Species differences in budburst responses in woody plants of North America

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Introduction

Materials and Methods

0.1 Field sampling

We combined data from two growth chamber studies using branch clippings of North American deciduous woody plants. The first used samples taken from two eastern populations—Harvard Forest, Massachusetts, USA (42.55°N, 72.20°W) sampled from XX-XX, and St. Hippolyte, Quebec, Canada (45.98°N, 74.01°W) sampled from XX-XX. The second growth chamber study spanned two western populations—Smithers (54.78°N, 127.17°W) and E.C. Manning Park (49.06°N, 120.78°W), British Columbia, Canada, respectively sampled from October 19th to 23rd and 26th to 28th, 2019.

We observed budburst for 47 species spanning our two transects, with 28 species at our eastern transect and 22 species at our western transect, of which 3 species occurred at both transects. We selected dominant forest species at each population, maximizing the number of species occurring across sites. Of the species in our eastern transect, 13 were shrubs and 15, while in our western forest community we sampled 18 shrub and 4 tree species. At all sites, we tagged between 15 and 20 healthy, mature, individuals for each species in the summer prior to the growth chamber study. We collected between 1-16 cuttings from six to 20 tagged plants, depending on the size of the individual, with samples being taken using a pole pruner from the ground. We kept samples cold during sampling and immediately placed them in water upon returning from the field. Our eastern study was conducted at the Arnold Arboretum, Boston, MA, USA, while we conducted the western study at the University of British Columbia, British Columbia, Canada. We used daily weather data from local weather stations to calculate the field chilling experienced by samples prior to collection (see Table XX).

Growth chamber study

To estimate effects of chilling, forcing and photoperiod, we used growth chambers with two levels of chilling—with either no additional chilling or 30 days at 4°C for our eastern study, and 30 days or 70 days of chilling at 4°C for our western study (all dark)—after which plants were moved to one of two levels of forcing—a cool regime of 15°C:5°C and a warm regime of 20°C:10°C—crossed with two photoperiods of either 8 or 12 hours—for a total of 8 distinct treatments. This design was similar for

both eastern and western species, except for thermoperiodicity in forcing treatments. In our eastern study, we set the duration of forcing temperatures to correspond with the photoperiod treatments, leading to a confounding effect in the thermoperiodicity. In our western study, we applied forcing temperatures for 12 hours independent of the duration of the photoperiod treatments. We had 8 replicates per treatment, with the exception of *Sorbus scopulina*, for which we we only had six replicates per treatment. Further details regarding the re-cutting of samples, changing water in containers, rotation through chambers, and similar are presented in Flynn and Wolkovich (2018).

Phenological observations of budburst were assessed using the BBCH scale, adapted for our specific species (Finn et al., 2007). We observed each sample every 1-3 days for 86 days. We recorded all phenological stages up to full leaf expansion (defined as code 17 by Finn et al. (2007)), but here we present the response in budburst date, defined as the day of budbreak or shoot elongation (defined as code 07 by Finn et al. (2007)). In total, our eastern study spanned 82 days, with over 19320 phenological observations. The western study in turn spanned 113 and we made 47844 phenological observations across all samples.

Statistical Analysis

To test for differences in cue responses across species and populations, we used a phylogenetic mixed effect model with partial pooling across species. This approach allowed us to account for both the evolutionary relatedness of our species, and estimate the species level cue responses and differences across sites. Species phylogenetic relatedness was obtained by pruning the **?** megatree of angiosperms. We included all cues as continuous variables in our model. To account for the greater amounts of field chilling experienced in our eastern study, we converted our chilling treatments to chill portions for each individual population using local weather station data and the chillR package (Luedeling2020). Chill portions were calculated for both the chilling experienced in the field prior to sampling and during the experiment in our 4°C chambers. We also converted our forcing temperature to the mean daily temperature in each treatment to account for differences in thermoperiodicity between the two studies (cite Dan's paper?). Finally, we z-scored each cue and site using two standard deviations to allow results across parameters to be directly compared. In addition to these overall predictors, we included all two-way interactions between cues and between cues and sites:

$$\hat{y}_i \sim \text{normal}(\alpha_{sp_i} + \beta_{site2} + \beta_{site3} + \beta_{site4} +$$
$$\beta_{chill_{sp_i}} + \beta_{force_{sp_i}} + \beta_{photo_{sp_i}} +$$
$$\beta_{force \times chilling_{sp_i}} + \beta_{chilling \times photo_{sp_i}} + \beta_{photoperiod \times chilling_{sp_i}} +$$
$$\beta_{force \times site2_{sp_i}} + \beta_{force \times site3_{sp_i}} + \beta_{force \times site4_{sp_i}} +$$
$$\beta_{chilling \times site2_{sp_i}} + \beta_{chilling \times site3_{sp_i}} + \beta_{chilling \times site4_{sp_i}} +$$
$$\beta_{photoperiod \times site2_{sp_i}} + \beta_{photoperiod \times site3_{sp_i}} + \beta_{photoperiod \times site4_{sp_i}})$$

$$\beta_{force_{sp}} \sim \text{normal}(\mu_{force_{sp}}, \sigma_{force}^2)$$

$$\ldots$$

$$\beta_{photoperiod \times site4} \sim \text{normal}(\mu_{photoperiod \times site4}, \sigma_{photoperiod \times site4}^2)$$

We included the phylogenetic effect as a variance covariance matrix in the parameterization of the normal random vector:

$$\boldsymbol{\alpha} = [\alpha_1, \ldots, \alpha_n]^T \text{ such that } \boldsymbol{\alpha} \sim \text{multi-normal}(\mu_\alpha, \boldsymbol{V}), \tag{1}$$

$$\tag{2}$$

The timing of budburst prior to evolution is represented as root values of the means of the multivariate normal distributions. For more detail on this method of phylogenetic modeling, see Morales-Castilla et al (XXXX).

We used semi-informative priors for each of our model parameters, validating the model code using test data prior to analysis. Our model produced $\hat{R}$ values close to one, well mixed chains, and n_{eff} values that exceeded 10% of the model iterations. Our model was fitted using the Stan language (Stan Development Team, 2018) using the rstan package (version) in R (R Development Core Team, 2017) (version).

We present model estimates, relative to baseline conditions of low chilling, low forcing, and short photoperiod treatments, and—when relevant—for the Smithers population.

Results

On average, we observed species budburst 28.1 (uncertainty interval: 45, 15.2) days after the start of our forcing and photoperiod treatments (all estimates are given as mean $\pm$ 90 uncertainty intervals, henceforth 'UI,' and budburst dates given as relative to baseline conditions, see methods for more details). But some species, such as *Aronia melanocarpa*, budburst as early as day 13.6, and as late as day 52.1 for *Quercus velutina*. Overall we found species budburst to be strongly phylogenetically structured (λ of 0.8, UI: 0.9, 0.6) and that all cues led to an advance in budburst date. Of the three cues chilling had the strongest effect (-15.2, UI: -17.3, -13.2) and photoperiod weakest (-3.6, UI: -4.3, -3.0, Fig. 1). But we did find a strong interaction between forcing and chilling (9.1, UI: 7.6, 10.5), which produced a delaying effect. The delaying effects of low chilling on budburst can therefore be offset by high forcing conditions (Fig. 2 and see Table 1 for model output).

Overall we found population effects were small compared to differences between cues. Across all species, the was considerable overlap in the responses of our four populations to each cue (Fig. 1). As such, low forcing, chilling, and shorter photoperiods all produced later estimated budburst dates compared to higher temperature and longer photoperiod treatments across all populations (Fig. 3 a-c, shown for baseline conditions) and across transects (Fig. 3, see Table 1 for model output). Populations were similar in their overall budburst dates, but eastern populations were marginally earlier (34.1, UI: 40.7, 27.1) compared to our western populations (46.5, UI: 52.7, 39.4, see also Table 1 for model output).

Budburst of individual species showed distinct differences in their timing and the relative importance of cues. Both chilling and forcing responses varied with budburst, with later budbursting species having slightly stronger responses to each cue respectively (Fig. 4). Despite our expectations that species responses should differ across the canopy layer, with shrubs being less sensitive to cues than tree species,

we did not find strong differences across these two functional groups (Fig. S3). While many of our earliest species were shrubs with weaker responses to chilling and forcing, like *Cornus stolonifera*, other shrubs, including *Menziesia ferruginea* and *Symphoricarpos alba*, exhibited the opposite response and budburst relatively late (Fig. S3). Individual tree species, such as *Quercus velutina* did have stronger chilling and photoperiod responses as predicted, and *Fagus grandifolia* produced the strongest photoperiod response of all our focal species. But overall there were no clear differences between the cue responses of trees and shrubs across our four forest communities

