

# False spring damage on temperate tree seedlings is amplified with winter warming

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## Introduction

1. The timing of spring in temperate deciduous forests shapes plant and animal communities and influences ecosystem services from agriculture to carbon sequestration to forest management.
  - (a) With warming temperatures in the Northern Hemisphere, spring phenology (i.e., budburst and leafout) is advancing.
  - (b) Budburst in trees and shrubs requires three cues: (1) over-winter cold temperatures (chilling), (2) warming spring temperatures (forcing) and (3) longer daylengths (photoperiod).
  - (c) As budburst and leafout are strongly cued by temperature, species ranges and growing seasons are highly susceptible to change with climate-induced warming (Chuine *et al.*, 2001).
2. And though the Northern Hemisphere is getting warmer, climate change is affecting general temperature trends but extreme weather events (e.g., polar vortexes) are still occurring.
  - (a) One such weather event is known as a ‘false spring’, which is when temperatures drop below freezing (Schwartz *et al.*, 2002, i.e., below -2.2°C) after budburst has initiated.
  - (b) Freezing tolerance in a plant steadily decreases after budburst begins until the leaf is fully unfolded, with leafout being the most susceptible to false spring damage (Lenz *et al.*, 2016).
  - (c) Thus, temperate plants are at risk of false springs and have evolved to minimize risk through myriad strategies, with the most effective being avoidance: plants must exhibit flexible spring

phenologies in order to maximize growth and minimize spring freeze damage by timing budburst effectively.

- (d) The rate of budburst, or the duration of vegetative risk (Chamberlain *et al.*, 2019), is a risky time for a plant and if a false spring occurs during this time, a plant is at an increased risk of experiencing an additional false spring as freezing temperatures can slow the rate of budburst (Augspurger, 2009).
  - (e) Seedlings and saplings generally initiate budburst before the canopy trees in order to benefit from the increased light levels (Augspurger, 2008; Vitasse *et al.*, 2013), which potentially puts understory species and individuals at greater risk (Vitasse *et al.*, 2014).
  - (f) False springs can be very damaging, with reports of trees taking 16-38 days to refoliate after leaf loss from freezing temperatures (Augspurger, 2009, 2013; Gu *et al.*, 2008; Menzel *et al.*, 2015).
  - (g) Such damage can have cascading effects to pollinators (Boggs & Inouye, 2012; Pardee *et al.*, 2017), nutrient cycling and carbon uptake as well as forest recruitment (Hufkens *et al.*, 2012; Klosterman *et al.*, 2018; Richardson *et al.*, 2013)
  - (h) False springs are predicted to increase in certain regions as climate change progresses, thus understanding the impacts of false springs on forests is essential for forest management strategies and climate forecasting (Kral-O'Brien *et al.*, 2019).
3. With climate change advancing, chilling is predicted to decrease as winter temperatures warm, potentially impacting phenology and, ultimately, growth.
- (a) Deciduous trees and shrubs require a certain number of chilling hours in order to leave the endodormancy phase (Charrier *et al.*, 2011).
  - (b) Endodormancy is the period of winter when temperate trees are inhibited from growing, regardless of the outdoor environment.
  - (c) Ecodormancy begins after the chilling requirement has been met and is the period of time when growth can occur but the external environment is not conducive to growth (e.g. too cold) (Basler & Körner, 2012).
  - (d) This two-phase sequence of dormancy helps protect temperate plants against stochastic warm spells in the winter and reduce the risk of a false spring (Basler & Korner, 2014).
4. Winter warming could impact the quality of budburst (Cleland *et al.*, 2007; Bonhomme *et al.*, 2009).
- (a) Optimal chilling accumulates between 0°C to 4°C, which is also the optimal temperature for starch degradation into sugar accumulation (Tixier *et al.*, 2019).

