

Differential sensitivity to the environment between flower and leaf
buds drive phenological sequence variation in woody plants

or

The physiology of flower-leaf sequences in deciduous woody plants:
differential sensitivity to the environment drives long-term variation
and future shifts with climate change

D.M. Buonaiuto ^{1,2,a}, E.M. Wolkovich³

Abstract

The order and duration of vegetative and reproductive phenological growth in the spring is an important fitness character for deciduous woody plants in the temperate zone. These flower-leaf sequences (FLSs) appear to be shifting with climate change, but the magnitude and impact of future shifts are difficult to predict without an improved understanding of the phenological coordination between flower and leaf buds with each other and the environment. We used long term field observations and growth chamber manipulations to characterize the phenological responses of flower and leaf buds to variable climate conditions for a suite of temperate woody species. We found that flower and leaf buds respond with differential sensitivity to temperature and day length, and that these differences dictate the direction and magnitude of FLS shifts with climate change. The potential for FLS shifts vary across species, and are likely to compromise the reproductive performance of some wind-pollinated, flowering-first species as global climate continues to change.

Introduction

Phenological sequences, the temporal relationships among distinct life-cycle events and transitions, strongly influence plant fitness (???). Among deciduous woody plants, the relative timing of flower and leaf development, or flower-leaf sequences (FLSs), may be particularly consequential to fitness in temperate regions (?). In these regions, flowering prior to leaf development is common among species in the and may facilitate efficient pollen transfer for wind-pollinated species (?), increase floral visibility in biotically-pollinated taxa (?), improve hydraulic functioning (??) or facilitate early flowering (?).

Long-term phenological observations over the last several decades indicate that FLSs are shifting with anthropogenic climate change, suggesting that some of these critical functions of FLSs may be at risk. However observed FLS shifts vary among species, which may put some species at greater risk while benefiting others (?). The effects of FLS shifts on woody plant fitness depends on their direction and magnitude and predicting these extent shifts will require a deeper inquiry of into the fundamental biological and environmental mechanisms that control FLSs.

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 in the spring (??) Yet, high levels of reported inter-annual variation in FLSs (?) indicate that there must be differences in how these cues influence phenological activity in floral and leaf buds.

While there are also developmental, architectural and physiological constraints to FLS variation, research has shown that the flower and leaf buds of many spring flowering woody species of the temperate zone are relatively independent (), suggesting that FLS variation is strongly influenced by the differences in cue utilization among flower and leaf buds, yet the specifics of these responses have not been thoroughly investigated. Identifying these differences is a necessary step for predict-

ing the direction and magnitude, and ultimately fitness impacts, of FLS shifts with climate change.

In this study, we integrate long-term field observations with experimental climate manipulations to compare the phenological response to changing environmental conditions between flower and leaf buds. We then leverage these data to make generalized projections for how FLSs may shift with future climate change. Finally, we interpret these predictions in the context of the functional hypotheses of FLS variation to assess how FLS shifts may impact the performance of some woody plants.

Physiological hypotheses for FLS variation

We are not aware of any previous studies that systematically compare the phenological responses of flower and leaf buds within individuals of wild species. However, because FLS variation has implications for fruit production, several studies have investigated these responses in tree crops (see ???). While crop phenology is often quite different than that of even related species wild species, this work in tree crops provides several hypotheses that can serve as starting points for an investigation in wild species.

There are two major hypotheses regarding the underlying physiology that structure FLS variation. Below we briefly review each hypothesis and their predictions for how FLS patterns would be expected to vary or shift in 1) long-term field observations, 2) growth chamber studies, and 3) with climate change.

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Precocity hierarchy

Some authors have suggested that reproductive and vegetative buds respond similarly to most environmental cues, but have consistently different forcing requirements for the commencement of phenological activity (?). Under this hypothesis, the first phenophase in the FLS always has a consistently lower heat sum requirement than the second—therefore any observed FLS variation is a product of climate variation during the interphase. This suggests that years in which it is cooler during the interphase heat sums accumulate more slowly, resulting in a longer interphase. Conversely, warmer years should reduce the interphase duration.

This hypothesis predicts that heat sums during interphase will be consistent (Fig. 1a) across years or environmental conditions, even though there may be substantial variation in the duration of the FLS interphase measured in calendar time. Further, the order of phenological events comprising the FLS cannot switch (though the duration of the interphase may vary substantially). At the cue level, the precocity hierarchy relies on flower and leaf buds sharing the same cues and that photoperiod and chilling cues are very similar in magnitude, but magnitude of the forcing cues, varies such that the second phase in the FLS requires more forcing (the sensitivity ($\delta days/\delta T$) of the second phase in the FLS to forcing should always be higher than the first). When extended to climate change impacts, the precocity hierarchy hypothesis predicts that if spring temperatures

increase with climate change, FLS interphases will decrease.

