments designed to isolate all three environmental cues have the potential to disentangle the two hypotheses. Studies aiming to differentiate these hypotheses can look for two different signatures. The key signature of the FHH is that the sensitivity to forcing ( $\Delta$  day of phenological event/  $\Delta^\circ\text{C}$ ) of the second phase in the phenological sequence is always greater than that of the first phase with sensitivity differences being inversely proportional to the difference in thermal requirement among bud types (2x in our simulations; 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 (Fig. 1 b.), but there is no characteristic pattern as to the strength or direction of this divergence (e.g., leaves may require longer or shorter photoperiods than flowers, or more or less chilling).

The clarity of these signatures from each hypothesis, however, may be obscured in certain situations. First, a species' buds could be both differentially sensitive to photoperiod or chilling (supporting the DSH) and also respond to forcing in a forcing hierarchy (that is, both hypotheses can operate at once, see Fig. 1 c.). Second, the differential sensitivity framework can generate the signature of the FHH when other cues are at high levels due to interactions between the chilling or photoperiod response and the thermal requirement, which make forcing the dominant phenological cue once other cue requirements have been met (see Fig. 1 b., chill x force interaction). When this happens the FHH would effectively be a special case of the DSH.

While these two hypothesized mechanisms may produce similar phenology patterns under current conditions, differentiating them is important, as they have substantially different im-

plications regarding the potential for FLS shifts with climate change. The FHH 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 FLS interphases will decrease, but given the relative auto-correlation of spring temperatures (Di Cecco & Gouhier, 2018), these shifts should be muted. If FLSs are structured by a forcing 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.

Climate change would also drive FLS shifts under the DSH, but the trajectory of these shifts are more complicated to predict. The DSH suggests that there could be strongly localized or regional 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 the FHH and DSH hypotheses in 10 temperate shrub and tree species. Using a full-factorial growth-chamber experiment we manipulated chilling, forcing and photoperiod cues for flower and leaf buds. We then leveraged these data to examine how FLSs may shift with climate change.



# Materials and Methods

## Growth chamber study

We sampled plant material from Harvard Forest in Petersham, MA, USA (42.5314°N, 72.1900°W) on 25 October 2017, just as most individuals dropped their leaves, to capture the period immediately after most plants in the area entered endo-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 cuttings per individual for a total of 48-56 per species), consisting of a mix of deciduous shrubs, understory and canopy trees commonly found in mesic hardwood forests of the eastern United States. The use of branch cuttings to study woody plant phenology in artificial environments is common (Ettinger *et al.*, 2020), and these methods have been shown to match whole-plant phenological patterns (Vitasse & Basler, 2014; Primack *et al.*, 2015). We selected species displaying variation of the three major FLS patterns; species for which flowers appear before leaves (flowering-first; *Acer rubrum*, *Betula alleghaniensis*, *Comptonia peregrina*, and *Corylus cornuta*), species for which flowers appear with their leaves (concurrent; *Acer pensylvanicum*, *Acer saccharum*, *Ilex mucronata*, *Prunus pensylvanica*, *Vaccinium corymbosum*), and species for which flowers appear after leaf development (leafing-first; *Ilex verticillata*, *Prunus virginiana*, *Viburnum acerifolium*). Because we expected that other characteristics such as pollination syndrome, bud type, and growth habit were likely to impact FLS sensitivity, we made sure that the species we chose exhibited variation in these traits as well (see Tab. S1 for details and full species list). We included several congeners in our study, explicitly choosing species with different FLS patterns to mitigate phylogenetic non-independence in our trait of interest (FLS) (Revell, 2010).

We transported all cuttings to the Arnold Arboretum in Boston, MA (USA) where they were re-cut in water to prevent callousing and minimize embolism and placed in 500 ml Erlen-

meyer flasks with distilled water.

We randomly assigned cuttings to a fully crossed set of eight experimental treatments; two levels of chilling (30 vs 60 days 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 twigs 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 assessed three phenological phases: leaf budburst (BBCH phase 07), leafout (BBCH phase 15) and first flower open (BBCH 60). While leaf budburst is the first stage of vegetative expansion and therefore most directly comparable to first flowering, we also included leafout in our observations because several of the functional hypotheses for FLS variation are predicated on developing leaves be sufficiently large enough to impact canopy structure, which would only happen at later stages of leaf development.

At the conclusion of this period we assessed all individuals that did not undergo budburst and excluded 56 dead twigs from our analyses.

## Data analysis

To assess the phenological sensitivity of each phase, we fit mixed-effects 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. We modeled the effects of environmental parameters on flower opening, leaf budburst and leafout 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. Two species, *Betula allegheniensis* and *Acer saccharum*, produced no flowers in our trial, likely because flowering branches were out of reach during our field sampling, so we excluded them from our analysis. In total, our analyses included 464 twigs from 10 species.

The models we fit appear below:

$$y_{[i]} \sim N(\alpha_{sp[i]} + \beta_{forcing_{sp[i]}} + \beta_{chilling_{sp[i]}} + \beta_{photoperiod_{sp[i]}} + \beta_{forcing \times chilling_{sp[i]}} + \beta_{forcing \times photoperiod_{sp[i]}} + \beta_{chilling \times photoperiod_{sp[i]}} , \sigma_y^2)$$

Where  $y_{[i]}$  is either the day of the experiment of leaf budburst, day of first flower opening, day of leafout or FLS interphase length, and  $\sigma_y^2$  the error. We modeled the intercept ( $\alpha$ ) and each slope ( $\beta$ ) 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 the hypothesis that the FHH is a special case of the DSH that occurs when other cue (i.e., chilling and photoperiod) requirements are met, we re-ran our leaf budburst and flowering models on a subset of our data which included both levels of the 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 slopes and intercept.

