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

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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. In our study, FLS shifts were largest in wind-pollinated species that flower before leafing. If these results turn out to be generalizable to more wind-pollinated woody species, FLS shifts may drive fitness declines in these

taxa as climate change continue to alter woody plant phenology in the decades to come.

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 phenolog-

ical 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.

For example, 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 (Milleron *et al.*, 2012), which would ultimately impact population structure and gene flow across a landscape.

Predicting FLS shifts requires identifying the proximate mechanisms that drive FLS variation. 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 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 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 (i.e., climate observed in observational studies), the FHH and DSH may produce similar phenological patterns but experiments designed to disentangle all three cues should differentiate between the two. Studies aiming to differentiate these hypotheses can look for two different signatures. The key signature of the FHH is that the sensitivity to forcing (Δ day of phenological event/ Δ°C) of the second phase in the phenological sequence is always greater than

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that of the first phase with sensitivity differences being inversely proportional to the difference in thermal requirement among buds (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, but there is no characteristic pattern as to the strength or direction of this divergence. (Fig. 1 c.)

However, the clarity of two signatures of each hypothesis may be obscured in certain situations. First, a species' buds could be both differentially sensitive to photoperiod or chilling and respond to forcing in a forcing hierarchy (Fig. 1 b.). Second, the differential sensitivity framework can generate this signature of the FHH when secondary cues are at high levels due to interactions between the chilling response and thermal requirement (Fig. 1 c., chill x force interaction). When this happens the FHH would be, effectively, a special case of the DSH.

While the hypotheses may be indistinguishable under current field conditions, they have different implications 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 with 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 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.

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 repro-

ductive 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 FHH 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 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), consisting of a mix of deciduous shrubs, understory and canopy trees commonly found in mesic hardwood forests of the eastern United States (see tab. S1) for species list). We transported all cuttings to the Arnold Arboretum in Boston, MA where they were re-cut in water to prevent callousing and cavitation and placed in 500 ml Erlenmeyer flasks with distilled water.

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°: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 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.

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The models we fit appear below:

$$y_{[i]} \sim N(\alpha_{sp_{[i]}} + \beta_{forcing_{sp_{[i]}}} + \beta_{chilling_{sp_{[i]}}} + \beta_{photoperiod_{sp_{[i]}}} + \beta_{forcingxchilling_{sp_{[i]}}} + \beta_{forcingxphotoperiod_{sp_{[i]}}} + \beta_{chillingxphotoperiod_{sp_{[i]}}}, \sigma_y^2)$$

Where $y_{[i]}$ is either the day of the experiment of leaf budburst, day of first flower opening or FLS interphase length, and σ_y^2 the error. We modeled the α and each β 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 FHH 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 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 model slopes and intercept.

We fit all models using the R package "brms" (Bürkner, 2018). We ran each model on four chains with 4000 iterations and 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 $\hat{R}$s 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 October - 15 April, measured in Chill Hours (Weinberger, 1950)) at Harvard Forest is 1170.7, approximately 63% of the difference between our low and high chilling treatment (Tab. S2). Thus, our low chilling treatment represents a feasible estimate for a decrease in chilling with climate change and our high chilling treatment approximates a reasonable increase. We should note that our low photoperiod treatment (8 hours of daylight) is well below the photoperiod experienced at Harvard Forest, but given that the photoperiod effects are expected to be small, we chose more extreme values in order to robustly estimate an effect (i.e., increasing statistical power). For this reason, our climate change projections for FLS variation are based on our high photoperiod treatment alone.

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

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

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

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

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

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

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species common to both datasets (Fig. S1), and found them to be comparable. Given the variable dynamics of shifts in environmental forcing and chilling with climate change over time and space, these projections should not be treated as absolute predictions of the magnitude of FLS shifts with climate change. Instead, we provide these projections 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.