Differential sensitivity to multiple cues

Flower and leaf buds may differ in the strength of their phenological responses to the same environmental cues. For example, ? found that in peach cultivars, vegetative buds responded more strongly to chilling exposure and had lower heating requirements than reproductive buds. Given this hypothesis, both the order of phenophases in a given FLS and the duration of the interphase depends on combinations of cues.

Generalities regarding FLS variation are more elusive, because forcing, chilling and photoperiod do not perfectly correlate over a growing season yet, because of this variability, this hypothesis predicts that both the calendar time and heat sums of the FLS interphase will vary among years. (Fig. 1b). Unlike the procicity hierarchy hypothesis, extreme climate years may even drive switches in the order of FLS phenophases. When considering cue use, this hypothesis predicts that different bud types will have marked differences in their sensitivities to each cue environmental cue (Fig. 1d). With strong cue use differences among bud types, there will be strongly localized effects of climate change on FLSs. While on average the climate is warming, chilling and forcing may increase or decrease at different locations and on different time scales (?). Shifts in FLS variation will depend on the direction and rate of change in cues at specific locations and the differential sensitivity of reproductive and vegetative phenology to cue combinations.

1 Methods:

Data simulations:

To better understand how the two hypotheses for FLS physiology would manifest in real data, we performed simple data simulations. First we generated 10 year of temperature data in which mean

daily temperatures during the inter-phase period varied substantially among years. We applied a fixed heat sum threshold (F^*) for the interphase to represent the precocity hierarchy hypothesis while letting the interphase F^* value vary inter-annual to represent changes in other cues for the differential sensitivity hypothesis. For each hypothesis, our simulations record the number of days to reach the F^* (the days between the first and second phenophases) in each year.

Next we simulated

Analysis of field observations:

We obtained 24 years of phenological data for a suite of woody species growing at Harvard Forest, in Petersham, MA (?) from the Harvard Forest Data Archive (?). For each individual tree, we calculated the duration of the FLS interphase in each year by subtracting the calendar day of budburst from the calendar day of flower opening. To calculate the growing degree days of the interphase we obtained daily temperature measurements from the Shaler Meteorological Station at Harvard Forest (). The temperature data collection at this station ended in 2004, so we only analyzed the phenology data through this season. For each individual tree in each year, we used the R package “chillR” () to calculate the Growing Degree Days accumulated during the interphase using a base temperature of 5 °C. Because flowers were not observed in all years for all species, we analyzed the data for the seven most well represented species in the dataset. To characterize the interannual variation in the FLS interphase for each of these species, we calculated the mean and standard deviation interphase length in both calendar time and growing degree days for each year of the data.

Growth chamber study

We sampled all plant material used in this experiment from Harvard Forest in Petersham, MA. On October 25, 2016, immediately after most plants in the area entered dormancy but before they could accumulate any significant chilling in the field, we collected branch cuttings from 7-13 indi-

viduals 12 woody plant species (4-12 cutting per individual for a total of 48-56 per species). The species consisted of a mix of deciduous shrubs, understory and canopy trees commonly found in mesic hardwood forests of the eastern United States (see S1 for species list). We transported all cuttings to the the Arnold Arboretum in Boston, MA where they were re-cut in water to prevent callousing and cavitation and place in 500 ml Erlenmeyer flasks with distilled water.

We randomly assigned cuttings to a full set of eight experimental treatments; 2 levels of chilling (4 vs 8 weeks at 4°C), 2 levels of temperature (24°C:18°C warm vs 18°C:12°C cool) and 2 levels of photoperiod (12 vs 8 hours). We alternated day/night temperature periodicity on a 12 hour schedule to reduce co-variation with photoperiodicity. We re-cut all twig and changed the water every 7-10 days and rotated all treatments between growth chambers every two weeks to minimize chamber effects. We made phenological observations every 2-3 days using the modified BBCH scale for woody plants () for 3 month following release from chilling conditions. In this period we assess three phenological phases: budbreak (BBCH phase 07), leaf unfolding (BBCH phase 15) and first flower open (BBCH 60). At the conclusion of this period we assessed all individuals that did not undergo budbreak and excluded any dead individuals for analysis.

Analysis of growth chamber study

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.

