Supporting Information: Experimental designs for testing the interactive effects of temperature and light in ecology and the problem of periodicity

February 24, 2022

OSPREE table of interactive studies

TO DO

Estimating the effects of periodicity co-varience mathmatically

[See comments in tex file.]

When experimental designs co-vary thermo- and photoperiodicity, they may incorrectly estimate effects of temperature, photoperiod and (for experiments with crossed designs) the interaction between photo and thermo-periodicity. While robustly testing the effect of this covariation requires additional experiments, you can solve for its potential effect based on the geometry of the experimental design given several assumptions.

If we assume that the effects of photoperiod and forcing (alone) are linear and additive (no FxP interaction), then the treatments and their results occupy a three-dimensional plane with temperature on the x axis, photoperiod on the y axis and the response on the z axis. Given our assumptions we can solve for the expected response if temperature and photoperiod were uncoupled.

To show this, we follow the design of Flynn & Wolkovich (2018) as an example. We used summed temperature (degree hours), to reflect the duration and intensity of temperature, on the x axis, photoperiod (hours) on the y axis and the response (δ days to budburst) on the z axis. [The combination of the co-varied treatments and responses produces a parallelogram in space, and we effectively solve for it as a rectangular—orthogonal—set of treatments.] Given the equation for a 3D plane and our knowledge of three sets of the coordinates:

$$ax + by + cz + d = 0 \tag{1}$$
$$200a + 8b + 0 + d = 0 \tag{2}$$
$$240a + 12b + 4.5c + d = 0 \tag{3}$$
$$320a + 8b + 9c + d = 0 \tag{4}$$

A, we can solve for the effects if we had been able to decouple the treatments. In this example, given that we know the δ days to budburst for 200 degree hours (temperature) at 8 hours of photoperiod, as well as 240 degree hours (temperature) at 12 hours of photoperiod, we essentially solve for the δ days to budburst given orthogonal treatments (e.g., 200 degree hours (temperature) at 12 hours of photoperiod; 240 degree hours (temperature) at 8 hours of photoperiod).

Algebraically, we simply solve for each unknown in turn. We begin by setting z to zero:
$$200a + 8b + 0 + d = 0 \text{ and solving for d yields:} \tag{5}$$
$$-200a - 8b = d \tag{6}$$

Now, we can solve for c (in terms of a and b) using our solution for d:
$$240a + 12b + 4.5c + (-200a - 8b) = 0 \tag{7}$$
$$-40/4.5a + 4/4.5b = c \tag{8}$$

And similarly then solve for a given our last set of coordinates and solutions for d and c (not shown). This yields:
$$a = \frac{1}{5}b \tag{9}$$
$$c = -\frac{8}{3}b \tag{10}$$
$$d = -48b \tag{11}$$
$$\tag{12}$$

Putting these back into the equation for a plane yields an overall equation to estimate the response z (days) for any combination of x (temperature) and y (photoperiod):
$$\frac{1}{5}bx + by - \frac{8}{3}bz - 48b = 0 \tag{13}$$
$$\frac{3}{40}x + \frac{3}{8}y - 18 = z \tag{14}$$
$$\tag{15}$$

Comparing:
$$\frac{3}{40} * (200) + \frac{3}{8} * (8) - 18 = 0 \tag{16}$$
$$\frac{3}{40} * (200) + \frac{3}{8} * (12) - 18 = 3 \tag{17}$$
$$\tag{18}$$

Shows that—given our assumptions—the estimated photoperiod effect that may be due to temperature is 3 days.

Modeling methods

To estimate the effects of impact of photo-thermo period co-variance we obtain two data files from two published phenology studies Flynn & Wolkovich (2018) and Buonaiuto & Wolkovich (2021). Both studies used dormant twigs collected from Harvard Forest in Petersham, MA, USA (42.5314N, 72.1900W) and exposed them to comparable treatments levels (12 vs. 8 hrs photoperiod, 20/10 vs. 15/5 day/night T (Flynn & Wolkovich, 2018) 24/18 vs. 18 vs, 12 (Buonaiuto & Wolkovich, 2021) in full factorial growth chamber treatments. We subset each date set to include only matching phenological observations ("leaf expansion" or BBCH 11 (Finn *et al.*, 2007)), species. This data subset included 713 observations ($n_{Flynn\&Wolkovich~(2018)} = 384, n_{Buonaiuto\&Wolkovich~(2021)} = 329, n_{species} = 7$).

We estimated the effect of study design on parameter with a Bayesian hierarchical mixed effect model using weakly informative priors. While both experiments were designed to test for interactions between photoperiod and forcing, we did not include this interaction term in our model in order to match the assumption of non-interactivity in the math. We included forcing, photoperiod, study and their interactions as main effects, and species as a random effect. We ran the model using the R package "brms" (Bürkner, 2018) on 4 chains with 2000 iterations and warmup of 1000 iterations for a total of 4000 posterior per parameter. The model is written below.

$$leafexpansion_{[i]} \sim N(\alpha_{sp_{[i]}} + \beta_{study} + \beta_{forcing} + \beta_{photoperiod} + \beta_{forcingxstudy} + \beta_{photoperiodxstudy}, \sigma_y^2)$$

We modeled the intercept (α) at the species level using the formula:

$$\alpha_{x_{sp}} \sim N(\mu_x, \sigma_x^2)$$

We note that the because the subset of overlapping data between the two studies is considerably smaller than it is not surprising that the relative effect sizes of photoperiod and forcing estimated here differ from those reported in the full, published studies.

References

Buonaiuto, D.M. & Wolkovich, E.M. (2021) Differences between flower and leaf phenological responses to environmental variation drive shifts in spring phenological sequences of temperate woody plants. *Journal of Ecology* **109**, 2922–2933.

Bürkner, P.C. (2018) Advanced bayesian multilevel modeling with the r package brms. *R Journal* **10**, 395–411.

	Estimate	Est.Error	Q2.5	Q25	Q75	Q97.5
Intercept	57.35	4.98	47.40	54.25	60.46	67.39
PHOTO	-3.28	0.38	-4.03	-3.54	-3.03	-2.51
FORCE	-1.32	0.15	-1.61	-1.42	-1.21	-1.02
study	12.22	2.49	7.47	10.50	13.96	17.02
PHOTO:study	2.27	0.66	1.02	1.82	2.73	3.57
FORCE:study	-1.74	0.39	-2.49	-2.00	-1.47	-1.00

Table S1: Main effect estimates from a Bayesian hierarchical model comparing coupled and uncouple experimental design. In this case, interactions with study represent the uncoupled estimates.

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.