Our results depict the relative importance of phenological cues to variation across species, but we found the effect of cues on budburst to only explain a small portion of the species variability in budburst or the order of species budburst within community assemblages. The differences in the cue responses between our earliest and latest budbursting species was relatively small (Fig. 4), contrary to our prediction of stronger cue requirements—especially chilling cues—for later budbursting species. For example, an early (shrub) species, such as *Lyonia ligustrina* had chilling and forcing cues of -15.4 and -10.4 respectively, which are comparable to the estimates of much later species, such as *Quercus alba* with estimated chilling and forcing responses of -16.6 and -10.4. Yet our model predicts these species to have a 15.9 day difference in day of budburst. This highlights how the timing of budburst across species could mostly be attributed to species level differences outside of cue responses (Fig. 5). Cues explained only 67.0 percent of variation for eastern species and 60.6 for western, with the intercept explaining the rest (Fig. 5).

Discussion

1. Across all species, relative importance of cue = varied = unique temporal niches (Fig. S2)

2. Large portion of budburst due to spp differences - need more research to understand this, if not cues what causes it? Could introduce traits and foreshadow the third chapter

3. The experimental design (which is impressive) combined with your models means that most other things some models would assign to the intercept are assigned to site, species, chill, force etc. here... and still the intercept is big. Wow!!

4. Suggests factors other than species cues and architectural growth driving budburst.

1   Tables and figures

Table 1: Summary output from a phylogenetic Bayesian model in which species are partially pooled and phylogeny is included on the intercept. The model includes photoperiod and site as dummy variables, while the forcing and chilling effect is included as continuous. Parameter estimates were used to estimate day of budburst for sites under low cue conditions and for Smithers only when relevant.

|	mean	sd	5%	95%
Intercept	12.51	3.14	7.40	17.60
Phylogenetic effect	0.79	0.12	0.60	0.90
Forcing	-9.55	0.74	-10.70	-8.30
Photoperiod	-3.62	0.41	-4.30	-3.00
Chilling	-15.21	1.25	-17.30	-13.20
Manning Park	2.09	0.36	1.50	2.70
Harvard Forest	-6.04	1.03	-7.80	-4.40
St. Hippolyte	-8.71	0.97	-10.30	-7.10
Forcing x photoperiod	0.23	0.71	-1.00	1.40
Forcing x chilling	9.06	0.90	7.60	10.50
Photoperiod x chilling	-0.67	0.90	-2.20	0.80
Forcing x Manning Park	-1.76	0.77	-3.00	-0.50
Photoperiod x Manning Park	0.58	0.79	-0.70	1.90
Chilling x Manning Park	-0.36	1.60	-3.00	2.20
Forcing x Harvard Forest	3.81	1.22	1.80	5.80
Photoperiod x Harvard Forest	-1.96	0.86	-3.30	-0.60
Chilling x Harvard Forest	9.97	2.03	6.60	13.40
Forcing x St. Hippolyte	5.25	1.19	3.20	7.20
Photoperiod x St. Hippolyte	-2.13	0.84	-3.50	-0.70
Chilling x St. Hippolyte	8.65	1.70	5.90	11.50
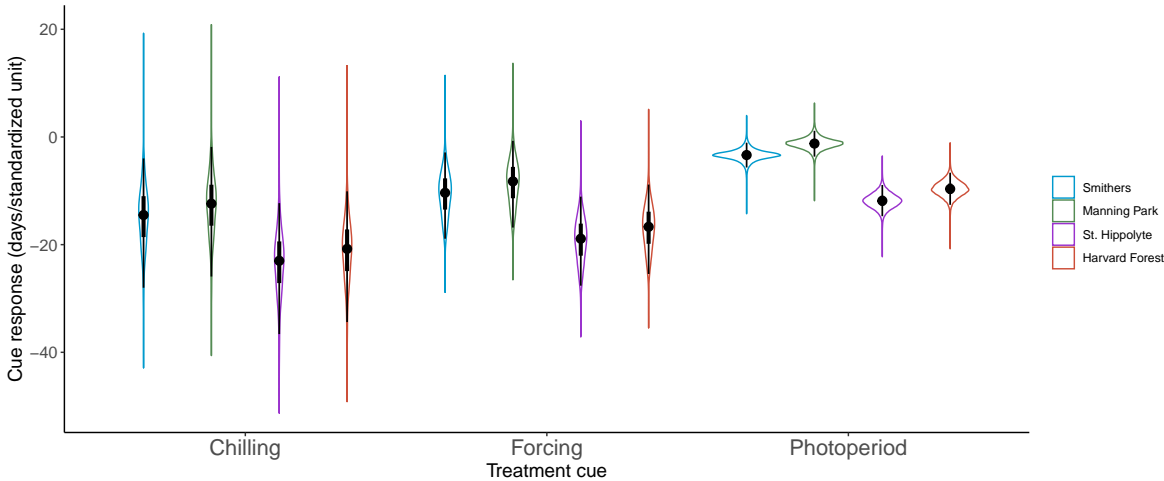
Figure 1: Posterior distributions of estimated chilling, forcing, and photoperiod responses with site level effects for individual populations. Black circles represent the median cue response, thicker black lines the 50% uncertainty interval, while the thinner black line the 90% uncertainty interval. The y-axis spans the entire range of the data. Cues were z-scored using two standard deviations.