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

Results

Growth chamber study

Both flower and leaf bud phenology advanced with higher forcing and longer chilling duration (flowers: chilling effect: -21.2, forcing effect: -19, leaves: chilling effect: -30.4, forcing effect: -17.8; Fig. 2, Tab. S3,Tab. S4), but increases in both of these cues together offset these advances (flowers: force x chill effect: 7, leaves: force x chill effect: 12.4; Fig. 2, Tab. S3,Tab. S4). 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. 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 both bud types. For both bud types, chilling and forcing were the dominant cues, while increasing photoperiod produced a

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more muted phenological response (Fig. 2, Tab. S3,Tab. S4).

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: 5.8x, photoperiod x forcing: 2.2x) (Fig. 2, Tab. S3,Tab. S4). Across all species both bud types displayed a relatively proportionate advance with increased forcing. (Fig. 2, Tab. S3,Tab. S4).

While there was significant variation among species in their strength of their response to forcing between bud types, we did find the characteristic sensitivity pattern of the FHH to be widespread in our experiment (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 we 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 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. **??**).

Climate change predictions

Our models predict that both flower and leaf phenology will advance in most of our generalized scenarios, but 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 projections showed that FLS interphases were more

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strongly influenced by changes in chilling duration than increased forcing alone.

The direction and magnitude of shifts in FLS interphases depended on species and the specifics of FLS phase order. Under some warming scenarios, our model predicted that FLS interphases for some species may effectively disappear or the order of phenophases in the FLS may switch (Fig. 4, Fig. S2). 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 projections were *Comptonia peregrina* and *Corylus cornuta* (Fig. S2). In all of our climate change scenarios, the FLS interphase was dramatically reduced in these taxa.

Discussion

Our experiments support the hypothesis that flower-leaf sequences are structured by differenital 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 results 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). Yet, under the high chilling and photoperiod treatments, we found that FLSs for most species followed the predicted sensitivity pattern of the FHH, with the sensitivity to forcing of the second phase of the FLS proportionately higher than that of the first phase (Fig.3, Tab. **??**). This may explain why the two FLS hypotheses

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have been difficult to distinguish under historic field conditions where in most locations chilling requirements were frequently met (Gauzere *et al.*, 2019). Predicting FLS shifts will require accurate estimations of the differential sensitivity to climate between flower and leaf bud, and because this is difficult to do under contemporary field conditions, there is a need for generalizing principles to anticipate the impacts of FLS shifts.

Reconciling the differential sensitivity and the forcing 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 FHH, and offers insight into why these hypotheses have been difficult to differentiate in the past. Our data show that the FHH 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 FHH, 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 FHH 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 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).

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). The results of our study in wild species are consistent with experimental manipulations of tree-crop phe-

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nology 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 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, 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

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contribute to population structure in the long term.

The implications of strong differential sensitivity to photoperiod among bud types for climate change are more difficult to characterize. 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 phenologucal shifts by a minimum several weeks before the experienced photoperiod of plants would change substantially ()(Us, in review). Because these dynamics are more extreme than most studies suggest (Thackeray *et al.*, 2016), we modeled climate change scenarios with a constant photoperiod in our FLS projections. Yet it is important to note that at high latitudes where photoperiod changes more rapidly over the season, temperature driven phenological shifts may significantly alter the experienced photoperiod of plants, which could mute or amplify the FLS shifts captured in our projections. This may be particularly important as species shift their distribution pole ward 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. S2).

These differences suggest that FLS shifts will impact some species more than others and successful forest conservation and management programs may require identifying the species that are most

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vulnerable to extreme FLS shifts. To aid this effort, there is a strong need for generalizing principles to identify species with potential for consequential FLS shifts with climate change. While our one study doe not begin to represent the taxonomic diversity of a temperate forest, we identified several patterns in the FLS responses of our multi-species experiment to 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 S2). 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 particular 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 (¡i¿prunus spinosa¡/i¿ 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.

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Franklin, D.C. (2016) Flowering while leafess 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. & 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.

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.

Jackson, S.T. & Lyford, M.E. (1999) Pollen dispersal models in quaternary plant ecology: Assumptions, parameters, and prescriptions **65**, 39–75.

