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 separate growth chamber studies using branch clippings of North American deciduous woody plants. The first was conducted in 2015 using samples taken from two eastern populations—Harvard Forest, Massachusetts, USA (42.55°N, 72.20°W) and St. Hippolyte, Quebec, Canada (45.98°N, 74.01°W). For details on the sampling and experimental set up of our eastern populations, see Flynn and Wolkovich (2018). The second growth chamber study was conducted over the winter of 2019-2020 and spanned two western populations—E.C. Manning Park (49.06°N, 120.78°W) and Smithers (54.78°N, 127.17°W), British Columbia, Canada.

For our western study, samples were collected for 21 dominant woody species at both sites, of which 19 species occurred in both population. At each population, we tagged 20 healthy, mature, individuals for each species in the summer of 2019 and returned for sample collection in October 2019. Depending on individuals size, we collected between 1-10 cuttings from each tagged plant, with samples being taken using a pole pruner from the ground. We kept samples cold during sampling and transportation back to the University of British Columbia, British Columbia, Canada.

Western growth chamber study

We replicated the general study design and methods used by Flynn and Wolkovich (2018) for the growth chamber study of western species. Our experiment consisted of eight replicates for each species across eight experimental treatments, with the exception of *Sorbus scopulina*, for which we we only had six replicates per treatment. Our treatments included two levels of forcing—a cool regime of 15°C:5°C and a warm regime of 20°C:10°C—two levels of chilling—30 or 70 days at 4°C—and two photoperiods of either 8 or 12 hours. We used daily weather data from local weather stations to calculate the field chilling experienced by samples prior to collection and used chill portions as our chilling treatments.

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

38 phenological stages up to full leaf expansion (defined as code 17 by Finn et al. (2007)), but here we
39 present the response in budburst date, defined as the day of budbreak or shoot elongation (defined as
40 code 07 by Finn et al. (2007)). In total we made 47844 phenological observations across all samples.
41

Statistical Analysis

43 To test for differences in cue responses across species and populations, we used a phylogenetic mixed
44 effect model with partial pooling across species. This approach allowed us to account for both the
45 evolutionary relatedness of our species, and estimate the species level cue responses and differences
46 across sites. Species phylogenetic related was obtained by pruning the **?** megatree of angiosperms.
47 Forcing and chill portions were included as continuous variables, and photoperiod and site as dummy
48 variables. In addition to these overall predictors, we included all two-way interactions between cues
49 and cues and sites:
50

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

51
52

$$
\begin{aligned}
\beta_{force_{sp}} &\sim \text{Normal}(\mu_{force_{sp}}, \sigma^2_{force}) \\
&\dots \\
\beta_{photoperiod \times site4} &\sim \text{Normal}(\mu_{photoperiod \times site4}, \sigma^2_{photoperiod \times site4})
\end{aligned}
$$

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

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

$$
\tag{2}
$$

55 The timing of budburst prior to evolution is represented as root values of the means of the multivariate
56 normal distributions. For more detail on this method of phylogenetic modeling, see Morales-Castilla
57 et al (XXXX).
58

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

63   2017) (version).

64

65   In our figures of model estimates, we visualized the baseline conditions, which we define as our low
66   chilling, low forcing, and short photoperiod treatments, and when relevant for our Smithers population.

Results

68   On average, we observed species budburst 28 (45, 15.2) days after the start of our forcing and photope-
69   riod treatments (all estimates are given as mean $\pm$ 90 uncertainty intervals and budburst dates given
70   as relative to baseline conditions, see methods for more details). But some species, such as *Aronia*
71   *melanocarpa* budburst as early as day 14 to as late as day 52 for *Quercus velutina*. Overall we found
72   species budburst to be strongly phylogenetically structured (λ of 0.8, UI: 0.9, 0.6) and that all cues led
73   to an advance in budburst date. Of the three cues chilling had the strongest effect (-15.2, UI: -17.3,
74   -13.2) and photoperiod weakest (-3.6, UI: -4.3, -3.0, Fig. 1). But we did find a strong interaction
75   between forcing and chilling (9.1, UI: 7.6, 10.5), with produced a delaying effect. The delaying effects
76   of low chilling on burburst can therefore be offset by high forcing comditions (Fig. 2 and see Table 1
77   for model output).