We fit all models using the R package “brms” (). We ran each model on four chains with 4000 iterations with a 3000 iteration warm up for a total of 4000 sampling iterations. In both models We used weakly informative priors and increasing the priors 5-fold did not affect the model results.

The models we fit appear below:
(Write models here)

Climate change predictions

To apply our model results to general climate change projections we chose our environmental treatments in this experiment to reflect historic and future conditions at Harvard Forest. Our low forcing treatment approximates average spring temperature (March/April) at the site while our high temperature treatment reflects a 5 °C increase. Average field chilling (measured in Utah units) at Harvard Forest is X, approximately 60% of the difference between our low and high chilling treatment. Thus, our low chilling treatment represents a feasible estimate for a decrease in chilling with climate change and our high chilling treatment approximate 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:

- The historical range of FLS (low forcing, 60 % chilling)
- FLS shifts with spring warming only (high forcing, 60 % chilling– equivalent to 6.5 weeks of chilling treatment)
- FLS shifts with warming and increased chilling ((high forcing, 100 % chilling– equivalent to 8 weeks of chilling treatment)

- FLS shifts with warming and decreased chilling ((high forcing, 0 % chilling– equivalent to 4 weeks of chilling treatment)

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 the range of possibilities vary based on individual characteristics of plant species and the specific climate dynamics.

Results

Field Observations

We found that for individuals at Harvard Forest both the calendar time and heat sums accumulated during the FLS interphase varied significantly across years. For *Acer rubrum*, the species with the most FLS observations in the data set, the calendar days of the interphase varied from X-Y and GDD of the same period from Z-A 2.

We found that the magnitude of GDD variation during interphase differed among species, but almost all had patterns that were more reflective of the differential sensitivity hypothesis (Fig. ??). We found similar patterns in the interphases of other floral-leaf sub-phases (eg. flower and leaf budburst) and observed that the order of these phenophases switch periodically in some species over time (Fig. ??) lending further support to the differential sensitivity hypothesis.

Growth chamber study

We found that that flower and leaf buds response to environmental cues with differential sensitivity (Fig. 3). Specifically, while both bud types had a proportionate response to forcing, leaf buds were more sensitive to chilling. At low levels of chilling and forcing, flower buds tended to advance

which increasing photoperiod while leaf buds were delayed. Leaf buds were more sensitive to cue interactions, demonstrating stronger responses to increasing in multiple cues than flower buds. While the order of the FLSs remained consistent across treatment combinations in most species, we found that one species, *Vaccinium corymbosum* switched FLS order across chilling treatments (Fig. 5).

Climate change predictions

Our model predicted that both flower and leaf phenology will advance in most of our generalized scenarios for most species, but shifts in FLS vary depending on how forcing levels change relative to chilling duration (Fig. 4). As would be expected from the significant differences in sensitivity between flowering and leafing phenology in our model, the FLS interphase was more strongly influenced by changes in chilling exposure than increased forcing alone. The direction and magnitude of shifts in the FLS interphase depended on species and the specifics of FLS order, with flowering-first and concurrent species tending show more profound alterations to FLS patterns than leafing-first ones. Under some warming scenarios, our model predicted that the FLS interphase effectively disappear or the order of phenophases would switch (see Fig. 4b-f).

Discussion

Outline

1. summarize results
2. compare results to crops
3. FLS shifts sensitive to chilling and chilling trends vary by location
4. Integrate magnitude with hypotheses
 - hysteranthous wind pollination and strong shifts- bad outcome
 - synanthous insect pollinated, fairly strong shifts- unclear outcome

- seranthous insect pollinated, weak shifts

5. qualify photoperiod impacts

Our analysis of long term field observations and experimental results suggest that flower and leaf buds are differentially sensitive to the primary environmental cues of spring phenology. Specifically, vegetative buds are more sensitive to chilling and cue interactions, and flower buds more sensitive to photoperiod. This differential sensitivity generates the high level of inter-annual FLS variation observed in nature, and will dictate the direction and magnitude of FLS shifts with climate change.

Our findings in support of the differential sensitivity hypothesis are consistent with much of the tree crop literature. Like studies in peaches (??) we found that the heat requirements for phenological activity were dictated by cue combinations, with leaf buds responding more strongly to chilling than flower buds. Similarly, we also found that like peaches, flowers in some species (eg *Vaccinium corybosum*) tend to emerge before the leaves at low chilling levels. We found no crop literature that evaluated the differential sensitivity of flower and leaf buds to photoperiod. However, consistent with our findings, genetic work in the model genus *Populus* suggests that flowering may be under stronger photoperiodic control than leafing ().