253 We fit all models using the R package “brms” (Bürkner, 2018). We ran each model on four  
 254 chains with 4000 iterations and a 3000 iteration warm up for a total of 4000 posterior draws  
 255 for each parameter. In all models we used weakly informative priors and increasing the priors  
 256 5-fold did not affect the model results. We assessed model performance through ensuring  $\hat{R}$ s  
 257 were between 1 and 1.01 and bulk and tail effective sample sizes were high (1800-2800 for  
 258 most parameters, but as low as 800-900 for some). We present 50% credible intervals in  
 259 figures because they are the most computationally stable (Gelman *et al.*, 2013), but provide  
 260 other intervals in the Supporting Information (Tab. S3, Tab. S4, Tab. S5). All of our  
 261 estimates of phenological sensitivity ( $\Delta$  day of phenological event/  $\Delta$  environmental cue) are  
 262 scaled by treatment level (chilling:  $\Delta$  30 days, forcing:  $\Delta$  6°C, photoperiod:  $\Delta$  4 hours; thus,  
 263 for example, when we report a forcing effect of -19 days it means a change of -19 days given  
 264 a 6°C temperature difference).

265

## 266 **Climate change scenarios**

267 To apply our model results to general climate change scenarios we chose environmental treat-  
 268 ments in the experiment to broadly reflect historic and future conditions at our sampling site.  
 269 Our low forcing treatment approximated average spring temperature (March/April) at the  
 270 site while our high temperature treatment reflects a 6 °C increase. Average field chilling  
 271 (calculated from 15 October - 15 April, measured in Chill Hours, Weinberger, 1950) at Har-  
 272 vard Forest is 1170.7, approximately 63% of the difference between our low and high chilling  
 273 treatment (Tab. S2). Thus, our low chilling treatment represents a feasible estimate for  
 274 a decrease in chilling with climate change and our high chilling treatment approximates a  
 275 reasonable increase (Luedeling, 2012). Our low photoperiod treatment (8 hours of daylight)  
 276 is well below the photoperiod experienced at Harvard Forest, but given that the photoperiod

effects are expected to be small (Laube *et al.*, 2014), we chose more extreme values in order to robustly estimate an effect (i.e., increasing statistical power). For this reason, our climate change scenarios for FLS variation are based on our high photoperiod treatment alone.

We used our flower, leafout and leaf budburst models to project for each species in our study:

1. FLSs under average environmental conditions (low forcing, 45 days of chilling)
2. FLS shifts with spring warming only (high forcing, 45 days of chilling)
3. FLS shifts with warming and increased chilling (high forcing, 60 days of chilling)
4. FLS shifts with warming and decreased chilling (high forcing, 30 days of chilling)

To validate our predictions, we compared our FLS interphase model estimates of “average” condition FLS interphases to long term phenological records from Harvard Forest (O’Keefe, 2015) for five species common to both datasets (Fig. S1), and found them comparable.

Given the variable dynamics of shifts in environmental forcing and chilling with climate change over time and space (Luedeling *et al.*, 2011), these scenarios should not be treated as absolute predictions of the magnitude of FLS shifts with climate change. Instead, we provide these scenarios to identify general trends in how FLSs could shift with warming and demonstrate that the range of possibilities vary based on characteristics of plant species and the specific climate dynamics.

Finally, we characterized FLS shifts for three broad FLS groups (flowering-first, concurrent, leafing-first) by extracting all sampling posterior estimates from our main models using the R package tidybayes (Kay, 2020), and grouping them by FLS pattern. We also include the

species-specific estimates on which these grouped estimates are based in Fig. S3.

## Results

### Growth chamber study

Flowering, leaf budburst and leafout advanced with higher forcing and longer chilling duration (flowering: chilling effect: -21.2 days, forcing effect: -19.0 days, leaf budburst: chilling effect: -30.4 days, forcing effect: -17.8 days, leafout: chilling effect: -39.7 days, forcing effect: -32.6 days; Fig. 2, Fig. S2, Tab. S3, Tab. S4—all sensitivity estimates are scaled by treatments; see “Data analysis” section above), but increases in both of these cues together offset these advances (flowering: force x chill effect: 7.0 days, leaf budburst: force x chill effect: 12.4 days, leafout: force x chill effect: 21.4 days; Fig. 2, Fig. S2, Tab. S3, Tab. S4). Leaf and flower phenologies diverged in their responses to photoperiod, with flower phenology advancing at longer photoperiod and leaf phenology remaining unaffected (leafout) with photoperiod changes, or delaying (budburst) at longer photoperiods when the other two cues were at low levels (Fig. 2, Fig. S2, Tab. S3, Tab. S4). As seen in the interactions between photoperiod and chilling and photoperiod and forcing, increasing chilling or forcing with longer photoperiod advanced the phenology of all three phases. For flowering, leaf budburst and leafout, chilling and forcing were the dominant cues, while photoperiod produced a more muted phenological response (Fig. 2, Fig. S2, Tab. S3, Tab. S4).

While leaf and flower phenological responses to environmental cues were qualitatively similar, the strength of their responses to each cue differed substantially. Leaf budburst and leafout responded more strongly to chilling than flowering (budburst: 1.4x, leafout: 1.9x),

and had stronger responses to all cue interactions (leaf budburst: forcing x chilling: 1.8x, photoperiod x chilling: 5.8x, photoperiod x forcing: 2.2x, leafout: forcing x chilling: 3.1x, photoperiod x chilling: 2.4x, photoperiod x forcing: 1x; Fig. 2, Fig. S2, Tab. S3, Tab. S4). Across all species, both leaf budburst and flowering displayed a similar magnitude advance with increased forcing, while leafout responded comparatively much stronger (Fig. 2, Fig. S2, Tab. S3, Tab. S4).

Our results did not show the characteristic sensitivity pattern of the FHH across most species in our experiment (Fig. 2, Fig. S2, see Fig. 1,a,b.), though there was significant variation among species in the relative strength of their response to forcing between flowering and leaf budburst. However, when we re-ran our flowering and leaf budburst 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 9/10 species followed the predicted pattern of the FHH, with the second phases of the FLS showing stronger sensitivity to forcing than the first phases (Fig. 3, Tab. S5).

## Climate change scenarios

Our models project that flowering, budburst and leafout will advance in our generalized future scenarios. FLS shifts depended strongly on how forcing levels change relative to chilling exposure (Fig. 4). Given the significant differences in sensitivity to chilling between flowering and leafing phenology we found in our experiment, our scenarios showed that FLS interphases will be more strongly influenced by changes in chilling duration than increased forcing alone.

