

Supplemental materials for Chilling outweighs photoperiod and forcing cues for temperate trees in experiments, but not in natural systems

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Supplemental Methods

The Observed Spring Phenology Responses in ALxperimental Environments (OSPREE) Database

To identify phenology experiments that manipulated forcing, chilling, and/or daylength, we conducted two Web of Science searches on XXX date with the following terms:

1. TOPIC = (budburst OR leaf-out) AND (photoperiod or daylength) AND temperature*, which yielded 85 publications
2. TOPIC = (budburst OR leaf-out) AND dorman*, which yielded 193 publications

We reviewed all these papers and included studies in the OSPREE database using the following criteria:

1. Focal species were woody plants
2. Quantitative estimates of budburst (include flowering and vegetative buds) were measured and available by scraping tables and/or figure sin the publication. Response variables measured included days to budburst, percent budburst, height,... what else is in respvar. simple?
3. Forcing, chilling, and photoperiod treatments could be quantitatively identified.
4. Studies were conducted in controlled environment chambers, not purely in situ.

The resuting database includes 13,000 rows of data across 85 studies, 41 years, and 227 species. For this paper, we focus on studies measuring days to budburst. This subset of OSPREE includes XX rows of data across XX studies, XX years, and XX species (Fig. S1).

Some species are only represented in one dataset in the OSPREE database, making it impossible to differentiate between species and treatment statistical effects for these taxa. To address this, we combined species found in only one study into “complexes” at the level of genera. Thus our taxonomic units of analysis are “species complexes”; either species represented in >1 dataset or generic complexes combing multiple species that are each singly represented in the dataset. Species represented in only one dataset with no congeners in other datasets were excluded from our analysis.

Models

We fit three models: the main budburst model, fit to all studies in OSPREE that measured days to budburst; the latitude model, which included only studies that XXX, and the weinberger model, which included only species used in both experiments that employed Weinberger methods and those that did not. We defined Weinberer studies as those with two or more field sample dates, each two or more weeks apart.

The models were fit using the programming languages **Stan** (Carpenter et al., 2017)(www.mc-stan.org), accessed via the *rstan* package (version 2.17.3) in R (R Development Core Team, 2017; Stan Development Team, 2018), version 3.4.2. Stan provides efficient MCMC sampling via a No-U-Turn Hamiltonian Monte Carlo approach (more details can be found in Gelman et al. (2014) and in Carpenter et al. (2017)). We validated that our models using test data, then fit the following models:

1. Main budburst model:

$$y_i = N(\alpha_{sp[i]} + \beta_{forcing_{sp[i]}} + \beta_{photoperiod_{sp[i]}} + \beta_{chilling_{sp[i]}} + \epsilon_i, \epsilon_i \sim N(0, \sigma_y^2)$$

The α and each of the three β coefficients were modeled at the species level, as follows:

$$\begin{aligned} \alpha_{sp} &\sim N(\mu_\alpha, \sigma_\alpha) \\ \beta_{forcing_{sp}} &\sim N(\mu_{forcing}, \sigma_{forcing}) \\ \beta_{photoperiod_{sp}} &\sim N(\mu_{photoperiod}, \sigma_{photoperiod}) \\ \beta_{chilling_{sp}} &\sim N(\mu_{chilling}, \sigma_{chilling}) \end{aligned}$$

2. Latitude model:

3. Weinberger model:

$$y_i = N(\alpha_{sp[i]} + \beta_{forcing} + \beta_{photoperiod} + \beta_{chilling} + \beta_{weinberger} + \beta_{forcing:weinberger}\beta_{photoperiod:weinberger} + \beta_{chilling:weinberger})$$

$$\alpha_{sp} \sim N(\mu_\alpha, \sigma_\alpha)$$

For all models, we choose weakly informative priors; increasing the priors three-fold did not change the model results.

We ran four chains simultaneously, each with 1 500 warm-up iterations followed by 2 500 sampling iterations, yeilding 4 000 posterior samples for each parameter. We assessed model performance through \hat{R} close to 1 and high n_{eff} (4 000 for most parameters, but as low as 1 057 for several parameters) as well as visual consideration of chain convergence and posteriors (Gelman et al., 2014).

In our figures we show means \pm XX% credible intervals from our models, because of our focus here is on the most likely value for each parameter (e.g., estimated response to forcing) and because they are computationally stable (Gelman et al., 2014; Carpenter et al., 2017). See tables XX other XX% credible intervals.

Quick review of what standardized units are (We used a standardized scale to allow comparisons of the three cues ...)