We found that species differ in the direction and magnitude of their FLS shift with variable climate conditions. Several species had FLSs that were relatively robust to changing environments. These species, *Ilex verticillata*, *Prunus pensylvanicum*, *Prunus virginiana*, and *Viburnum acerifolium*, all share a strongly leafing-first FLS, with the fairly long FLS interphase. These species all also have mixed buds, so there may be relatively strong physiological constraints on their FLS. With climate change, it may be that any functional impacts of FLS shifts specifically will be minor compared to those that come about from phenological shifts in general such as alterations to the growing season (), increased risk of frost or pest damage (), and phenological mismatch ().

For all other species, there were significant shifts in FLS under varying climate conditions. The

direction and magnitude shifts largely depend on how forcing and chilling shifted relative to each other. In many northern temperate locations, chilling is projected to increase over the next several decades (?), while in other locations, it may rapidly decrease. These divergent trajectories are likely to increase the population level variation in FLS.

Importantly, the impact of these FLS shift depends not only on the magnitude of the shift, but also on the adaptive function of FLS, which is also likely to differ between taxa in this study and in temperate forest communities in general. Our study identified several species with significant FLS variation under different treatment conditions and fairly substantial FLS shifts with climate change. For *Acer pensylvanicum*, *Vaccinium corymbosum* and *Ilex mucronata*, which typically begin to produce leaves shortly before flowers open, increased forcing coupled with a reduction in chilling advanced flowering more than vegetative budburst, resulting in a switch to concurrent or flowering-first FLS. The performance implications for such shifts are not clear. If these species rely on visual pollinators, this shift may increase pollination success, but as of yet, there is little evidence for the pollinator visibility hypothesis of FLS. In accordance with the water dynamics hypothesis, this shift could theoretically reduce hydraulic demand on the plant as a whole by partition the phenological activity of these two tissues. However, this emerging flowering-first phenotype may not confer much hydraulic advantage given that our model projects that the FLS interphase would still be very short (a few days) and that it is unlikely that plants are water limited in the spring season in the temperate zone (?).

Shifts in FLS may be most consequential for wind-pollinated taxa. There is growing evidence that a flowering-first FLS is an important adaptation for wind pollination, reducing barriers for airborne pollen transfer(?). For example, ? quantified the amount of pollen impacted on non-floral structures, estimating that a single branch with leaves would intercept more than double than what was impacted on a bare branch. This suggests that a reduction in the FLS interphase may dramatically reduce reproductive success of flowering-first, wind-pollinated taxa.

297 In our study, the two species with the most significant FLS shifts across treatment combinations
 298 were the flowering-first, wind-pollinated species *Comptonia peregrina* and *Corylus cornuta*. In all of
 299 our climate change scenarios, the FLS interphase was dramatically reduced in these taxa. Given the
 300 hypothesized function of FLS in wind pollinated species, and the observed direction and magnitude
 301 of FLS shifts in our experiment, it may be that these species, and flowering-first, wind-pollinated
 302 taxa in general, may face particular risk for reproductive performance reductions from FLS shifts.

303

304 However, we did not observed this trend in the other wind-pollinated, flowering first species in our
 305 study *Acer rubrum*, which maintained a fairly large and consistent FLS interphase in each of the
 306 treatment combinations in our study, as well as in our climate change projections. While the low
 307 sample size in our study for these (or this one?) species warrants caution in interpreting this find-
 308 ing, it may reflect biological differences between these species. *Acer rubrum* is a canopy tree while
 309 the other two species are low growing, understory shrubs. These species are also phylogenetically
 310 distant (). Additionally, the genus *Acer*, is ambophilous(?), and there is evidence that even *Acer*
 311 *rubrum* which is considered largely wind-pollinated may still rely on insects for pollination as well
 312 (). These species also have very different floral morphologies (access dejong to describe this better).

313

314 The particulars of the floral morphology in *Corylus* and *Comptonia* and their relatives in the
 315 temperate dominant Betulaceae, Juglandaceae and Fagaceae, and Salicaceae families may further
 316 increase the functional impact of FLS shifts. The taxa are generally monoecious and protandrous,
 317 with staminate flowers proceeding pistillate ones to promote out-crossing (). Staminate flowers
 318 are born on separate catkins, while pistillate flowers emerge from within leaf buds and temporally
 319 constrained leaf phenology. Thus, a reduction in the FLS interphase correlates with a reduction in
 320 dichogamy, increasing the likelihood of self-pollination and ultimately inbreeding depression ().

