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) As budburst and leafout are strongly cued by temperature, species' ranges and growing season lengths are highly susceptible to change with climate-induced warming (Chuine *et al.*, 2001).
   (c) These advancements in spring phenology is leading to increased carbon uptake across temperate forests, which are essential carbon sinks that combat the negative effects of climate change (Keenan *et al.*, 2014).
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) These weather events can in turn have big impacts on plant development each spring.
   (b) One such 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.
   (c) Damage from false spring events 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).

(d) It has been reported that it can take up to 16-38 days for plants to refoliate after leaf loss from a false spring (Augspurger, 2009, 2013; Gu *et al.*, 2008; Menzel *et al.*, 2015), which would lead to additional false springs in a season (Augspurger, 2009).

(e) 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. Warmer winters directly impact one of the major cues plants use to time budburst: over-winter cold temperatures (chilling), (in addition to warming spring temperatures (forcing) and longer daylengths).

   (a) Many temperate plants have evolved chilling requirements to avoid leafout during warm snaps in the middle of the winter, but with climate change chilling requirements may not be met.

   (b) If chilling is not met, plants may leaf out much slower or incompletely, which can in turn affect freeze tolerance.

   (c) Thus, understanding the interplay of warming winters and false spring risk is critical to predict how temperate forests will change in the future.

4. This interaction between winter chilling and false springs may be especially critical at the plant community level as species have likely evolved along a trade-off of risking spring frosts for early access to resources.

   (a) Ideally, individuals would evolve to require high levels of chilling to delay budburst and ultimately diminish false spring risk but competition for nutrients, water and light resources in the early spring pushes individuals to leafout earlier.

   (b) Young trees and understory species generally initiate budburst before the canopy trees to benefit from higher light levels (Augspurger, 2008; Vitasse *et al.*, 2013), which potentially puts these species and individuals at higher risk of freeze damage (Vitasse *et al.*, 2014).

   (c) Thus, successful forest recruitment requires seedlings and saplings to minimize false spring risk while maximizing growth.

   (d) Species typically leafout in a similar sequence, with understory species leafing out earlier and higher canopy trees leafing out last but many studies are predicting substantial shifts in chronological order and reassembly of species' leafout with climate change (Roberts *et al.*, 2015; Laube *et al.*, 2013).

   (e) As warming alters winter temperatures and false spring prevalence, phenological cues and their interactions are anticipated to change, which could greatly alter competition and recruitment among forest species for early season resources and ultimately impact species diversity and carbon uptake in temperate forests.

5. Here, we assessed the effects of over-winter chilling length and false springs on seedling phenology and growth across eight temperate tree and shrub species.
   (a) Individuals were exposed to different levels of over-winter chilling and then half of the individuals were exposed to a false spring event.
   (b) Individuals were observed 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, physical leaf characteristics 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., *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 mantained 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 $\mu mol/m^2/s$.

   (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 (Chamberlain *et al.*, 2019) 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 over 14 hours (Figure 1).

   (e) After 8am the following day, individuals were collected at 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.

   (c) The average chlorophyll content was calculated and then converted to mg/cm^2 using the atLEAF CHL PLUS conversion tool.

   (d) We measured leaf thickness using a Shars Digital Micrometer (scale works to 0.001mm) and leaf toughness in Newtons using a Shimpo Digital Force Gauge 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 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 and (8) total biomass.

(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) 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.

(c) Increasing priors three-fold did not impact our results.

(d) 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.

(e) 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).

(f) We additionally assessed chain convergence and posterior predictive checks visually (Gelman *et al.*, 2014).

Results

1. False springs and chilling durations impacted individual phenology.

   (a) Individuals exposed to the false spring treatment had longer durations of vegetative risk for the four weeks and slightly for the six weeks of chilling cohort (2.97 $\pm$ 0.79 and 1.53 $\pm$ 1.14, respectively; Figure 3**a** and Table S1).

   (b) Longer chilling treatments reduced the duration of vegetative risk, especially in the eight weeks of chilling cohort (-2.67 $\pm$ 1.14; Figure 3**a**) and Table S1).

   (c) Additionally, individuals exposed to a false spring that received eight weeks of chilling did not experience major changes in their durations of vegetative risk (0.92 $\pm$ 1.08), Figure 3**a** and Table S1).

   (d) With increases in chilling duration, the growing season length decreased for individuals exposed to six and eight weeks of chilling (2.48 $\pm$ 4.87 for six weeks and -9.66 $\pm$ 5 for eight weeks; Figure 3**b**) and Table S2).

2. False springs affected physical leaf traits.

   (a) Leaf chlorophyll content decreased under false spring conditions with increases in chilling, especially with eight weeks of chilling (-1.45 $\pm$ 1.16 for six weeks and -2.03 $\pm$ 1.07 for eight weeks; Figure S2) and Table S3).

(b) Leaf toughness decreased across all chilling treatments under false spring conditions (-0.05 ± 0.02 for four weeks of chilling, -0.09 ± 0.03 for six weeks of chilling and -0.08 ± 0.03 for eight weeks of chilling; Figure 4**a** and Table S4).

(c) Additionally, leaf thickness decreased across four and eight week chilling durations under false spring conditions, but there was little change for the six weeks of chilling cohort (-8.9 ± 3.74 for four weeks of chilling, -3.5 ± 5.31 for six weeks of chilling and -15.78 ± 5.25 for eight weeks of chilling; Figure 4**b** and Table S5).