Forecasting with the OSPREE model

We selected sites in Europe where temperature and budburst have been monitored since the 1950s (the Pan European Phenology Project, <http://www.pep725.eu>, PEP). We extracted mean temperature data from 1951 through 1961 (pre-warming time period) and used these values as baseline data. We then investigated model predictions of budburst given different levels of warming (from 1-7 °C) above this baseline, including altered chilling and forcing as well as potential declines in photoperiod due to advancing phenology. We did this for one common European species: *Betula pendula* (silver birch) at all latitudes and longitudes included in the PEP database between 1951 and 1961. We also did this for another common European species, *Fagus sylvatica*, for a subset of sites where it occurred with *B. pendula*, in order to compare budburst responses of these two species when they experience the same baseline climate and warming levels.

To understand how experimental temperature, photoperiod, and budburst sensitivity compare to past and current conditions in nature, we used data from the PEP database. We summarized forcing, chilling, and budburst day for two common species: *Betula pendula* (silver birch) and *Fagus sylvatica* (European beech) during a pre-warming time-period (1950-1980) and post-warming period (1981-2014?).

Supplemental Results/Discussion

1. Surprising species-specific responses:
 - (a) Positive responses to forcing: *Fagus*, *Acer*-complex, *Fraxinus* complex, *Cornus alba*.
 - (b) Positive Responses to chilling for *Tilia* and *Salix*. have positive response to chilling with chill portions model.

Understanding declines in temperature sensitivity in European long-term data

As our model results (when applied to realistic forcing, chilling and photoperiod conditions in Europe) do not predict a dramatic decline temperature sensitivity, we tested whether observed declines could instead be due to a statistical artifact of the method commonly used to estimate temperature sensitivity (a linear regression of annual budburst date versus mean or other aggregated metrics of spring temperature yielding estimates in days/°C). If warming produces systematically warmer daily temperatures this method will inherently estimate lower sensitivities, because the ‘days’ unit will effectively have increased in the thermal time it represents.

To test this hypothesis we compared observed trends with simulations. First, we collated PEP 725 (CITE) data for *Betula pendula* for all sites with leafout data each year from two 10-year time-periods: 1951-1960, which represented a period before significant anthropogenic warming (citeIPCC) and 2001-2010, a period with significant warming. We used leafout data (BBCH=11; which is defined as “leaf unfolding (first visible leaf stalk” in the PEP725 database)) instead of budburst (BBCH=7; defined as “Beginning of sprouting”) as leafout data are far more common in the PEP 725 database. Next, we simulated budburst data with constant cues. For this, we did not include any chilling or photoperiod cues, but simply assumed budburst occurred after a certain summation of heat, estimated via growing degree days with a base temperature of 0°C. We then estimated temperature sensitivity (days/°C) and the difference in these estimates given different levels of spring warming. For the simulations showed here we used a GDD requirement of 150, a base mean spring temperature of 6°C with a variance of 3°C and estimated temperature sensitivity for 10-year periods for 45 simulated sites (these values were chosen to best match the PEP 725 data, but note that the general findings are robust to other combinations of these parameter values).

As expected temperature sensitivity estimates for *Betula pendula* from PEP 725 declined across the two time periods in step with warming daily temperatures. Across the sites studied here we estimated a decline of 0.8 ± 0.3 days/ $^{\circ}\text{C}$ (comparing 2000-2010 and 1950-1960) and 1.1°C warming; this estimate was very similar to simulations given constant cues and 1°C warming (Fig. S4). Additionally, in both the real and simulated data variance in leafout date declined, as would be expected if plants use a thermal sum threshold of forcing to leaf out and warming produces systematically warmer days: in the PEP 725 data we found a decline in leafout variance of 58%, compared to a decline of 37% in the simulations. Additionally we found little change in accumulated chilling (1 September - 1 March of each year) in the PEP 725 data across the two time points (2247 ± 31 Utah units in 1950-1960, compared to 2236 ± 20 Utah units in 2000-2010), further suggesting that shifts in chilling do not explain the declining sensitivities. Simple plots of the chilling and forcing required for budburst suggest very low chilling is often required to dramatically increase the forcing required for budburst (Fig. fig:pepgddchill).

May need to move back to main text ...

The paucity of studies directly manipulating chilling—which our results suggest has the greatest effect on budburst—suggests a major gap in current research. While many studies (X out of Y here) directly manipulated forcing, far fewer directly manipulated chilling (Z out of Y). Our model highlights how the choice of chill units can affect model estimates and associated forecasts (reference supplemental figure with utah units vs chill portions in main manuscript). Given the limited manipulation of chilling in studies, we estimated chilling for all studies by combining chilling from the field (i.e., chilling before plants were sampled) and experimental chilling into two widely used metrics of chilling: Utah and Chill portions (Dennis, 2003). We found the effects of chilling and other cues remain qualitatively consistent across the two chilling units, though chilling and photoperiod estimates were slightly lower using chill portions compared to Utah (cite supplemental table comparing estimates with both units).