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

Kay, M. (2020) *tidybayes: Tidy Data and Geoms for Bayesian Models*. R package version 2.1.1.

Kling, M.M. & Ackerly, D.D. (2020) Global wind patterns and the vulnerability of wind-dispersed species to climate change. *Nature Climate Change* **10**, 868–875.

Linkosalo, T., Lappalainen, H.K. & Hari, P. (2008) A comparison of phenological models of leaf

bud burst and flowering of boreal trees using independent observations. *Tree Physiology* **28**, 1873–1882.

Man, R., Lu, P. & Dang, Q.L. (2017) Insufficient chilling effects vary among boreal tree 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. & Zust, 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., 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.

Niklas, K.J. (1985) The aerodynamics of wind pollination. *The Botanical Review* **51**, 328–386.

O'Keefe, J. (2015) *Phenology of Woody Species at Harvard Forest since 1990.* Harvard Forest Data Archive: HF003., Petersham, MA, USA.

20

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 –.

Post, E.S., Pedersen, C., Wilmers, C.C. & Forchhammer, M.C. (2008) Phenological sequences reveal aggregate life history response to climatic warming **89**, 363–370.

Rathcke, B. & Lacey, E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* **16**, 179–214.

Savage, J.A. (2019) A temporal shift in resource allocation facilitates flowering before leaf out and spring vessel maturation in precocious species. *American Journal of Botany* **106**, 113–122.

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.

WAY, D.A. & MONTGOMERY, R.A. (2015) Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment* **38**, 1725–1736.

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

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

483 Zhang, X., Tarpley, D. & Sullivan, J.T. (2007) Diverse responses of vegetation phenology to a

484   warming climate. *Geophysical Research Letters* **34**.

485 Ziello, C., Böck, A., Estrella, N., Ankerst, D. & Menzel, A. (2012) First flowering of wind-pollinated

486   species with the greatest phenological advances in europe. *Ecography* **35**, 1017–1023.
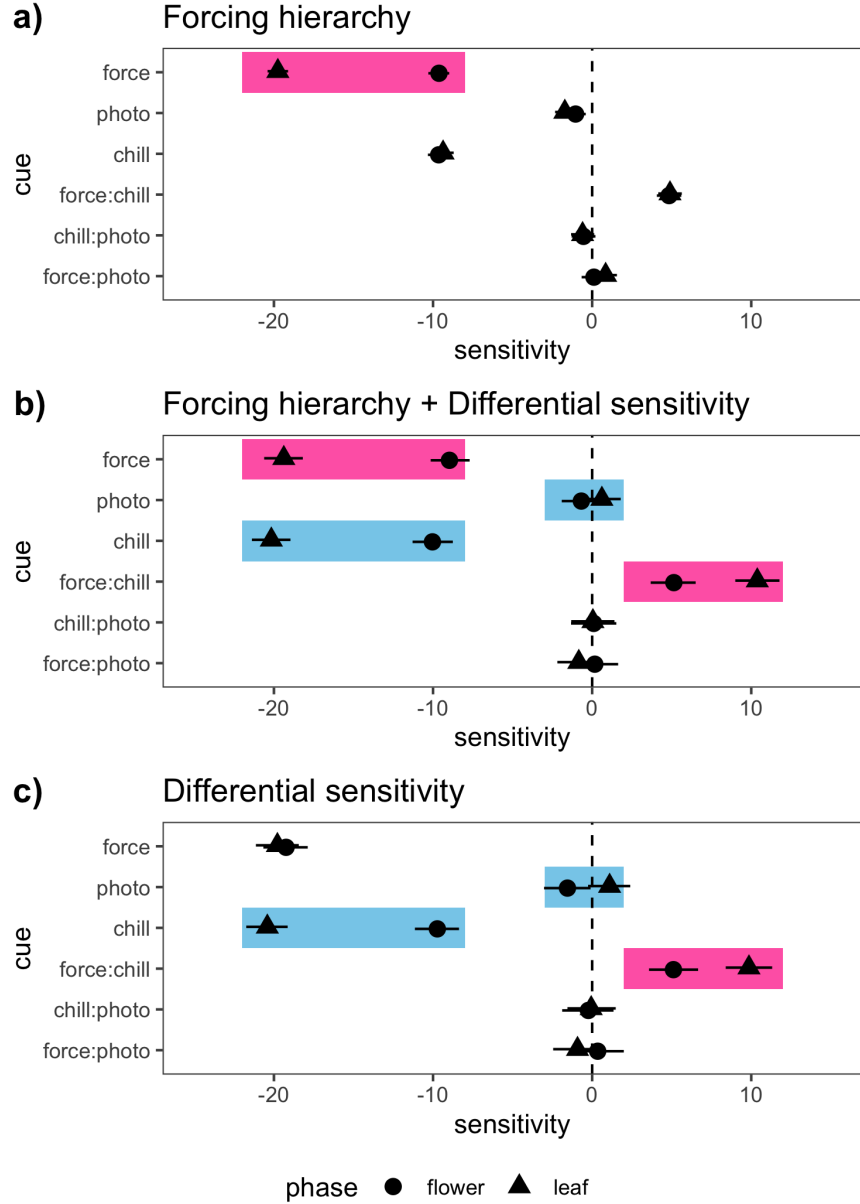
487 # Figures