- (b) Sugar accumulation over the winter increases cold hardiness but is also crucial for dormancy break (Tixier, Aude and Roxas, Adele Amico and Godfrey, Jessie and Saa, Sebastian and Lightle, Dani and Maillard, Pauline and Lampinen, Bruce and Zwieniecki, Maciej A., 2017; Tixier *et al.*, 2019).
  - (c) Decreases in chilling temperatures from climate change could affect budburst timing (Nanninga, Claudia and Buyarski, Chris R and Pretorius, Andrew M and Montgomery, Rebecca A, 2017), as well as the synchrony of budburst within a population or even within an individual (Sanz-Pérez, V. and Castro-Díez, P. and Valladares, F., 2009).
  - (d) And, reduced chilling—especially if there are fewer cold nights with warming—could impact a plant’s tolerance of freezing temperatures throughout the winter (Charrier *et al.*, 2011).
  - (e) With decreased freezing tolerance, plants are susceptible to damage to leaf tissue, canopy dieback, xylem embolism and injury to the shoot apical meristem, all of which could greatly reduce a plant’s ability to recover for the remainder of the growing season or even survive until the next season (Sakai & Larcher, 1987; Gu *et al.*, 2008).
5. Here, we assessed the effects of over-winter chilling length and false springs on seedling phenology and growth across ten temperate tree and shrub species.
- (a) Individuals were exposed to three levels of over-winter chilling.
  - (b) Once budburst was initiated, half of the individuals were exposed to freezing temperatures at -3°C to mimic a false spring event.
  - (c) Individuals were then put in a greenhouse for the remainder of the growing season to ask: (1) How does the accumulation of over-winter chilling hours and (2) how do false spring events impact phenology and growth?

## Methods

### Plant Selection and Material

1. We chose 10 temperate woody plant tree and shrub species with varying phenologies, that were not used as crops or ornamental species: *Acer saccharinum* L., *Alnus incana rugosa* L., *Betula papyrifera* Marsh., *Betula populifolia* Marsh., *Cornus racemosa* Lam., *Fagus grandifolia* Ehrh., *Nyssa sylvatica* Marsh., *Salix purpurea* L., *Sorbus americana* Marsh., and *Viburnum dentatum* L.
  - (a) We received 48 dormant bare root seedlings—each measuring 6-12 inches—for each species from Cold Stream Farm LLC (Freesoil, MI; 44°6’ N -86°12’ W) for a total of 480 individuals.

- (b) Upon receipt, plants were potted in POT INFO AND SOIL INFO HERE!! and placed in growth chambers at the Weld Hill Research Building of the Arnold Arboretum (Boston, MA; 42°17' N -71°8' W) at 4°C to maintain dormancy.
- (c) Two species—*Fagus grandifolia* and *Nyssa sylvatica*—were delivered as root cuttings rather than seedlings and had to be removed from the experiment resulting in eight total species and 384 individuals.
- (d) After all individuals had leafed out, all seedlings were up-potted to new pots (NEW POT SIZE HERE) and given fertilizer (FERTILIZER INFO HERE).

## Growth Chamber and Greenhouse Conditions

1. Individuals were randomly selected and placed in six experimental treatments: 4 weeks of chilling at 4°C x no false spring, 4 weeks of chilling at 4°C x false spring, 6 weeks of chilling at 4°C x no false spring, 6 weeks of chilling at 4°C x false spring, 8 weeks of chilling at 4°C x no false spring, 8 weeks of chilling at 4°C x false spring.
  - (a) While individuals were in the growth chamber under chilling conditions, photoperiod was maintained at eight hour days.
  - (b) Lighting within the chambers was provided through a combination of T5HO fluorescent lamps with halogen incandescent bulbs at roughly  $250 \mu\text{mol}/\text{m}^2/\text{s}$ .
  - (c) Individuals were rotated within and among growth chambers every two weeks to eliminate possible growth chamber effects.
2. Once chilling was completed, individuals were moved to a greenhouse with mean daytime temperature of 15°C and a mean nighttime temperature of 10°C.
  - (a) Photoperiod was set to 12 hour days throughout the spring until all individuals reached full leaf expansion.
  - (b) After all individuals reached full leaf expansion, greenhouse temperatures and photoperiods were kept ambient (see Supplemental Materials for more information).

## Phenology and False Spring Treatment

1. Phenology observations were taken every 2-3 days through full leaf expansion and then recorded weekly over the summer.

- (a) Budburst was denoted as BBCH stage 07, which is ‘beginning of sprouting or bud breaking’ and monitored until full leaf expansion (BBCH stage 19) in order to evaluate the duration of vegetative risk for each individual (Finn *et al.*, 2007).
- (b) For the individuals under the ‘false spring treatment’, once at least 50% of the buds were at BBCH stage 07 but the individual had not yet reached BBCH stage 19, they were placed in a growth chamber set to mimic a false spring event.
- (c) Individuals receiving the false spring treatment were placed in a growth chamber for 14 hours, starting at 6pm.
- (d) Temperatures in the growth chamber were ramped down: 6pm to 10°C, 8pm to 5°C, 10pm to 0°C, 12am to -3°C, 3am to 0°C, 4am to 5°C, 6am to 10°C and 8am temperatures rose again to 15°C (Figure XX).
- (e) After 8am the following day, individuals were collected and placed back in the greenhouse with all of the other plants.
- (f) Once all individuals reached full leaf expansion (BBCH stage 19), phenology observations were made weekly until August 1st, when observations were made every 2-3 days again to monitor fall phenology.
- (g) Individuals were monitored until complete budset, at which point they were harvested for biomass measurements.