An additional important limitation is the rarity of studies designed to test interactions. Interactions between these cues are widely expected (cites) and, when examined, often found (cites), we were unable to estimate interactions in our meta-analysis because very few studies design experiments to test for interactions between chilling, forcing, and photoperiod (cite table with number of interactions from coding challenge!). The few that do incorporate interactions generally use the Weinberger method, which is not designed to robustly tease out of the effects of multiple cues (cites, Tables, figs). Our estimated effects average over interactions (Gelman and Hill, 2006), but identifying them in future research will be critical to understanding and predicting budburst. For example, the most commonly observed interaction between chilling and forcing—that lower amounts of chilling increases forcing requirements for budburst (cite papers in the osprey database that interact chilling and forcing)—is the hypothesized cause of declining sensitivities in European trees (Fu et al., 2015; ?). As more data become available, it would allow additional tests of important interactions, such as how responses vary across latitudes (ref latitude figure).

Things we'd like to discuss in main paper but don't have room for

Photothermoperiodicity, for example, is an ongoing challenge: chamber studies may seek to replicate patterns in nature, pairing daylength and temperature treatments such that night temperatures are always cooler than day temperatures (e.g., cite studies that do this). This results in daylength treatments that differ in temperature conditions (and therefore chilling and forcing treatments) as well, however.

Chilling: Current common models for chilling (i.e., Utah which was developed for XX species, chill portions which was developed for XX species) are *hypotheses* for how chilling may accumulate to affect the process of dormancy release, but are likely to be inaccurate for many species. Our model highlights how the choice of

chill units can affect model estimates and associated forecasts (Figures 1,????, ??).

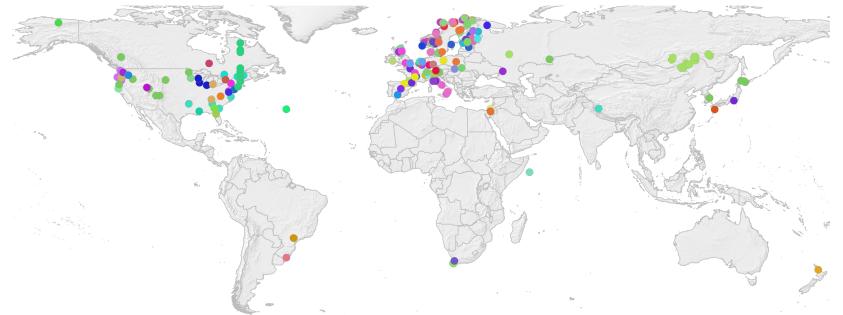
References

- Carpenter, B., A. Gelman, M. Hoffman, D. Lee, B. Goodrich, M. Betancourt, M. A. Brubaker, J. Guo, P. Li, and R. Allen. 2017. Stan: A probabilistic programming language. *Journal of Statistical Software* 76:10.18637/jss.v076.i01.
- Dennis, F. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. *HortScience* 38:347–350.
- Fu, Y. S. H., H. F. Zhao, S. L. Piao, M. Peaucelle, S. S. Peng, G. Y. Zhou, P. Ciais, M. T. Huang, A. Menzel, J. P. Uelas, Y. Song, Y. Vitassee, Z. Z. Zeng, and I. A. Janssens. 2015. Declining global warming effects on the phenology of spring leaf unfolding. *Nature* 526:104–107.
- Gelman, A., J. B. Carlin, H. S. Stern, D. B. Dunson, A. Vehtari, and D. B. Rubin. 2014. Bayesian Data Analysis. 3rd ed. CRC Press, New York.
- Gelman, A., and J. Hill. 2006. Data analysis using regression and multilevel/hierarchical models. Cambridge university press.
- R Development Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Stan Development Team. 2018. RStan: the R interface to Stan. R package version 2.17.3.

Supplemental Tables

Still need: Table 3S: a table that goes with Figure 3 (the 4-paneled 3D forecasting figure) that includes the mean budburst day of year, chilling estimates, mean winter temperature, and spring (forcing) temperatures for the 4 sites with no warming and with the 7 levels of warming.

Supplemental Figures



Ospree Locations

ashby62	cook00b	gianfagna85	heide11	lieten95	partanen05	sonstebry13
basler12	cook05	gomory15	heide12	lieten97	partanen98	spann04
basler14	cronje03	granhus09	heide77	linkosal06	pettersen71	spiers74
biasi12	dantec14	guak98	heide93	man10	pop2000	swartz81
biran73	darrow36	guerrero90	heide93a	manson91	ramos99	verheul07
boyer	devries82	gunderson12	howe95	morin10	rinne94	viheraaarnio06
bradford10	dumer84	guterman88	jones12	myking95	rinne97	webb78
caffarra11a	falus03	hawerroth13	junttila12	myking97	ruesink98	worrall67
calme94	falus90	hawkins12	karlsson03	nerd95	sanz-perez09	yazdaniba64
campbell75	falus96	heide01	kinet93	nienstaedt66	schnabel87	zohner16
cannell83	falus97	Heide03	kronenberg76	nishimoto95	skre08	
charrier11	fu13	heide05	lamb37	okie11	skuterud94	
chavarria09	gansert02	heide07	laube14a	pagter15	sogaard08	
ghelardini10	heide08	heide08	li05	partanen01	sonstebry09a	

Figure S1: Map of days to budburst experiments in the OSPREE database. Add different symbols for weinberger vs not?