78

79   Overall we found population effects were small compared to differences between cues. Across all species,
80   the was considerable overlap in the responses of our four populations to each cue (Fig. 1). As such, low
81   forcing, chilling, and shorter photoperiods all produced later estimates of budburst dates compared to
82   higher temperature and longer photoperiod treatments across all populations (Fig. 3 a-c, shown for
83   baseline conditions) and across transects (Fig. 3, see Table 1 for model output). Populations were
84   similar in their overall budburst dates, but eastern populations were marginally earlier (34.1, UI: 40.7,
85   27.1) compared to our western populations (46.5, UI: 52.7, 39.4, see also Table 1 for model output).

86

87   Budburst of individual species also show distinct differences in their timing and the relative impor-
88   tance of cues, independent of species architecture. Both chilling and forcing responses varied with
89   budburst, with later budbursting species having slightly stronger responses to each cue respectively
90   (Fig. 4). But we found similar responses for both shrubs and trees overall (Fig. S3). While many of
91   our earliest species were shrubs with weaker responses to chilling and forcing, like *Cornus stolonifera*,
92   other shrubs, including *Menziesia ferruginea* and *Symphoricarpos alba* exhibited the opposite response
93   and budburst relatively late (Fig. S3). Tree species, however, produced no strong trend overall. But
94   individual species, such as *Quercus velutina* did have stronger chilling and photoperiod responses as
95   predicted, and *Fagus grandifolia* produced the strongest photoperiod response of all our focal species.

96

97   While our results depict the relative importance of phenological cues to variation across species, trends
98   were weak and suggest other cues are driving species differences in budburst. Across the period of
99   budburst, gradients in species' responses are relatively weak gradients (Fig. 4). In examining species
100 responses, we find early shrub species, such as *Lyonia ligustrina*, to have as strong a chilling and
101 forcing cue as much later species, like *Quercus alba*, and yet they have a 15.9 day differences in their
102 mean estimated day of budburst. The timing of budburst across species could mostly be attributed
103 to species level differences (intercept) as opposed to cue responses (Fig. 5). For our western species,
104 these species level differences accounted for 60.6% of the estimated timing of budburst, while 67.0% of
105 the estimated timing of budburst of eastern species was due to their species intercepts (Fig. 5).

106

Discussion

108   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
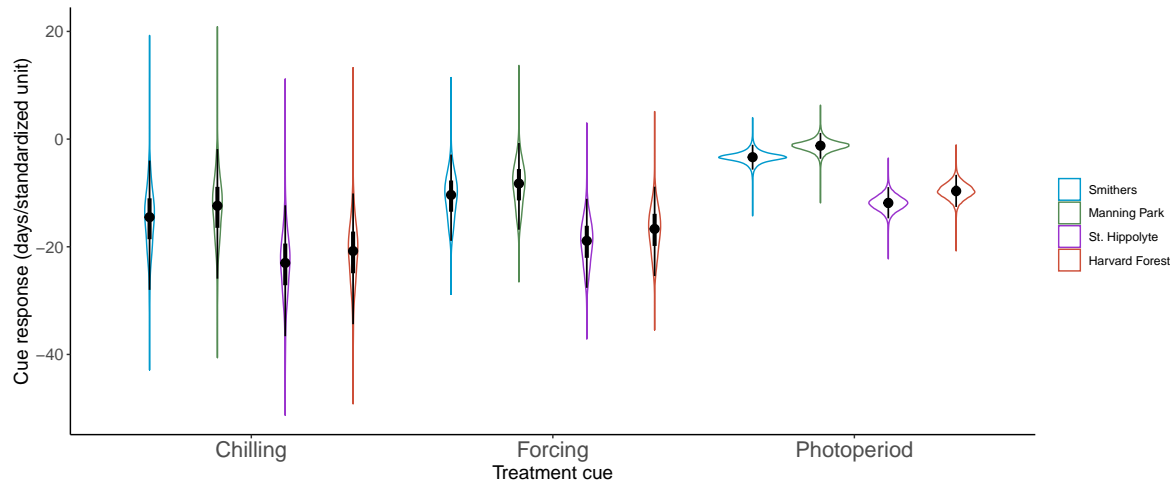
Figure 1: Posterior distributions of estimated chilling, forcing, and photoperiod responses with site level effects for individual populations. Black circles represent the median cue response, thicker black lines the 50% uncertainty interval, while the thinner black line the 90% uncertainty interval. The y-axis spans the entire range of the data. Cues were z-scored using two standard deviations.
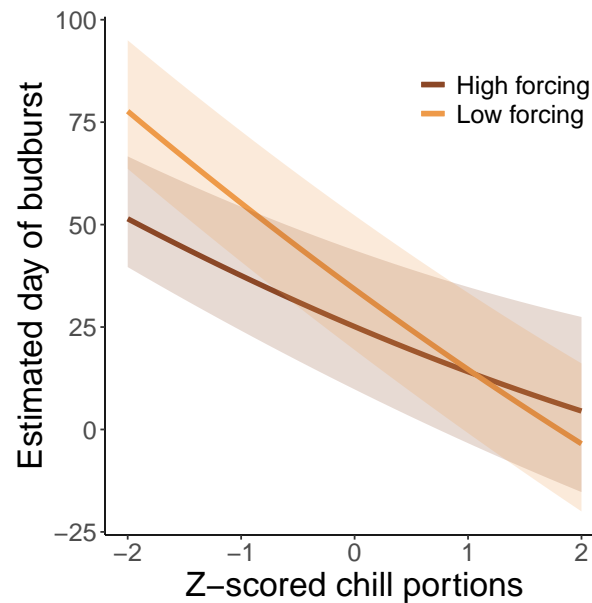