321 Much of the conversation around phenology and pollination in the context of global change has
 322 centered around trophic mismatches between pollinator and floral phenology (), which is of little

relevance to abiotically pollinated taxa. By contrast, we find evidence that the effect of FLS shifts with climate change may be particularly important for abiotically pollinated woody plants and the scope and impact of FLS shifts in these taxa should be explored in greater detail in the future.

Despite the fact that on our experiment we found photoperiod to be an important cue dictating FLS shifts, in our FLS projections with climate change we modeled climate change scenarios with a constant photoperiod. Climate change does not directly impact photoperiod, but warming does shift the time of year when plants become phenologically active, changing the photoperiod they experience. However, depending on the latitude, phenology would have to shift by at minimum several weeks before the experience photoperiod would change substantially (?). However, at high latitudes where photoperiod changes more rapidly over the season, the experienced photoperiod may mute FLS shifts captured in our projections. This may be particularly important as species shift their distribution poleward with climate change and begin to encounter novel photoperiod regimes ().

Conclusion:

Both our field observations and experimental study provide strong evidence that flower and leaf buds integrate the same environmental cues differently, maintaining variation in FLS. As climate change continues to alter these temperature cues, species with physiologically independent buds and strongly divergent temperature sensitivities among bud types will likely experience significant shifts in FLS. This shift may be particularly detrimental to flowering-first, wind-pollinated species that rely on a lengthy leaf-free period for pollination. Because of the prevalence of these taxa in temperate forests, the scope and impact of FLS shifts in these taxa remain a high research priority.

Supplement:

1. Species list

2. field plots for extra species

3. table with chilling comparisons

2 Figures

Figure 1: This figure predicts the field (a-b) and experimental (c-d) results under the order heat sums (a&c) and differential sensitivity (b & d) hypothesis

Figure 2: look there is high variation in acer rubrum, also need to remake this figure