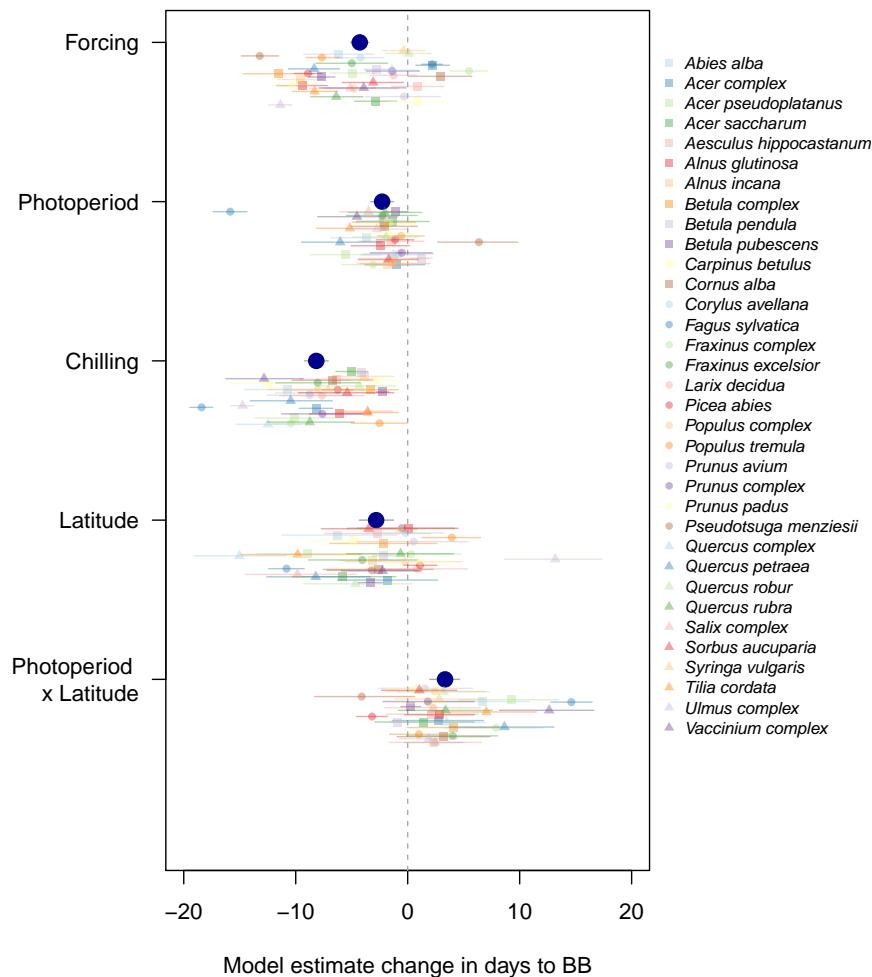


Figure S2: Estimates for effects of chilling exceeded estimates for forcing, photoperiod, latitude, and the interaction between latitude and photoperiod, for most species, in the latitude budburst model fit to centered data, including the subset of studies in OSPREE database that XXX. Here we show estimates from the model fit to centered data, enabling comparisons of effects sizes across predictors, and using Utah units to quantify chilling.

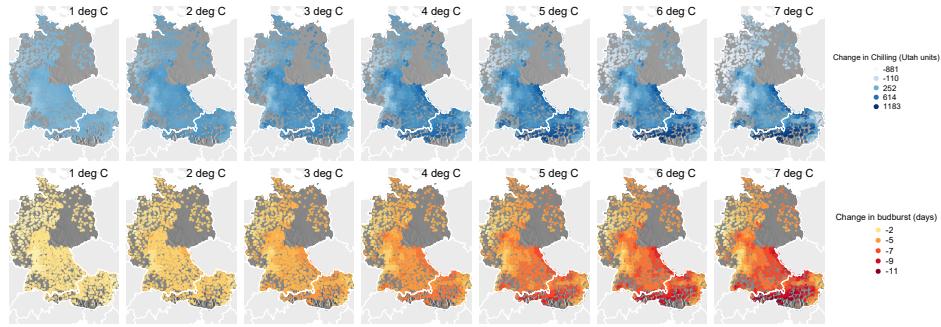


Figure S3: **Forecasted changes in chilling (top panel) and leafout for *Betula pendula* (bottom panel)**, in locations included in the PEP database, where phenology dates are known for the pre-warming time period (1951-1960). Changes in chilling and budburst are calculated relative to the mean chilling and budburst dates during this pre-warming timeperiod for each location.