Figure 2: Estimated day of budburst of first bud in response to chill portions and forcing, estimated for our defined baseline conditions.

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.

Flynn, D. F. B., and E. M. Wolkovich. 2018. Temperature and photoperiod drive spring phenology across all species in a temperate forest community. New Phytologist 219:1353–1362.
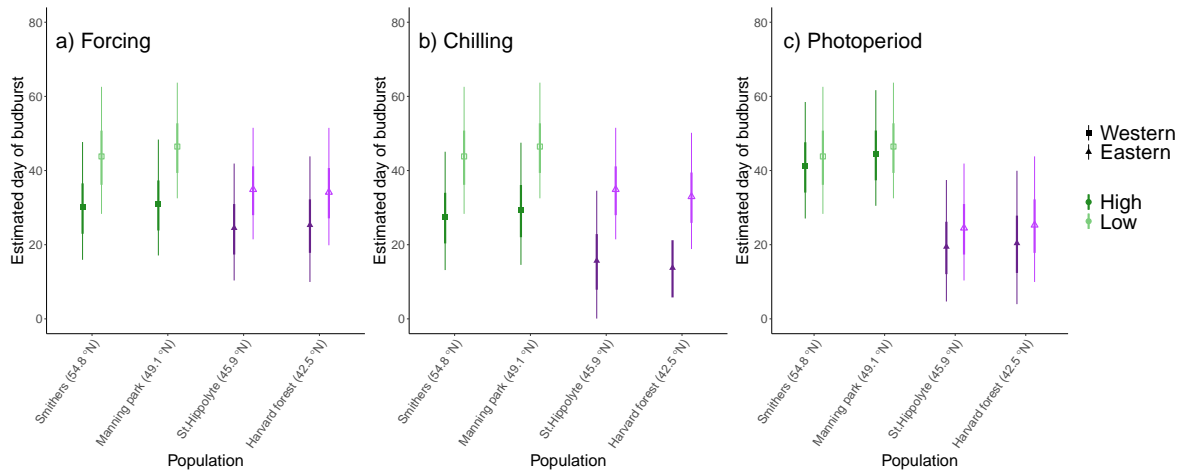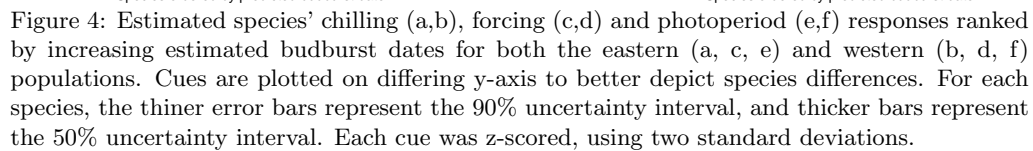
Figure 3: Estimated day of budburst of first bud in response to (a) forcing across populations under low chilling conditions and short photoperiods, (b) chilling across populations under low forcing and short photoperiods, and (c) across photoperiods under our baseline forcing and chilling conditions 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.