The direction and magnitude of shifts in FLS interphases varied among species, varying espe-

cially by whether or not they displayed a flowering-first FLS. Under some warming scenarios, flower-leafout interphases were strongly reduced in a number of taxa, and flower-budburst interphases for some species effectively disappeared or the order of phenophases in the FLS switched (Fig. 4, Fig. S3). Several species, *Acer rubrum*, *Ilex verticillata*, *Prunus pensylvanica*, *Prunus virginiana*, and *Viburnum acerifolium*, had FLSs that were relatively robust to changing environments. For other species, *Acer pensylvanicum*, *Vaccinium corymbosum* and *Ilex mucronata*, which typically begin to produce leaves shortly before flowers open, the magnitudes of projected FLS shifts were moderate. The two species with the most significant FLS shifts in both direction and magnitude across treatment combinations and climate change scenarios were the flowering-first, wind-pollinated shrubs *Comptonia peregrina* and *Corylus cornuta* (Fig. S3). In all of our climate change scenarios, the FLS interphase was dramatically reduced in these taxa.

## Discussion

Our experiment supports the hypothesis that flower-leaf sequences are structured by differential sensitivity (DSH) to the environment between flower and leaf phenology. Specifically, differences in the chilling response among flowering and leaf phenology was the strongest driver of FLS variation. We also found that under high chilling and long photoperiods, the FLSs of most species followed the predicted sensitivity pattern of the FHH, supporting the hypothesis that the FHH is likely a special case of the DSH that occurs when other phenological cues are met for both vegetative and flowering phenology. Together, these results explain why the two FLS hypotheses have been difficult to distinguish, and suggest that climate change has the potential to significantly disrupt FLSs as global warming alters historic chilling patterns across the temperate zone (Morin *et al.*, 2009).



## Reconciling the differential sensitivity and the forcing hierarchy hypotheses

The relationship we found between the FHH and the DSH in our experiment offers a path forward for reconciling previous studies that have favored one hypothesis over the other. Support for the FHH is most often associated with observational studies (e.g. Cosmulescu & Calusaru, 2020; Guo *et al.*, 2014). By contrast, experimental studies which manipulate chilling levels beyond historically observed minima in the field tend to support the DSH (e.g. Aslamarz *et al.*, 2009; Gariglio *et al.*, 2006). This pattern can be explained by the FHH being a special case of the DSH, if historic climate regimes make detection of the DSH in the field difficult.

Under historic climate regimes, seasonal chilling requirements were usually met (Gauzere *et al.*, 2019; Chuine *et al.*, 2016), making it difficult to detect any effect of chilling in observational studies, let alone differences in flower versus leaf responses. It would take larger reductions in chilling to reveal the signature of the DSH, which at present, rarely occurs in the field, but can be induced in artificial environments, which explains the discrepancy between these two methods. These dynamics are consistent with findings in other phenological studies that suggest simple growing degree models (which underlie the FHH) 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). 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 the magnitude of interannual FLS variation is likely to extend beyond historically observed reaction norms.

## Population-level implications of the DSH 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). Layered onto these variable shifts in chilling, spring warming from climate change also differs by location (Karmalkar & Bradley, 2017; Loarie *et al.*, 2009), suggesting that forcing may increase more rapidly in some places than others. These shifts in both chilling and forcing suggest 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.*, 2021) and our findings suggest that these population-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 could occur contemporaneously across a species’ range.

This increased population-level heterogeneity in FLSs that our models project has the potential to influence patterns of pollen dispersal across the landscape (Borycka *et al.*, 2017; Pace *et al.*, 2018). For example, in wind-pollinated species 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. In biotically-pollinated species FLS shifts may interact with other drivers of global change such as phenological mismatches with pollinators (Burkle *et al.*, 2013) or flowering asynchrony among populations (Cresti *et al.*, 2013; Zohner *et al.*, 2018) to impact gene flow, but more research is needed to determine if and how FLS variation affects the foraging behavior of pollinators. 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.

While changes in chilling and forcing from climate change may increase FLS variation, the climate change implications of strong differential sensitivity to photoperiod among flower and leaf phenology are more difficult to predict. Climate change does not directly impact photoperiod, but may alter the photoperiods plants experience as they approach their temperature optima for phenological activity. However, in most temperate regions, temperature would have to drive phenological shifts by a minimum of several weeks before the experienced photoperiod of plants would change substantially. Because these dynamics are more extreme than most studies suggest (Thackeray *et al.*, 2016) and the photoperiod effect we observed on our study was relatively weak, we modeled climate change scenarios with a constant photoperiod in our FLS scenarios. Such an approach may be useful at temperate and some boreal latitudes, but may not work at high latitudes, where photoperiod changes more rapidly over the season and thus temperature-driven phenological shifts may significantly alter the experienced photoperiod of plants. Our results suggest that such shifts could mute or amplify the FLS shifts, which 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

The impact of FLS shifts with climate change rely on both the function of FLS variation and the magnitude of FLS shifts. Just as previous analyses suggest that the function of FLS variation may differ among species (Buonaiuto *et al.*, 2021; Gougherty & Gougherty, 2018), our study highlights that the direction and magnitude of FLS shifts with climate change are also species-specific. We found that FLSs of some species were very sensitive to changing

climate conditions, while other remain fairly resilient (Fig. 4, Fig. S3).

The different FLS response patterns we observed across species may correlate with suite of interrelated anatomical, physiological and phenological traits. Just as pollination syndrome appears to be a major driver of differences in the function of FLSs in woody plants (Buonaiuto *et al.*, 2021), we also found significant differences in the magnitude and direction of FLS shifts between biotically and wind-pollinated species.

The species that maintained FLS structure across our climate change scenarios were biotically-pollinated. They also generally shared a strongly leafing-first FLS, with a fairly long FLS interphase (Fig 4). As expected, these species tended to have mixed buds so there may be strong physical constraints on their FLSs. Our models suggest that the FLSs of a subset of biotically-pollinated taxa, those classified with “concurrent”, are expected to shift under some scenarios (see Fig 4), but it is unclear if these FLS shifts would be large enough in magnitude to impact plant performance.