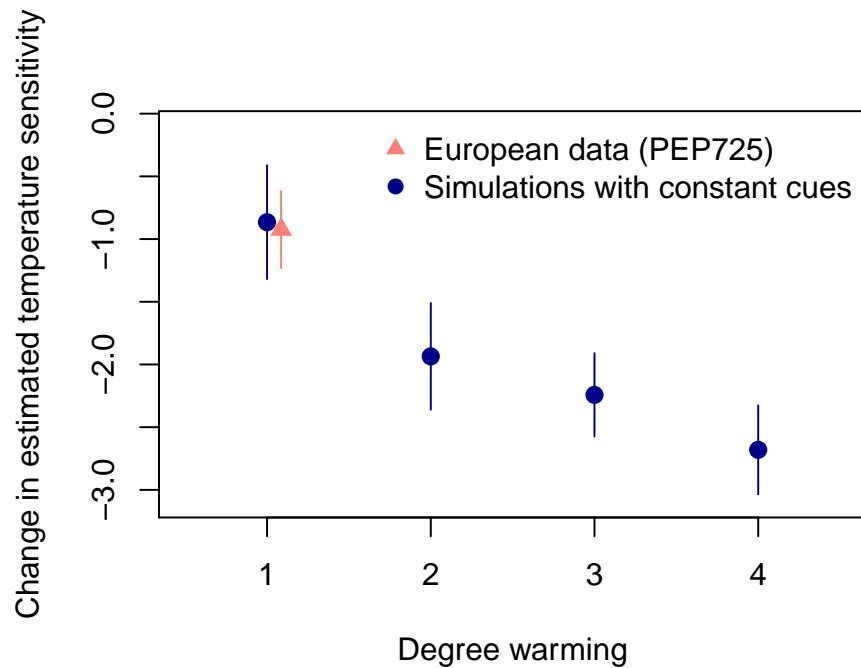


Figure S4: Declining sensitivities observed in long-term European data for a suite of common trees may be explained by a statistical artifact. We compared the sensitivity estimated from linear regressions of day of leafout versus mean spring temperature (estimated thus as days/ $^{\circ}\text{C}$) from PEP 725 data for *Betula pendula* from 45 sites ('European data') with estimated declines in simulations where the cues were held constant but spring temperatures warmed by 1-4 $^{\circ}\text{C}$ ('Simulations') and found the estimated temperature sensitivity measured as days/ $^{\circ}\text{C}$ declined even though the underlying cues had not changed, see *Understanding declines in temperature sensitivity in European long-term data* in Supplement for further details.

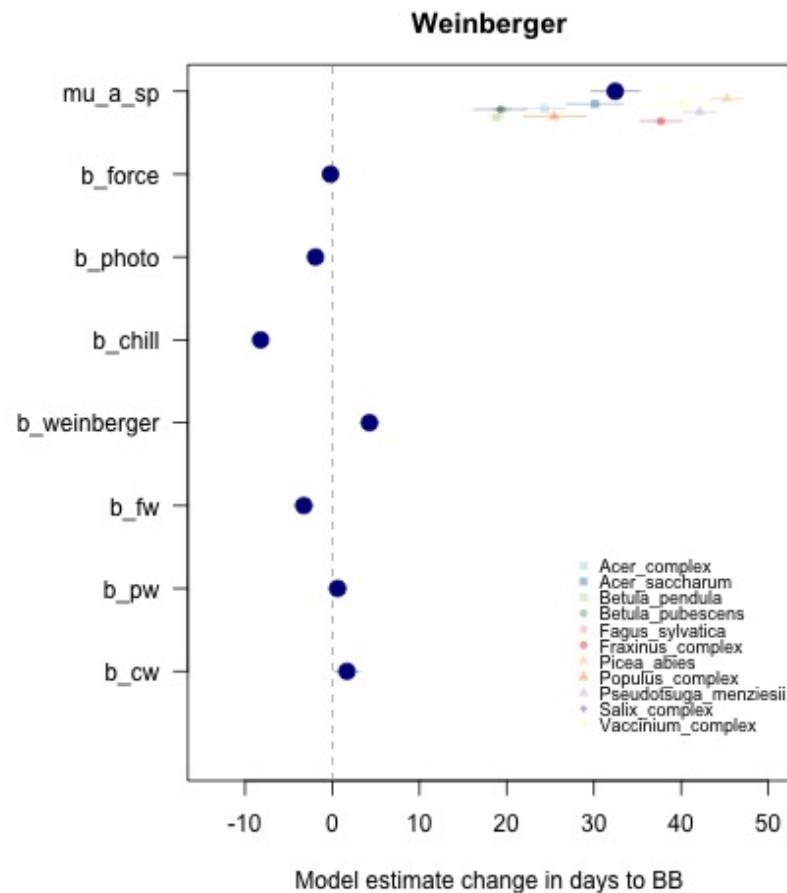


Figure S5: Comparision of estimated effects for environmental paremeters for overlapping species included in both Weinberger method studies and non-Weinberger method studies. The effect of chilling is estimated to be weaker, and the effect of forcing stronger in Weinberger studies.