## Growth measurements

1. Growth was closely measured throughout the entirety of the experiment.
  - (a) Height was measured three times throughout the growing season: the day an individual reached full leaf expansion, 60 days after full leaf out and when an individual reached complete budset.
  - (b) We measured the chlorophyll content of four leaves on each individual 60 days after full leaf out using an atLEAF CHL PLUS Chlorophyll meter ([https://www.atleaf.com/atLEAF\\_CHL\\_PLUS](https://www.atleaf.com/atLEAF_CHL_PLUS)).
  - (c) The average chlorophyll content was calculated and then converted to mg/cm<sup>2</sup> using the atLEAF CHL PLUS conversion tool (<https://www.atleaf.com/SPAD>).
  - (d) We measured leaf thickness using a Shars Digital Micrometer (<https://www.shars.com/products/measuring/micrometers/0-1-solid-metal-frame-electronic-outside-micrometer>) and leaf toughness in Newtons using a Shimpo Digital Force Gauge ([http://shimpoinstruments.com/force\\_gauges/fg-3000](http://shimpoinstruments.com/force_gauges/fg-3000)) on two leaves for each individual.

- (e) Additionally, we monitored damage to the shoot apical meristem, which consisted of complete damage or disruption of growth in the main stem and resulted in early dormancy induction or reliance on lateral shoot growth.
- (f) Finally, belowground and aboveground biomass were harvested after an individual reached complete budset to include leaves in our biomass calculations.
- (g) Belowground and aboveground plant material were separated and then put in a Shel Lab Forced Air Oven (<https://www.sheldonmanufacturing.com/shellabproducts/productid/SMO28HP2>) at 60°C for at least 4 days.

## Data analysis

1. Using Bayesian hierarchical models with the brms package (Bürkner, 2017), version 2.3.1, in R (R Development Core Team, 2017), version 3.3.1, we estimate the effects of chilling duration, false spring treatment and all two-way interactions as predictors on: (1) duration of vegetative risk, (2) growing season length, (3) total growth in centimeters, (4) chlorophyll content, (5) leaf thickness, (6) leaf toughness, (7) shoot apical meristem damage, (8) total biomass and (9) belowground to aboveground biomass ratio.
  - (a) Species are modeled hierarchically as grouping factors, which generates an estimate and posterior distribution of the overall response across the eight species used in our experiment.
  - (b) False spring treatment is written as ‘tx’ in the equation below and chilling was split into two binary predictors: ‘chill1’, which is denoted as ‘0’ for 4 weeks of chilling or ‘1’ for 6 weeks of chilling and ‘chill2’ which is ‘1’ for 8 weeks of chilling:

$$y_i = \alpha_{species[i]} + \beta_{tx_{species[i]}} X_{tx} + \beta_{chill1_{species[i]}} X_{chill1} + \beta_{chill2_{species[i]}} X_{chill2} + \beta_{txchill1_{species[i]}} X_{txchill1} + \beta_{txchill2_{species[i]}} X_{txchill2} + \epsilon_i$$

,

$$\epsilon_i \sim N(0, \sigma_y)$$

- (c) The  $\alpha$  and each of the five  $\beta$  coefficients are modeled at the species level, as follows:

$$\begin{aligned}
\alpha_{species} &\sim N(\mu_\alpha, \sigma_\alpha) \\
\beta_{tx_{species}} &\sim N(\mu_{tx}, \sigma_{tx}) \\
\beta_{chill1_{species}} &\sim N(\mu_{chill1}, \sigma_{chill1}) \\
\beta_{chill2_{species}} &\sim N(\mu_{chill2}, \sigma_{chill2}) \\
\beta_{txchill1_{species}} &\sim N(\mu_{txchill1}, \sigma_{txchill1}) \\
\beta_{txchill2_{species}} &\sim N(\mu_{txchill2}, \sigma_{txchill2})
\end{aligned}$$