By contrast, the species that were most sensitive to FLS shifts were monoecious, flowering-first, wind-pollinated shrubs (Fig 4, Fig S3). This result supports other evidence that the reproductive phenology of wind-pollinated species may be more sensitive to climate change compared to biotically pollinated taxa (Ziello *et al.*, 2012). In all scenarios, our projections suggest that the flower-leaf interphase will decrease for these species. Given this period is considered critical for successful pollination in these taxa (Whitehead, 1969), this pattern suggests that these species, and flowering-first, wind-pollinated taxa in general, may face particular risk for reproductive performance reductions with climate change.

However, there are several caveats to this prediction. The function of FLS variation in wind-

pollinated species suggests that leaves must create a substantial barrier to pollen movement. In our study, we observed both leaf budburst, a stage that is unlikely to contribute much to canopy structure, and leafout, a stage more likely to impact airflow through the canopy. It is unclear at what point in leaf development wind-pollination becomes impaired, so the effective duration of the FLS interphase that impacts wind pollination may be somewhere in between the two interphases we measured (flower-leaf budburst and flower-leafout) or beyond the interphases used in our projections. Secondly, in growth chamber experiments such as ours, it is difficult to evaluate the full duration of phenological episodes (Primack *et al.*, 2015). Shifts in the duration of flowering or period of leaf expansion could compensate for, or exacerbate the shifts in FLSs we observed.

Finally, many wind-pollinated species are monoecious or dioecious (Ackerman, 2000) and in several families (e.g. *Betulaceae*, *Juglandaceae*), male flowers occur in separate buds, while female flowers occur in mixed buds (Pope *et al.*, 2013). This suggests that the phenology of female flowers may be more tightly constrained by leaf phenology and if this is the case, even increases in the FLS interphases for flowering-first wind-pollinated taxa such as those observed in the field (e.g. Ma *et al.*, 2020) could adversely affect wind-pollination by disrupting floral synchrony of male and female flowers (Alizoti *et al.*, 2010; Mutke *et al.*, 2005; Elkassaby & Davidson, 1991).

While much of the public interest around phenology and pollination in the context of global change has centered around trophic mismatches between pollinator and floral phenology (Memmott *et al.*, 2007), our study identified the possibility that the effects 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. Our results suggest that species in this functional group should be considered a research priority for the study of spring phenological sequences in deciduous woody plants.

## **Acknowledgements:**

We thank C. de Keyser for generously helping to collect and prepare plant materials for the experiment. We also thank R. Lee for help with monitoring and maintaining the experiment.

## **Author Contributions:**

DMB and EMW conceived of the experiment; DMB collected the data and performed the statistical analyses, DMB and EMW wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## **Data Availability Statement:**

Data and code will be available on KNB (<https://knb.ecoinformatics.org/>) upon publication.

## References

- Ackerman, J.D. (2000) Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Plant Systematics and Evolution* **222**, 167–185.
- Alizoti, P.G., Kilimis, K. & Gallios, P. (2010) Temporal and spatial variation of flowering among *Pinus nigra* Arn. clones under changing climatic conditions. *Forest Ecology and Management* **259**, 786–797, Conference on Adaptation of Forests and Forest Management to Changing Climate with Emphasis on Forest Health, Umea, Sweden, AUG 25-28, 2008.
- 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.
- Basler, D. & Körner, C. (2012) Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology* **165**, 73–81.
- 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.
- Bukovac, Z., Shrestha, M., Garcia, J.E., Burd, M., Dorin, A. & Dyer, A.G. (2017) Why background colour matters to bees and flowers. *Journal of Comparative Physiology A* **203**, 369–380.
- Buonaiuto, D.M., Morales-Castilla, I. & Wolkovich, E.M. (2021) Reconciling competing hypotheses regarding flower–leaf sequences in temperate forests for fundamental and global change biology. *New Phytologist* **229**, 1206–1214.

- Burkle, L.A., Marlin, J.C. & Knight, T.M. (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* **339**, 1611–1615.
- 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., Lacomte, 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. *Journal of Agricultural Meteorology* **76**, –.
- 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**.
- Cresti, M., Aroca, R., Khanduri, V.P., Sharma, C.M., Kumar, K.S. & Ghildiyal, S.K. (2013) Annual variation in flowering phenology, pollination, mating system, and pollen yield in two natural populations of *schima wallichii* (dc.) korth. *The Scientific World Journal* **2013**, 350157.



- Di Cecco, G.J. & Gouhier, T.C. (2018) Increased spatial and temporal autocorrelation of temperature under climate change. *Scientific Reports* **8**, 14850.
- Di-Giovanni, F., Beckett, P.M. & Flenley, J.R. (1989) Modelling of dispersion and deposition of tree pollen within a forest canopy. *Grana* **28**, 129–139.
- Diggle, P.K. (1995) Architectural effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology and Systematics* **26**, 531–552.
- Donnelly, A. & Yu, R. (2021) Temperate deciduous shrub phenology: the overlooked forest layer. *International journal of biometeorology* **65**, 343–355.
- Elkassaby, Y. & Davidson, R. (1991) Impact of pollination environment manipulation on the apparent outcrossing rate in a Douglas Fir seed orchard. *Heredity* **66**, 55–59.
- Ettinger, A., Gee, S. & Wolkovich, E. (2018) Phenological sequences: how early season events define those that follow. *American Journal of Botany* **105**.
- Ettinger, A.K., Chamberlain, C.J., Morales-Castilla, I., Buonaiuto, D.M., Flynn, D.F.B., Savas, T., Samaha, J.A. & Wolkovich, E.M. (2020) Winter temperatures predominate in spring phenological responses to warming. *Nature Climate Change* **10**, 1137–1142.
- 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. & Thomson, J.D. (2009) Background complexity affects colour preference in bumblebees. *Naturwissenschaften* **96**, 921–925.
- 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.

Friedman, J. & Barrett, S.C.H. (2009) Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* **103**, 1515–1527.

Gariglio, N., González Rossia, D.E., Mendow, M., Reig, C. & Agusti, 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.

Gelman, A., Carlin, J., Stern, H., Dunson, D., Vehtari, A. & Rubin, D. (2013) *Bayesian Data Analysis*. Chapman and Hall/CRC.