References

Finn, G. A., A. E. Straszewski, and V. Peterson. 2007. A general growth stage key for describing trees and woody plants. Annals of Applied Biology 151:127–131.
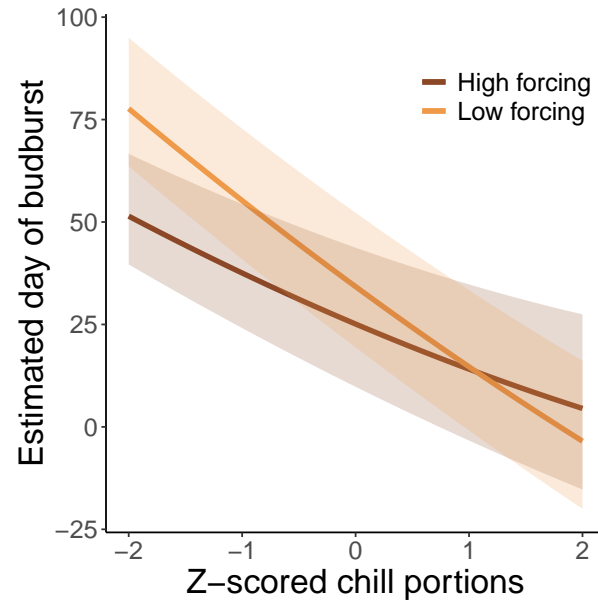
Figure 2: Estimated day of budburst of first bud in response to chill portions and forcing, estimated for our defined baseline conditions.
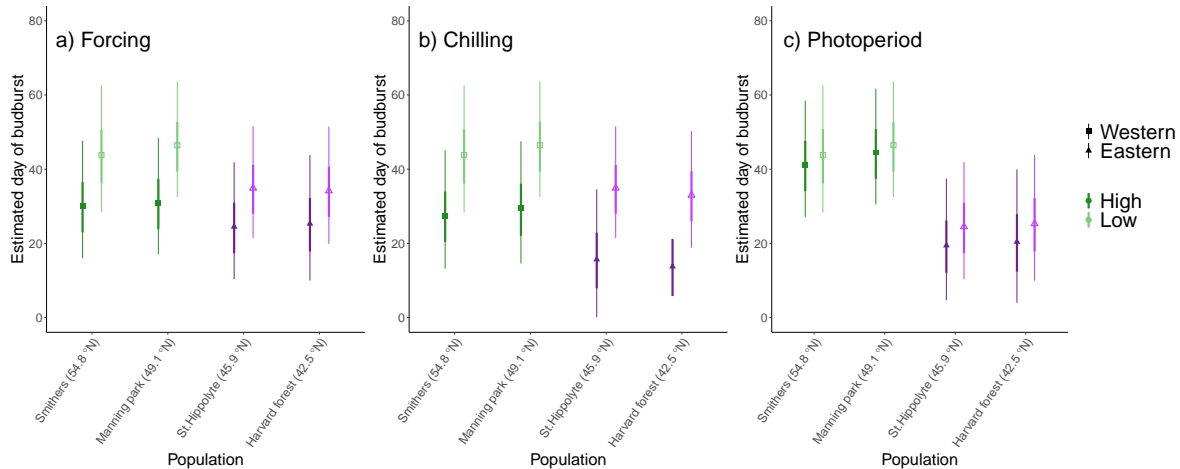

Figure 3: Estimated day of budburst of first bud in response to (a) forcing across populations under low chilling conditions and short photoperiods, (b) chilling across populations under low forcing and short photoperiods, and (c) across photoperiods under our baseline forcing and chilling conditions for species sampled from our four populations. The thin error bars represent the 90% uncertainty interval, while the thicker error bars represent the 50% uncertainty interval.