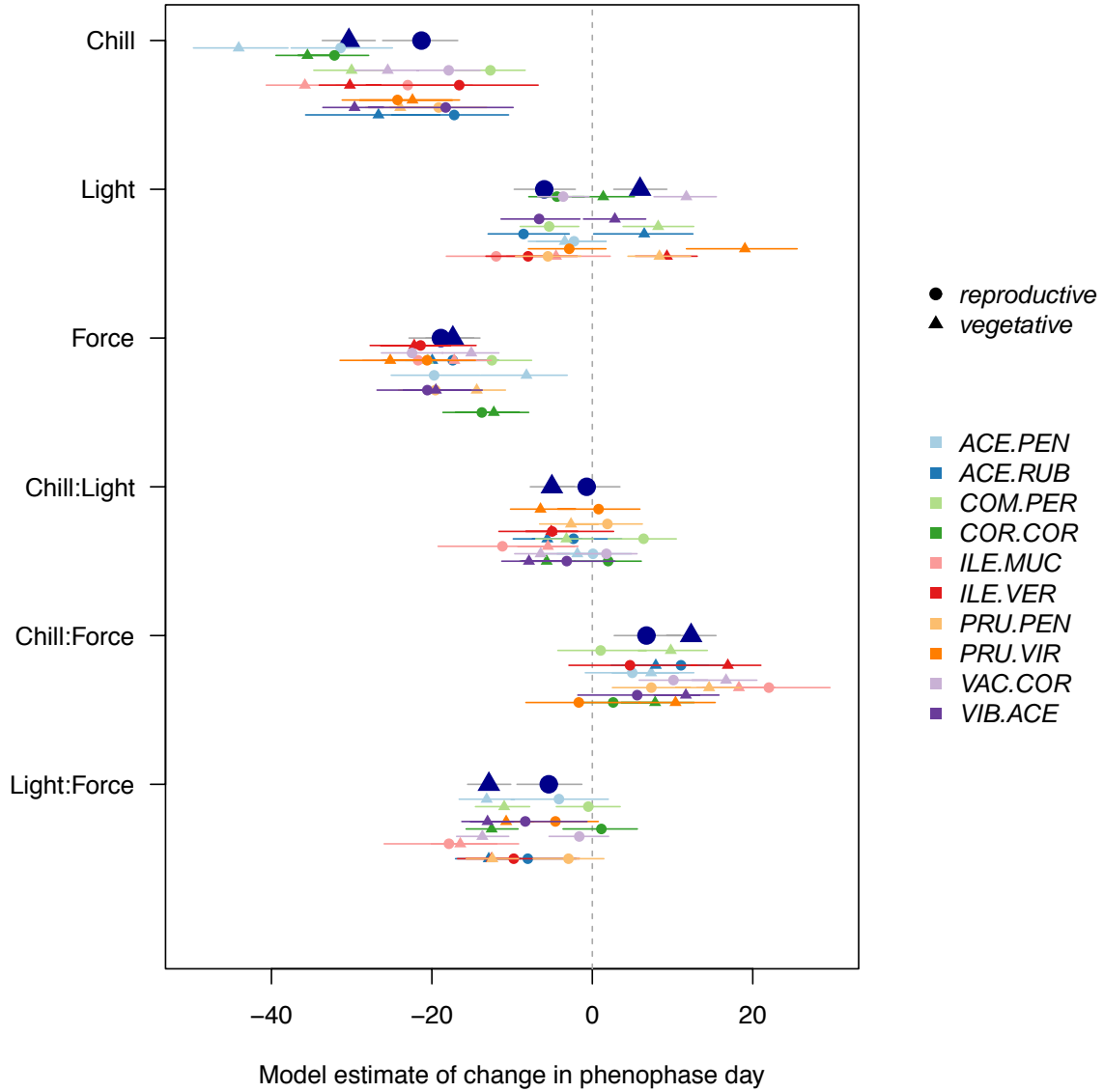


Figure 3: **Experimental results suggest differential sensitivity to environmental cues between flower and leaf buds.** Vegetative buds (circles) as more sensitive to chilling and interaction between chilling and forcing. Flower buds (triangles) advance with photoperiods increases but leaf buds appear to delay. These differential sensitivities have implications with how FLS patterns vary given environmental variation