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.

Janzen, D.H. (1967) Synchronization of sexual reproduction of trees within the dry season in central america. *Evolution* **21**, 620–637.

Karmalkar, A.V. & Bradley, R.S. (2017) Consequences of global warming of 1.5 °c and 2 °c for regional temperature and precipitation changes in the contiguous united states. *PLOS ONE* **12**, e0168697–.

- 609 Kay, M. (2020) *tidybayes: Tidy Data and Geoms for Bayesian Models*. R package version  
610 2.1.1.
- 611 Kling, M.M. & Ackerly, D.D. (2020) Global wind patterns and the vulnerability of wind-  
612 dispersed species to climate change. *Nature Climate Change* **10**, 868–875.
- 613 Körner, C. & Basler, D. (2010) Phenology under global warming. *Science* **327**, 1461–1462.
- 614 Laube, J., Sparks, T.H., Estrella, N., Höfler, J., Ankerst, D.P. & Menzel, A. (2014) Chilling  
615 outweighs photoperiod in preventing precocious spring development. *Global Change Biology*  
616 **20**, 170–182.
- 617 Linkosalo, T., Lappalainen, H.K. & Hari, P. (2008) A comparison of phenological models of  
618 leaf bud burst and flowering of boreal trees using independent observations. *Tree Physiology*  
619 **28**, 1873–1882.
- 620 Loarie, S.R., Duffy, P.B., Hamilton, H., Asner, G.P., Field, C.B. & Ackerly, D.D. (2009) The  
621 velocity of climate change. *Nature* **462**, 1052–1055.
- 622 Luedeling, E. (2012) Climate change impacts on winter chill for temperate fruit and nut  
623 production: A review. *Scientia Horticulturae* **144**, 218–229.
- 624 Luedeling, E., Girvetz, E.H., Semenov, M.A. & Brown, P.H. (2011) Climate change affects  
625 winter chill for temperate fruit and nut trees. *PLOS ONE* **6**, e20155–.
- 626 Ma, Q., Huang, J.G., Hänninen, H., Li, X. & Berninger, F. (2020) Climate warming prolongs  
627 the time interval between leaf-out and flowering in temperate trees: Effects of chilling,  
628 forcing and photoperiod. *Journal of Ecology* **n/a**.
- 629 Man, R., Lu, P. & Dang, Q.L. (2017) Insufficient chilling effects vary among boreal tree  
630 species and chilling duration. *Frontiers in Plant Science* **8**, 1354.

- Mehlenbacher, S.A. (1991) Chilling requirements of hazelnut cultivars. *Scientia Horticulturae* **47**, 271–282.
- Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007) Global warming and the disruption of plant–pollinator interactions. *Ecology Letters* **10**, 710–717.
- Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kuebler, K., Bissolli, P., Braslavska, O., Briede, A., Chmielewski, F.M., Crepinsek, Z., Curnel, Y., Dahl, A., Defila, C., Donnelly, A., Filella, Y., Jatcza, K., Mage, F., Mestre, A., Nordli, O., Penuelas, J., Pirinen, P., Remisova, V., Scheifinger, H., Striz, M., Susnik, A., Van Vliet, A.J.H., Wielgolaski, F.E., Zach, S. & Züst, A. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**, 1969–1976.
- Milleron, M., Lopez de Heredia, U., Lorenzo, Z., Perea, R., Dounavi, A., Alonso, J., Gil, L. & Nanos, N. (2012) Effect of canopy closure on pollen dispersal in a wind-pollinated species (*Fagus sylvatica* L.). *Plant Ecology* **213**, 1715–1728.
- Morin, X., Lechowicz, M.J., Augspurger, C.K., O’Keefe, J., Viner, D. & Chuine, I. (2009) Leaf phenology in 22 north american tree species during the 21st century. *Global Change Biology* **15**, 961–975.
- Mutke, S., Gordo, J. & Gil, L. (2005) Variability of mediterranean stone pine cone production: Yield loss as response to climate change. *Agricultural and Forest Meteorology* **132**, 263–272.
- O’Keefe, J. (2015) *Phenology of Woody Species at Harvard Forest since 1990*. Harvard Forest Data Archive: HF003., Petersham, MA, USA.
- Pace, L., Boccacci, L., Casilli, M., Di Carlo, P. & Fattorini, S. (2018) Correlations between weather conditions and airborne pollen concentration and diversity in a mediterranean high-altitude site disclose unexpected temporal patterns. *Aerobiologia* **34**, 75–87.

- Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37 EP –.
- Pope, K.S., Dose, V., Da Silva, D., Brown, P.H., Leslie, C.A. & DeJong, T.M. (2013) Detecting nonlinear response of spring phenology to climate change by bayesian analysis. *Global Change Biology* **19**, 1518–1525.
- Post, E.S., Pedersen, C., Wilmers, C.C. & Forchhammer, M.C. (2008) Phenological sequences reveal aggregate life history response to climatic warming. *Ecology* **89**, 363–370.
- Primack, R.B. (1987) Relationships among flowers, fruits, and seeds. *Annual Review of Ecology and Systematics* **18**, 409–430.
- Primack, R.B., Laube, J., Gallinat, A.S. & Menzel, A. (2015) From observations to experiments in phenology research: investigating climate change impacts on trees and shrubs using dormant twigs. *Annals of Botany* **116**, 889–897.
- Rathcke, B. & Lacey, E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* **16**, 179–214.
- Revell, L.J. (2010) Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution* **1**, 319–329.
- Tauber, H. (1967) Investigations of the mode of pollen transfer in forested areas. *Review of Palaeobotany and Palynology* **3**, 277–288.
- Thackeray, S.J., Henrys, P.A., Hemming, D., Bell, J.R., Botham, M.S., Burthe, S., Helaouet, P., Johns, D.G., Jones, I.D., Leech, D.I., Mackay, E.B., Massimino, D., Atkinson, S., Bacon, P.J., Brereton, T.M., Carvalho, L., Clutton-Brock, T.H., Duck, C., Edwards, M., Elliott, J.M., Hall, S.J.G., Harrington, R., Pearce-Higgins, J.W., Høye, T.T., Kruuk, L.E.B., Pemberton, J.M., Sparks, T.H., Thompson, P.M., White, I., Winfield, I.J. & Wanless,

S. (2016) Phenological sensitivity to climate across taxa and trophic levels. *Nature* **535**,  
241–245.

Vitasse, Y. & Basler, D. (2014) Is the use of cuttings a good proxy to explore phenological  
responses of temperate forests in warming and photoperiod experiments? *Tree Physiology*  
.