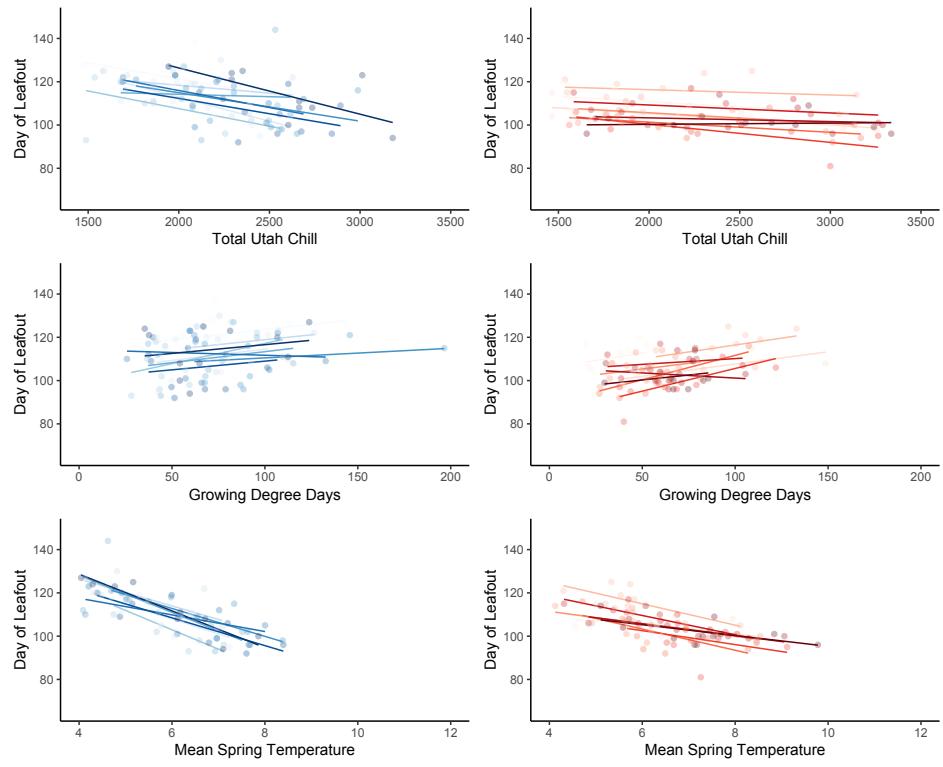


Figure S6: **Day of leaf out versus chilling, growing degree-days, and mean spring temperature** pre- (left panels, 1951-1961) and post- warming (right panels, 2000-2010) for PEP sites in Germany where *Betula pendula* phenology has been monitored for decades.

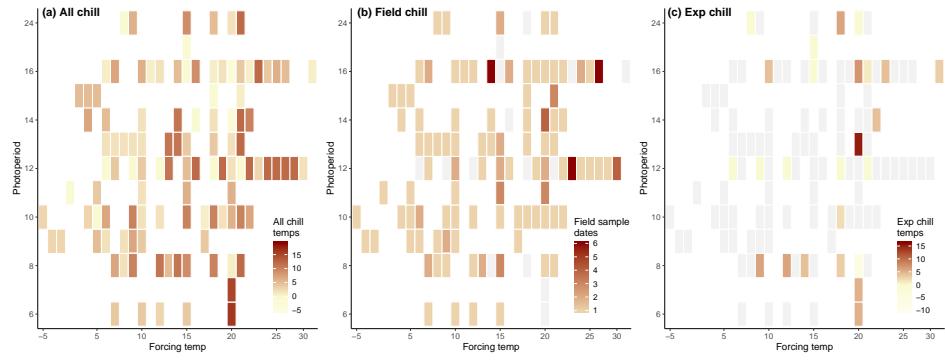


Figure S7: **Heatmaps of treatments ...** need to make sure this matches the data we have ended up using!