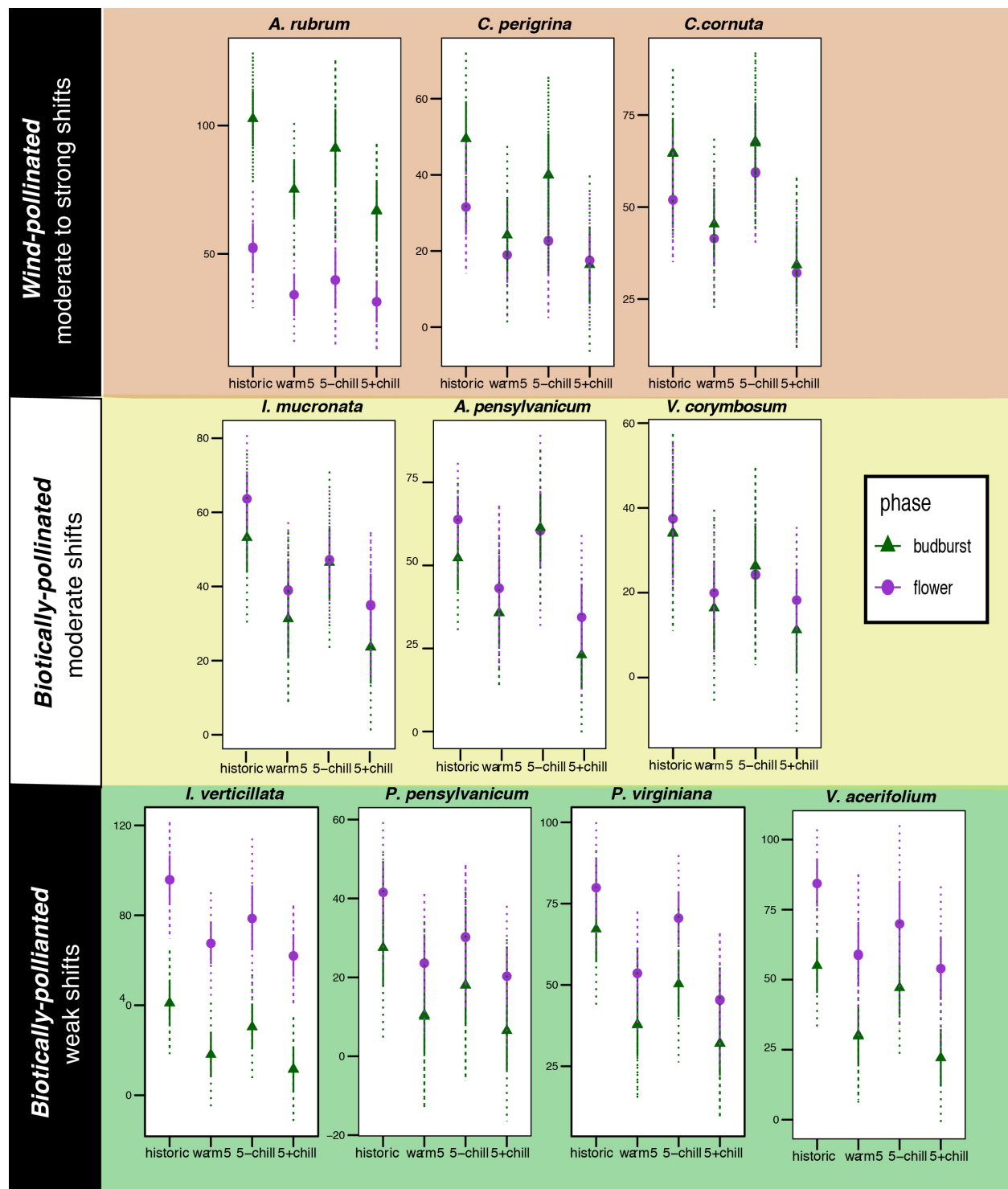


Figure 4: This shows our climate change predictions

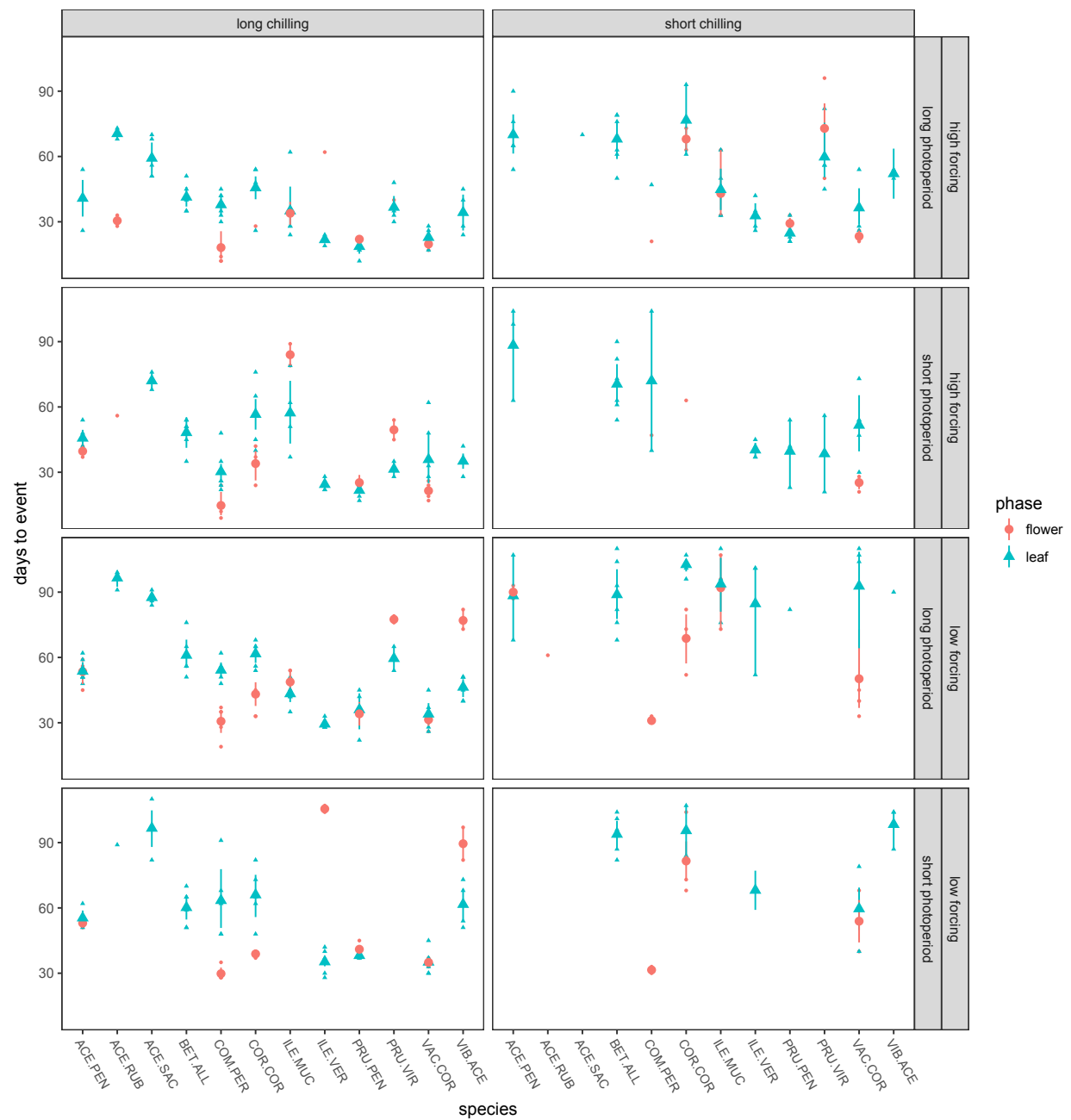


Figure 5: This figure need to be re-made using bbch 9 instead of 15, but should probably just show vaccinium or bounce to supplement