Way, D.A. & Montgomery, R.A. (2015) Photoperiod constraints on tree phenology, perfor-  
mance and migration in a warming world. *Plant, Cell & Environment* **38**, 1725–1736.

Weinberger, J.H. (1950) Chilling requirements of peach varieties. id - 19511602967. *Proceed-  
ings. American Society for Horticultural Science* **56**, 122–28.

Whitehead, D.R. (1969) Wind pollination in the angiosperms: Evolutionary and environ-  
mental considerations. *Evolution* **23**, 28–35.

Zhang, X., Tarpley, D. & Sullivan, J.T. (2007) Diverse responses of vegetation phenology to  
a warming climate. *Geophysical Research Letters* **34**.

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

Zohner, C.M., Mo, L. & Renner, S.S. (2018) Global warming reduces leaf-out and flowering  
synchrony among individuals. *eLife* **7**, e40214.

## Figures

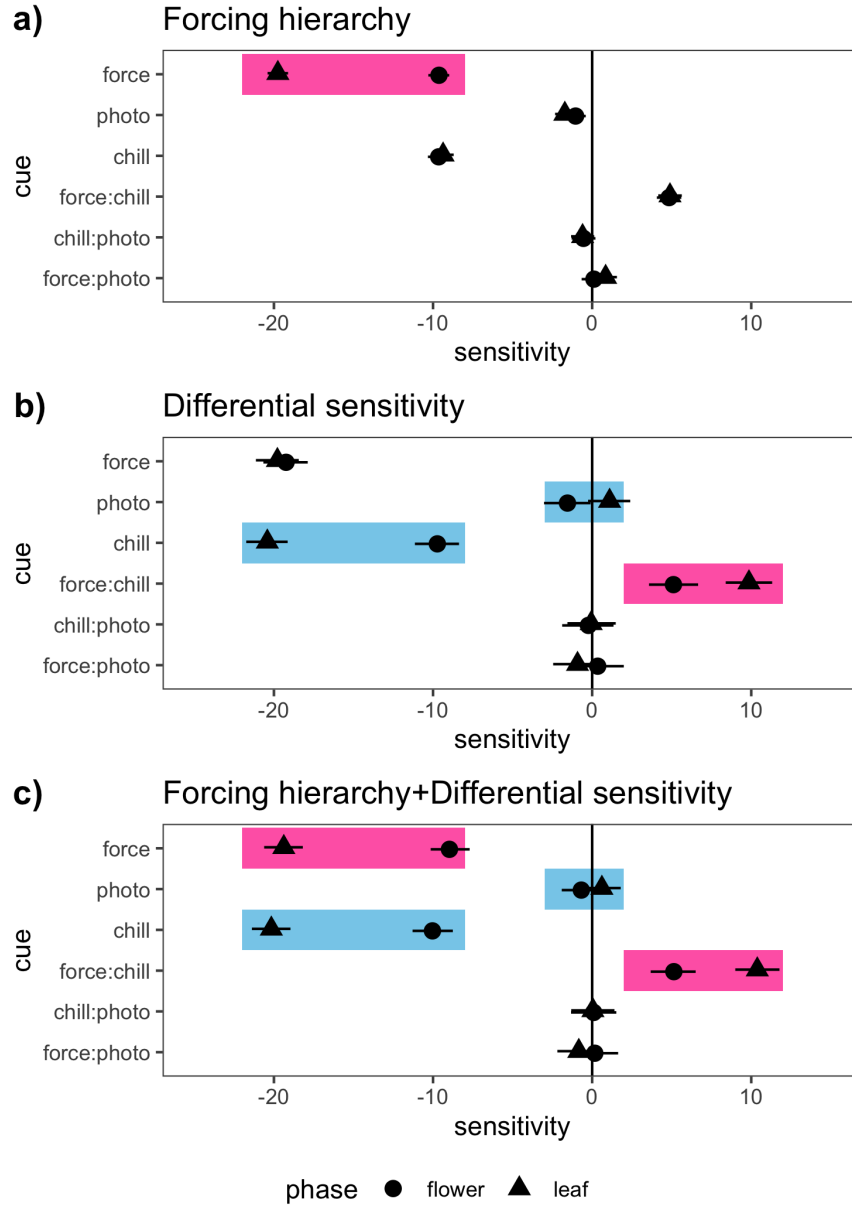


Figure 1: Characteristic sensitivity ( $\Delta$  day of phenological event /  $\Delta$  environmental cue) patterns of the phenological response to changing cue levels for the two major flower-leaf sequence hypotheses. **a)** displays a signature pattern of the forcing hierarchy hypothesis (FHH, pink boxes)—with the second phenophase in the sequence (in this case leafing) having a higher sensitivity to forcing than the first. **b)** Highlights a typical sensitivity pattern produced by the differential sensitivity hypothesis (DSH). **c)** Depicts a scenario where both the FHH and the DSH contribute to flower-leaf sequence variation. Here the characteristic forcing sensitivity of the FHH is still apparent but the differential sensitivity to chilling and photoperiod is seen as well (blue boxes). All plots above are based on simulations (see Supporting Information: Methods). Shapes indicate mean estimates and lines depict 95% credible intervals from Bayesian hierarchical models with advances in phenology shown as negative numbers, and delays in phenology as positive numbers.