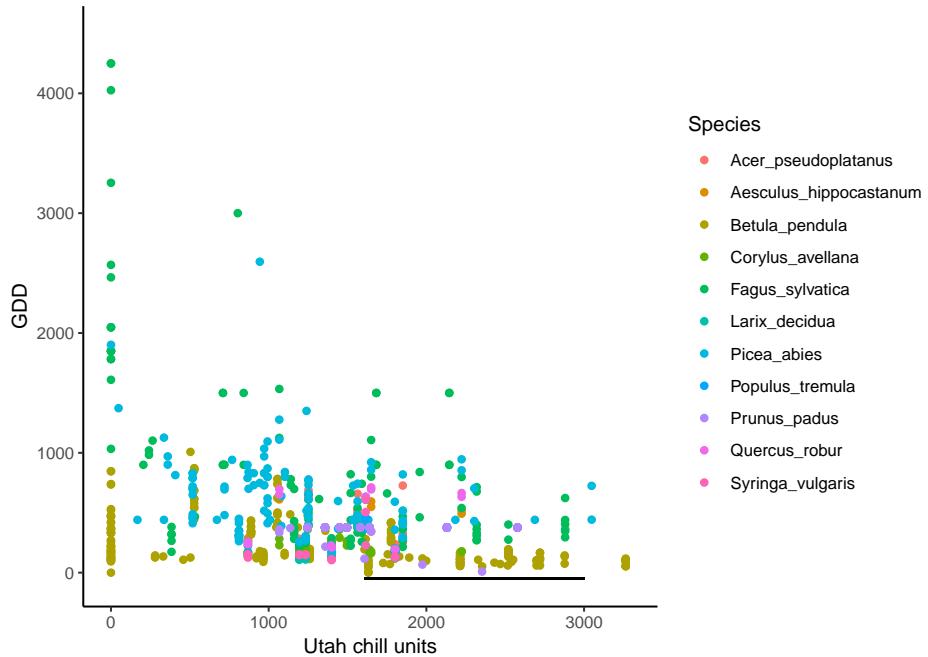


Figure S8: GDD (growing degree days) versus chill units at the time of budburst from the OSPREE database for common species in the PEP 725 long-term phenological database. The black line shows the range of chilling (10-90% quantiles) accumulated from 1 September to 1 March for 45 sites for *Betula pendula* (see also *Understanding declines in temperature sensitivity in European long-term data*). We calculated GDD here as the average daily forcing temperature multiplied by days to budburst.

Table S1: **Species included in the OSPREE database.** Could add to this: the range of forcing, chilling, and photoperiod treatments for each species, and perhaps the range of latitudes, in OSPREE.

spname	stnum	studies
<i>Abies.alba</i>	2	basler12,laube14a
<i>Abies.homolepis</i>	1	laube14a
<i>Acer.barbinerve</i>	1	zohner16
<i>Acer.campestre</i>	1	zohner16
<i>Acer.ginnala</i>	1	zohner16
<i>Acer.negundo</i>	1	laube14a
<i>Acer.platanoides</i>	1	zohner16
<i>Acer.pseudoplatanus</i>	3	basler12,basler14,laube14a
<i>Acer.saccharinum</i>	1	webb78
<i>Acer.saccharum</i>	3	calme94,laube14a,webb78
<i>Acer.tataricum</i>	1	laube14a
<i>Actinidia.deliciosa</i>	2	biasi12,guerrero90
<i>Aesculus.flava</i>	1	zohner16
<i>Aesculus.hippocastanum</i>	3	basler12,laube14a,zohner16
<i>Aesculus.parviflora</i>	1	zohner16
<i>Alnus.glutinosa</i>	2	heide93,myking98
<i>Alnus.incana</i>	2	heide93,zohner16
<i>Alnus.maximowiczii</i>	1	zohner16
<i>Amelanchier.alnifolia</i>	1	zohner16
<i>Amelanchier.florida</i>	1	zohner16
<i>Amelanchier.laevis</i>	1	zohner16
<i>Amorpha.fruticosa</i>	1	laube14a
<i>Aronia.melanocarpa</i>	1	zohner16
<i>Berberis.dielsiana</i>	1	zohner16
<i>Betula.alLEGhaniensis</i>	1	calme94
<i>Betula.lenta</i>	1	zohner16
<i>Betula.nana</i>	1	zohner16
<i>Betula.pendula</i>	10	heide93,li05,rinne97,basler12,laube14a,laube14b,linkosalo06,myking95,myking95,zohner16
<i>Betula.populifolia</i>	1	zohner16
<i>Betula.pubescens</i>	6	heide93,rinne94,caffarra11a,caffarra11b,myking95,myking97
<i>Buddleja.albiflora</i>	1	zohner16
<i>Buddleja.alternifolia</i>	1	zohner16
<i>Buddleja.daviddii</i>	1	zohner16
<i>Caragana.pygmaea</i>	1	zohner16
<i>Carpinus.betulus</i>	3	heide93a,laube14a,zohner16
<i>Carpinus.laxiflora</i>	1	zohner16
<i>Carpinus.monbeigiana</i>	1	zohner16
<i>Carya.cordiformis</i>	1	zohner16
<i>Carya.laciniosa</i>	1	zohner16
<i>Carya.ovata</i>	1	zohner16
<i>Castanea.sativa</i>	1	zohner16
<i>Cedrus.libani</i>	1	zohner16
<i>Celtis.caucasica</i>	1	zohner16
<i>Celtis.laevigata</i>	1	zohner16
<i>Celtis.occidentalis</i>	1	zohner16
<i>Cephalanthus.occidentalis</i>	1	zohner16
<i>Cercidiphyllum.japonicum</i>	1	zohner16
<i>Cercidiphyllum.magnificum</i>	1	zohner16
<i>Cercis.canadensis</i>	1	zohner16
<i>Cercis.chinensis</i>	1	zohner16
<i>Cladrastis.lutea</i>	1	zohner16
<i>Cornus.alba</i>	2	laube14a,zohner16
<i>Cornus.kousa</i>	1	zohner16
<i>Cornus.mas</i>	2	laube14a,laube14b
<i>Corylopsis.sinensis</i>	1	zohner16
<i>Corylopsis.spicata</i>	1	zohner16
<i>Corylus.avellana</i>	4	basler12,heide93,laube14a,zohner16
<i>Corylus.heterophylla</i>	1	zohner16
<i>Corylus.sieboldiana</i>	1	zohner16
<i>Decaisnea.fargesii</i>	1	zohner16
<i>Deutzia.gracilis</i>	1	zohner16
<i>Deutzia.scabra</i>	1	zohner16
<i>Elaeagnus.ebbingei</i>	1	zohner16
<i>Eleutherococcus.senticosus</i>	1	zohner16
<i>Eleutherococcus.setchuenensis</i>	1	zohner16
<i>Eleutherococcus.sieboldianus</i>	1	zohner16
<i>Euonymus.europaeus</i>	1	zohner16
<i>Euonymus.latifolius</i>	1	zohner16
<i>Fagus.crenata</i>	1	zohner16
<i>Fagus.engleriana</i>	1	zohner16
<i>Fagus.orientalis</i>	1	zohner16
<i>Fagus.sylvatica</i>	1	zohner16