Figure 1: Characteristic sensitivity (Δ day of phenological event/ Δ 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 phenophases in the sequence (in this case leafing) having twice the sensitivity to forcing than the first. **b)** depicts a scenario where both the FHH and the differential sensitivity hypothesis (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 observable as well (blue boxes). **c)** highlights a typical sensitivity pattern produced by the DSH without the FHH. All plots above are based on simulations (see Supplemental Methods). Points are the mean estimates and lines represent the 95% credible intervals from Bayesian hierarchical models.
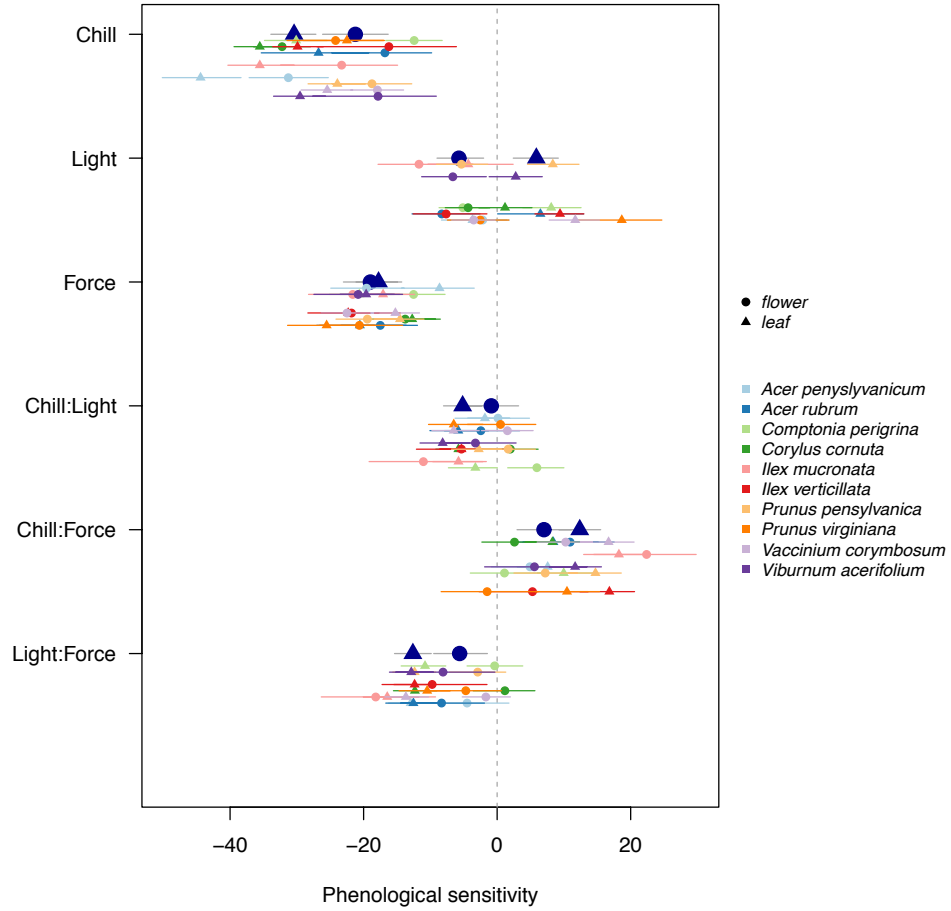
Figure 2: Effects of forcing temperature, chilling duration, and photoperiod on the leaf (circles) and flower (triangle) phenology of 10 temperate woody plant species. Shapes indicate mean estimates and lines depict 50% credible intervals from Bayesian hierarchical models with advances in phenology shown as negative numbers, and delays in phenology as positive numbers. Flower and leaf buds differ in sensitivity (Δ day of phenological event/ Δ environmental cue) to these environmental cues.