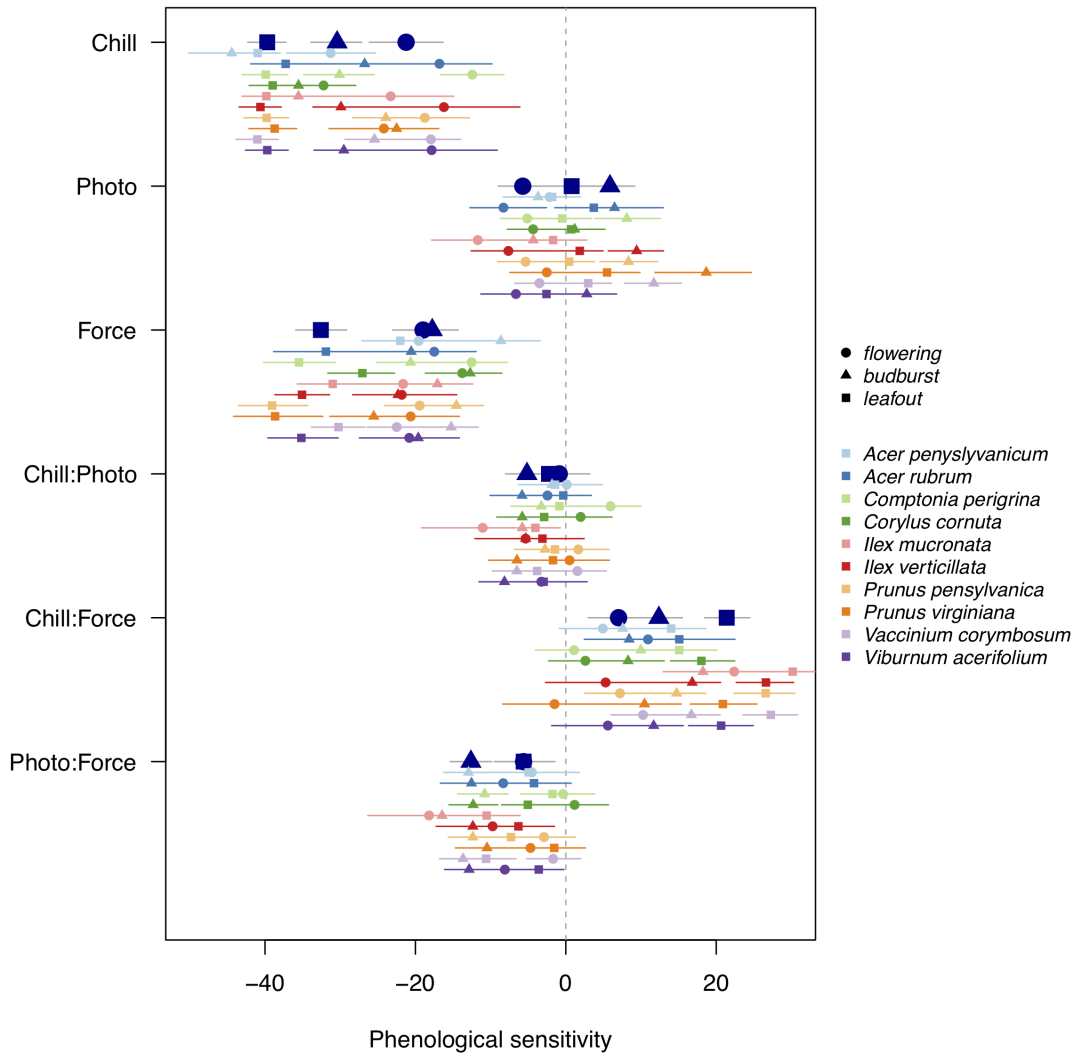


Figure 2: Effects of forcing temperature, chilling duration, and photoperiod on the leaf budburst (triangles), leafout (squares) and flowering (circles) phenology of 10 temperate woody plant species collected from Harvard Forest (Petersham, MA, USA). Shapes indicate mean estimates and lines depict 50% credible intervals (See Tab. S3, Tab. S4 for other intervals) from Bayesian hierarchical models with advances in phenology shown as negative numbers, and delays in phenology as positive numbers. Flower and leaf phenology differs in sensitivity ( $\Delta$  day of phenological event/  $\Delta$  environmental cue; 30 days chilling/6 °C forcing/4 hours photoperiod) to these environmental cues. See Fig. S2 for an alternative presentation of these results that depicts the difference between the mean estimates of each phase (shapes).