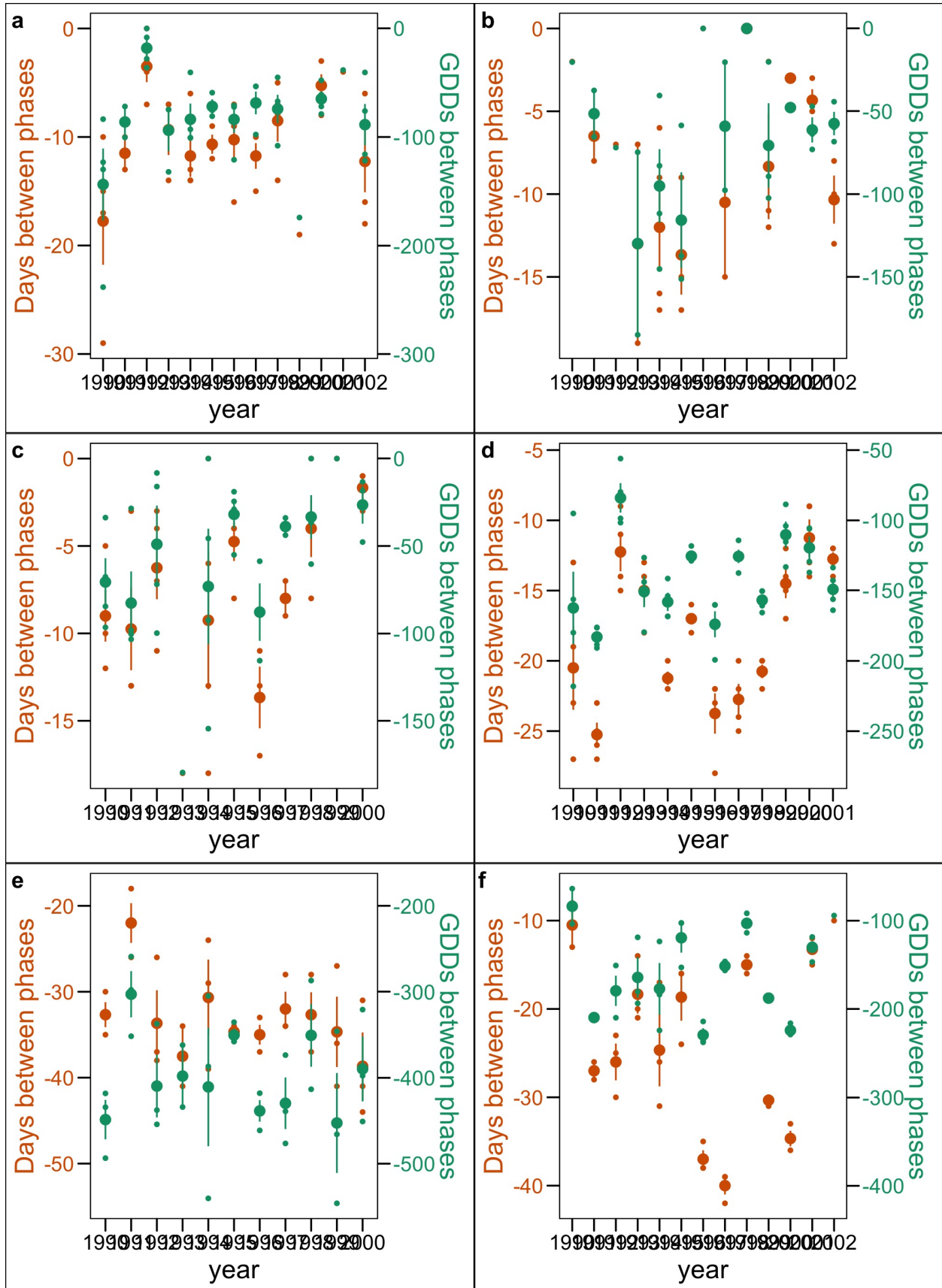


Figure 6: **Other species in HF**