Figure 3: Phenological sensitivity (Δ phenological event/ Δ C°) to forcing temperatures of flowerw and leaves from 10 temperate deciduous woody plants at long (12 hour) photoperiod and long chilling duration treatments (8 weeks at 4°). Points indicate mean estimates and lines depict 50% credible intervals from Bayesian hierarchical models. Stronger advances in phenology are shown as negative numbers, and delays in phenology as positive 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 well met for both bud types.
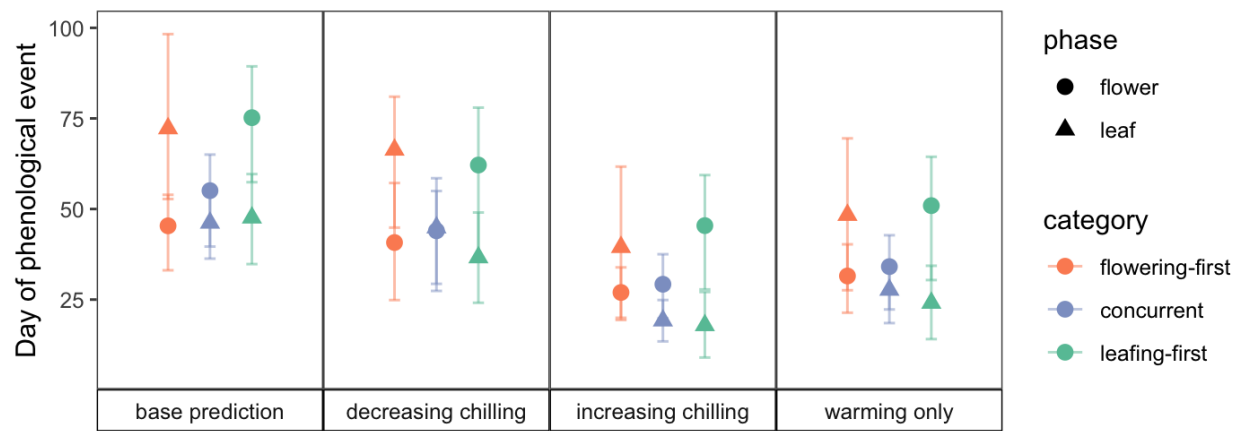
Figure 4: Projected shifts in flower-leaf sequences under current environmental conditions (base prediction) and three climate change scenarios differ among the three major FLS types. Predictions are based on species-level posterior estimates grouped by FLS category (flowering-first, concurrent, leafing-first) from Bayesian, hierarchical models comparing flower and leaf bud responses to variable chilling duration and forcing temperatures. Points represent the mean estimates and lines represent the 50% credible intervals. Our models predict that FLS shifts will be strongest is flowering-first species.