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

123 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.
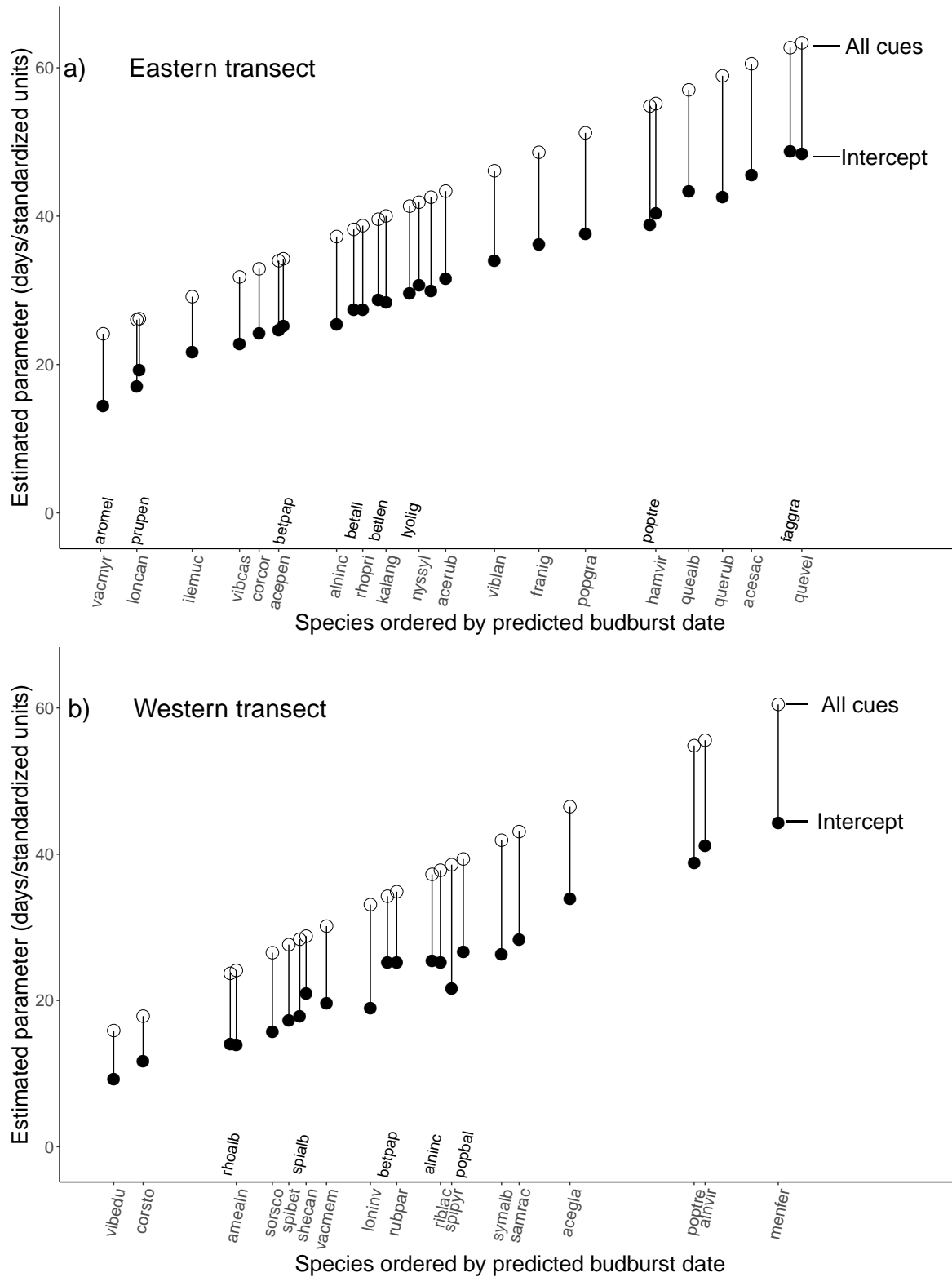
Figure 5: Estimated budburst, shown in blue, and species intercepts, shown in red, against species estimated budburst dates (e.g., quantitative estimate of species phenological order within community) for both the eastern (a) and western (b) populations.