142   Flynn, D. F. B., and E. M. Wolkovich. 2018. Temperature and photoperiod drive spring phenology
143   across all species in a temperate forest community. New Phytologist 219:1353–1362.

144   R Development Core Team. 2017. R: A language and environment for statistical computing. R
145   Foundation for Statistical Computing, Vienna, Austria .

146   Stan Development Team. 2018. RStan: the R interface to Stan. R package version 2.17.3.
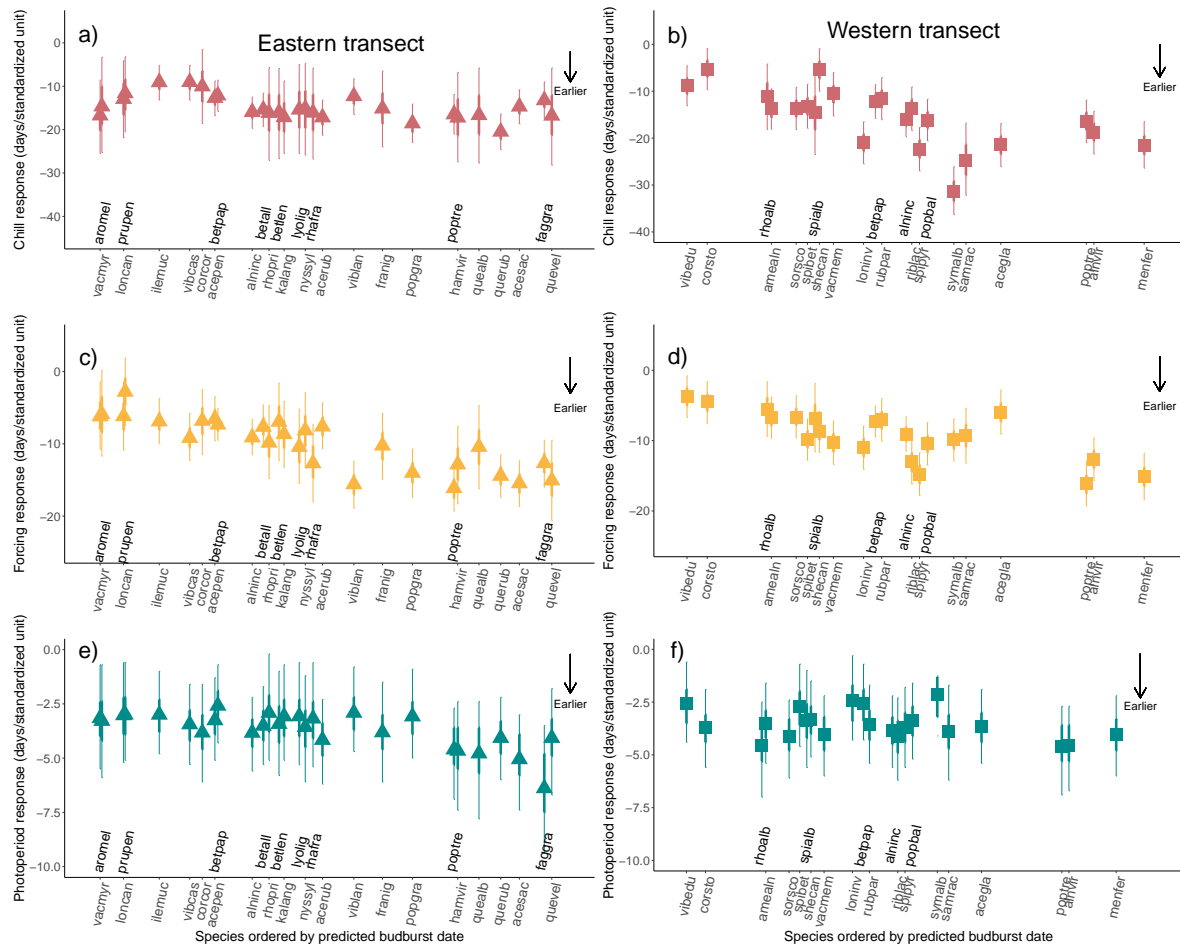
Figure 4: Estimated species' chilling (a,b), forcing (c,d) and photoperiod (e,f) responses ranked by increasing estimated budburst dates for both the eastern (a, c, e) and