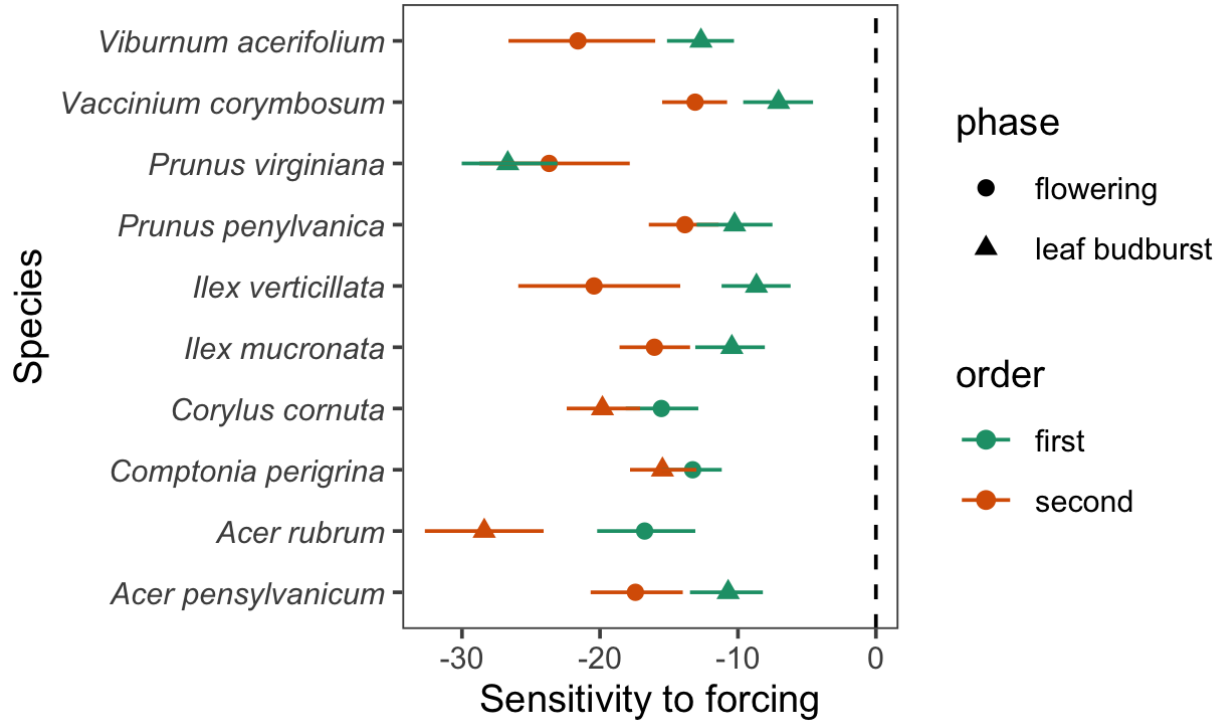


Figure 3: Phenological sensitivity ( $\Delta$  days of phenological event/  $\Delta$  6°C) to forcing temperatures of leaf budburst (triangles) and flowering (circles) phenology from 10 temperate deciduous woody plants at long (12 hour) photoperiod and long chilling duration treatments (60 days at 4°C). Shapes indicate mean estimates and lines depict 50% credible intervals (See Tab. S5 for other intervals) from Bayesian hierarchical models with advances in phenology shown as negative numbers. When photoperiod and chilling are high, most species follows the predicted pattern of the forcing hierarchy hypothesis (FHH), with the second phenophase of the sequence consistently more sensitive to forcing than the first. This result suggests that the FHH should be considered a special case of the differential sensitivity hypothesis (DSH) that occurs when the chilling and photoperiod requirements are met for both tissue types.

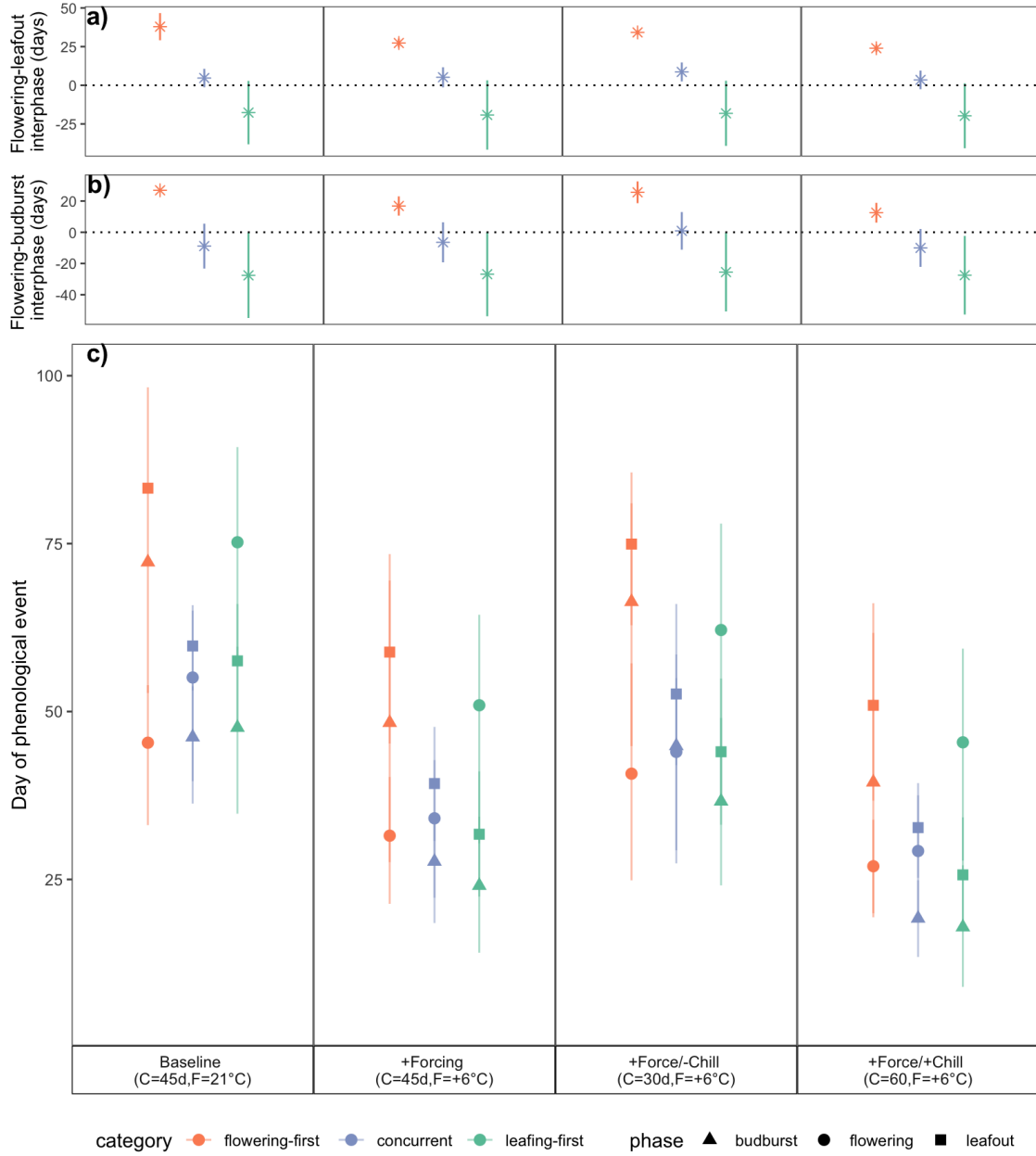


Figure 4: Projected shifts in flower-leaf sequences under current environmental conditions (Baseline) and three climate change scenarios (increase forcing, increase forcing/decrease chilling, increase forcing/increase chilling) predict that FLS shifts differ among the three major FLS types, and will be strongest in flowering-first species. Panels a) and b) show the mean time between flowering and vegetative phenological events (shapes) with 50% credible intervals (lines). Panel c) shows the predicted event day for each phase. Predictions are based on species-level posterior estimates grouped by FLS category (flowering-first, concurrent, leafing-first) from Bayesian hierarchical models comparing flowering (circles), leaf budburst (triangles) and leafout (squares) phenological responses to variable chilling duration and forcing temperatures. Shapes represent the mean estimates and lines represent the 50% credible intervals.