- (d) where  $i$  represents each unique observation,  $species$  is the species,  $\alpha$  represents the intercept,  $\beta$  terms represent slope estimates, and  $y$  is the phenology or growth measurement.
- (e) For the shoot apical meristem model, we used a Bernoulli distribution, which is modeled as:

$$\begin{aligned}
y_i &\sim \text{Binomial}(1, p) \\
\text{logit}(p) &= \alpha_{species[i]} + \beta_{tx_{species[i]}} X_{tx} + \beta_{chill1_{species[i]}} X_{chill1} + \beta_{chill2_{species[i]}} X_{chill2} \\
&\quad + \beta_{txchill1_{species[i]}} X_{txchill1} + \beta_{txchill2_{species[i]}} X_{txchill2} + \epsilon_i
\end{aligned} \tag{2}$$

- (f) The  $\alpha$  and each of the five  $\beta$  coefficients are modeled the same as the other models:

$$\begin{aligned}
\alpha_{species} &\sim N(\mu_\alpha, \sigma_\alpha) \\
\beta_{tx_{species}} &\sim N(\mu_{tx}, \sigma_{tx}) \\
\beta_{chill1_{species}} &\sim N(\mu_{chill1}, \sigma_{chill1}) \\
\beta_{chill2_{species}} &\sim N(\mu_{chill2}, \sigma_{chill2}) \\
\beta_{txchill1_{species}} &\sim N(\mu_{txchill1}, \sigma_{txchill1}) \\
\beta_{txchill2_{species}} &\sim N(\mu_{txchill2}, \sigma_{txchill2})
\end{aligned}$$

- (g) We ran four chains, each with 2 500 warm-up iterations and 4 000 sampling iterations for a total of 6 000 posterior samples for each predictor for each model using weakly informative priors.
- (h) Increasing priors three-fold did not impact our results.

- (i) We evaluated our model performance based on  $\hat{R}$  values that were close to one and did not include models with divergent transitions in our results.
- (j) We also evaluated high  $n_{eff}$  (4000 for most parameters, but as low as 1400 for a couple of parameters in the shoot apical meristem model).
- (k) We additionally assessed chain convergence and posterior predictive checks visually (Gelman *et al.*, 2014).

## Results

### 1. We found that false springs impacted both phenology and growth.

- (a) Individuals exposed to the false spring treatment had longer duration of vegetative risks (XX) at the beginning of the season and generally initiated dormancy earlier at the end of the season (XX), except for the cohort that received only 4 weeks of over-winter chilling, which had a consistent growing season length (Figure XX).
- (b) However, growing season lengths were shortest for the cohort that received 8 weeks of over-winter chilling (XX), but individuals had increased growth rates with increased over-winter chilling (XX).
- (c) Under 8 weeks of chilling, growth and biomass were maintained (XX) regardless of false spring treatment and were higher even with shorter growing season lengths.

### 2. Additional growth traits were affected differently.

- (a) Across all species and chilling treatments, individuals exposed to false springs experienced more damage to the shoot apical meristem (Figure XX and XX) and relied more on lateral shoot growth, rendering inefficient growth patterns.
- (b) Leaf chlorophyll content decreased under false spring conditions for the 6 weeks (XX) and 8 weeks (XX) of chilling cohorts.
- (c) Leaf toughness and leaf thickness both decreased across all chilling treatments under false spring conditions (XX for 4 weeks of chilling, XX for 6 weeks of chilling and XX for 8 weeks of chilling).
- (d) Shoot growth for the first 60 days of the growing season was highest for the 8 weeks of chilling cohort but false spring conditions reduced growth for the 6 and 8 weeks of chilling groups (XX and XX respectively).
- (e) Individuals exposed to false spring conditions had lower total biomasses for both the 4 weeks of chilling cohort (XX) and the 6 weeks of chilling cohort (XX), but the 8 weeks of chilling cohort



had higher total biomasses overall, regardless of whether or not there was a false spring treatment imposed (XX no false spring and XX for false spring conditions).

## Discussion

1. Thus, false springs reduce the length of the growing season when the chilling requirement is met.
2. This suggests chilling is more important for seedling growth than avoiding false springs.
3. With climate change and warming temperatures, over-winter chilling is anticipated to decrease and false springs are predicted to increase in certain regions.
4. This combination could greatly impact plant performance, survival and shape species distributions, ultimately affecting crucial processes such as carbon uptake and nutrient cycling.

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Tables and Figures