western (b, d, f) populations. Cues are plotted on differing y-axis to better depict species differences. For each species, the thiner error bars represent the 90% uncertainty interval, and thicker bars represent the 50% uncertainty interval. Each cue was z-scored, using two standard deviations, to make the results across cues directly comparable.
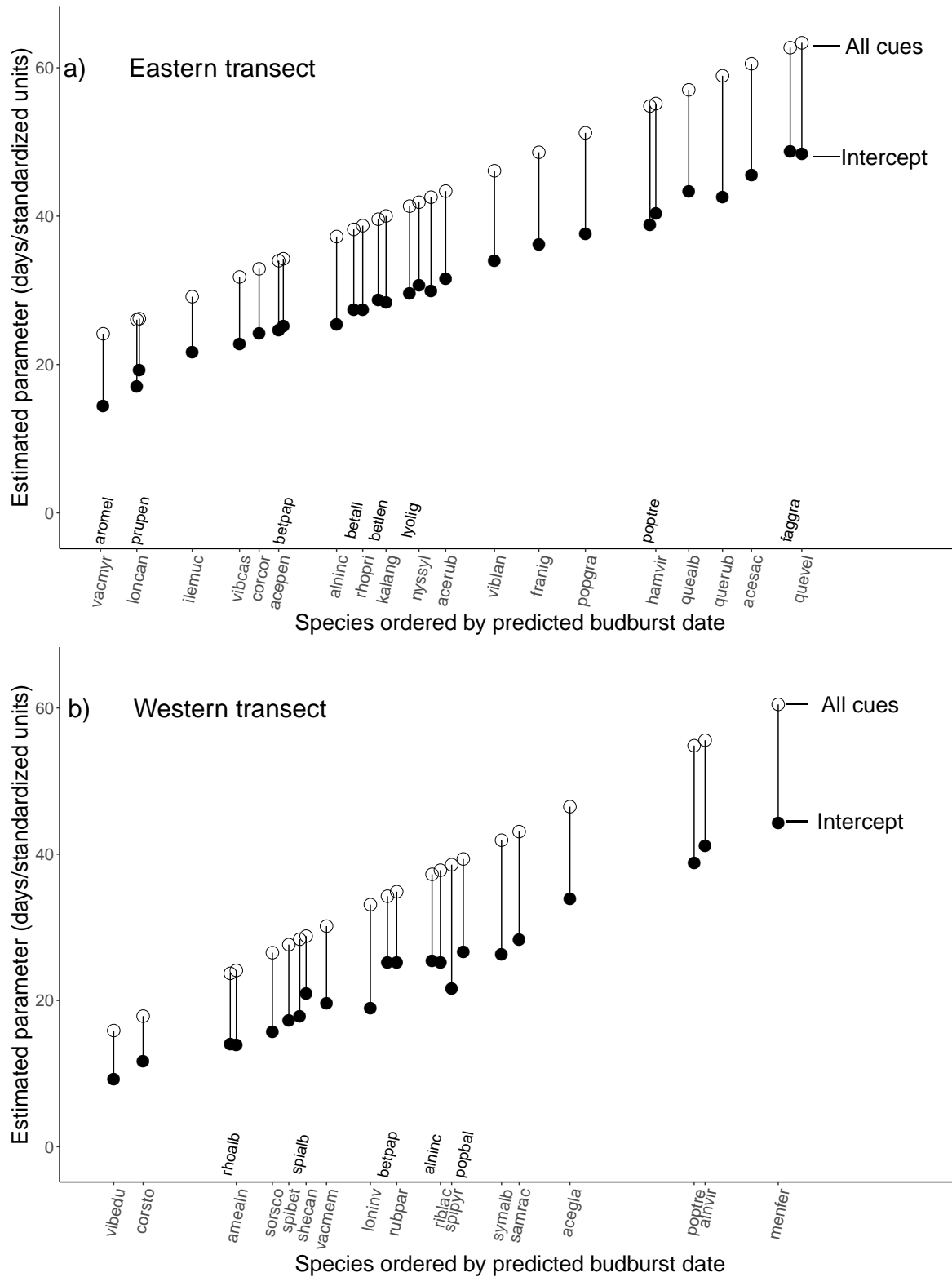
Figure 5: Estimated budburst, shown in blue, and species intercepts, shown in red, against species estimated budburst dates (e.g., quantitative estimate of species phenological order within community) for both the eastern (a) and western (b) populations.