Table S2: **Estimates from model fit with standardized predictors.** The model we present in the main text uses Utah units for chilling and includes studies that experimentally manipulated forcing and photoperiod. Using instead a model with chilling in Chill Portions (column two) results in quantitatively different species-level and overall estimates, though the results are qualitatively similar. We also present coefficients from a model including all species (i.e., with crops) with all treatment types. We present estimates and their 95 percent credible intervals from models in which the predictors have been standardized so that they are directly comparable,

parameter	utah.est	utah.ci	cp.est	cp.ci	allsp.est	allsp.ci
mu_a_sp	29.92	26.41-33.37	30.74	26.91-34.4	30.86	28.61-33.17
mu_b_force_sp	-4.37	-6.7-2.12	-4.8	-7.1-2.44	-6.15	-8.56-3.72
mu_b_photo_sp	-3.13	-5.63-0.77	-3.18	-5.39-1.03	-1.03	-2.31-0.2
mu_b_chill_sp	-8.87	-12.06-5.77	-8.2	-11.25-4.97	-8.04	-9.65-6.42
sigma_a_sp	9.41	7.21-12.2	10.17	7.69-13.29	14.42	12.67-16.42
sigma_b_force_sp	5.68	4.01-7.77	6.03	4.33-8.15	8.51	6.53-10.88
sigma_b_photo_sp	5.22	3.36-7.85	4.51	2.97-6.63	3.69	2.83-4.67
sigma_b_chill_sp	7.38	5.27-10.1	7.9	5.68-10.73	6.27	4.8-7.85
sigma_y	15.77	15.24-16.32	15.47	14.93-16	14.93	14.55-15.32
n_sp	39		39		205	

Table S3: **Estimates from models fit with predictors on their natural scales**, so that effect sizes can be readily interpreted in a meaningful way (e.g., change in days of budburst per degree C of warming for forcing temperature). The model we present in the main text uses Utah units for chilling. Here we also present coefficients from a model included all species, including crops, and all treatment types. We present estimates and their 95 percent credible intervals from models in which the predictors have been standardized so that they are directly comparable,

parameter	utah.est	utah.ci	cp.est	cp.ci	allsp.est	allsp.ci
mu_a_sp	62.89	54.9-70.93	67.02	58.05-75.76	62.57	57.64-67.38
mu_b_force_sp	-0.79	-1.16-0.42	-0.85	-1.25-0.45	-1.02	-1.29-0.76
mu_b_photo_sp	-0.53	-0.93-0.16	-0.54	-0.9-0.18	-0.14	-0.35-0.06
mu_b_chill_sp	-2.86	-3.78-1.95	-0.25	-0.33-0.16	-2.49	-2.92-2.06
sigma_a_sp	19.17	14.76-25.04	21.93	16.9-28.18	17.7	15.31-20.42
sigma_b_force_sp	0.91	0.63-1.25	0.99	0.69-1.41	0.71	0.57-0.88
sigma_b_photo_sp	0.77	0.49-1.14	0.7	0.46-1.02	0.59	0.45-0.74
sigma_b_chill_sp	2.06	1.46-2.82	0.2	0.14-0.28	1.24	0.95-1.57
sigma_y	15.83	15.28-16.39	15.53	15.01-16.08	15.16	14.77-15.58
n_sp	39		39		205	