3. False springs impacted growth habit but not total biomass.

   (a) Across all chilling treatments, especially for the four and eight week cohorts, individuals exposed to false springs experienced more damage to the shoot apical meristem (2.07 ± 0.97 for four weeks, 1.33 ± 1.42 for six weeks and 2.17 ± 1.31 for eight weeks; Figure 5**a** and Table S7)

   (b) Shoot growth over the growing season increased with eight weeks of chilling (11 ± 4.01) except growth was not affected under false spring conditions (4.73 ± 5.49; Figure S3) and Table S6)).

   (c) Individuals exposed to false spring conditions had slightly lower total biomasses when they were exposed to only four weeks of chilling (-3.45 ± 2.78) but there was very little change in total biomass under false spring conditions compared to the control for both the six weeks of chilling cohort (-3.62 ± 4.04) and the eight weeks of chilling cohort (2.88 ± 3.04; Figure 5**b**) and Table S8)).

4. False springs and chilling duration treatements resulted in some species-level differences but not phenological rank within the community.

   (a) Duration of vegetative risk decreased for most species with increasing chilling durations (i.e., the six and eight week cohorts), except for *Salix purpurea*, which experienced longer durations of vegetative risk with longer chilling durations, especially for six weeks of chilling (Figure 3**a**).

   (b) There was a lot of species-level variation with leaf thickness under the longer chilling durations, with *Sorbus americana* and *Viburnum dentatum* having thicker leaves with increases in chilling (Figure 4**b**).

   (c) All species experience meristem damage under false spring conditions except for *Betula populifolia* and *Sorbus americana* and *Viburnum dentatum* generally experienced meristem damage under all treatments (Figure 5**a**).

   (d) Order of leafout timing was consistent across all treatments, with *Salix purpurea* always being first to leafout, followed by *Betula papyrifera*, *B. populifolia* and *Cornus racemosa* and finally by *Alnus rugosa*, *Sorbus americana*, *Viburnum dentatum* and *Acer saccharinum* (Figure 2).

(e) *Viburnum dentatum* was the only species to change rank across treatments, though it consistently was grouped with the later-leafout group of species.

(f) Order of budset timing was also consistent across all treatments, with *Cornus racemosa* and *Sorbus americana* being first to set bud, followed by *Betula papyrifera* and *Acer saccharinum* and finally by *Viburnum dentatum*, *B. populifolia*, *Salix purpurea* and *Alnus rugosa* (Figure S1).

(g) *Acer saccharinum* was the only species to change rank across treatments, though it consistently was grouped with *Betula papyrifera* and *Viburnum dentatum*.

Discussion

1. Chilling length greatly influences spring phenology and can compensate for the detrimental effects of false springs.

   (a) False springs increase the duration of vegetive risk, leading to an increased risk in multiple false spring risks in one season.

   (b) But chilling can compensate for this increase in duration of vegetative risk: if the chilling requirement is met, the rate of budburst does not increase with a false spring event.

   (c) This suggests chilling is more important for seedlings in terms of exposure to multiple false springs.

   (d) With climate change and warming temperatures, over-winter chilling is anticipated to decrease and false springs are predicted to increase in certain regions.

   (e) This combination could greatly impact plant performance, survival and shape species distributions, ultimately affecting crucial processes such as carbon uptake and nutrient cycling.

2. False springs greatly impact the physical characteristics of the leaf.

   (a) With chlorophyll content, leaf toughness and leaf thickness decreasing, the quality of the leaf dwindles.

   (b) This reduction in quality could subsequently lead to an increase in herbivory risk (**?**).

   (c) Further studies that assess the secondary compounds and total phenolic content (Ayres, 1993; Webber & Mason, 2016) of the leaves exposed to false springs are needed to better understand the level of herbivory risk.

3. With sufficient chilling, false springs are less damaging to seedling growth.

   (a) However, false springs consistently impair shoot apical meristem growth, which can lead to relience on lateral shoot growth, rendering inefficient growth patterns.

(b) Shoot apical meristem damage—if significant within a stand—can lead to declines in recruitment (Rhodes & Clair], 2018).

(a) Growth chamber studies and climate model projections predict substantial shifts in species leafout order under climate change conditions (Roberts *et al.*, 2015; Laube *et al.*, 2013), other studies using long-term phenology observations suggest leafout phenology order is consistent across years (Wesołowski, Tomasz and Rowiński, Patryk, 2006).

   i. We are not seeing major shifts in species leafout order except for in *Viburnum dentatum*, which still leafs out within the later cohort of species across all treatments.

   ii. Therefore, we do not predict major reassembly of forest communities due to winter warming or false spring incidence.

(b) Understanding the impacts of false springs coupled with reduced over-winter chilling is essential for forecasting.

   i. Our findings have large implications for forest recruitment.

   ii. With over-winter chilling decreasing with climate change, seedlings are more at risk of sustaining damage from false spring events.

   iii. Understanding recruitment and inter- and intraspecific competition with false springs is crucial.

   iv. If individuals that initiate budburst earlier are more at risk of false spring exposure, this could lead to dieback of early-budbursting species in temperate forests with climate change.

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Tables and Figures

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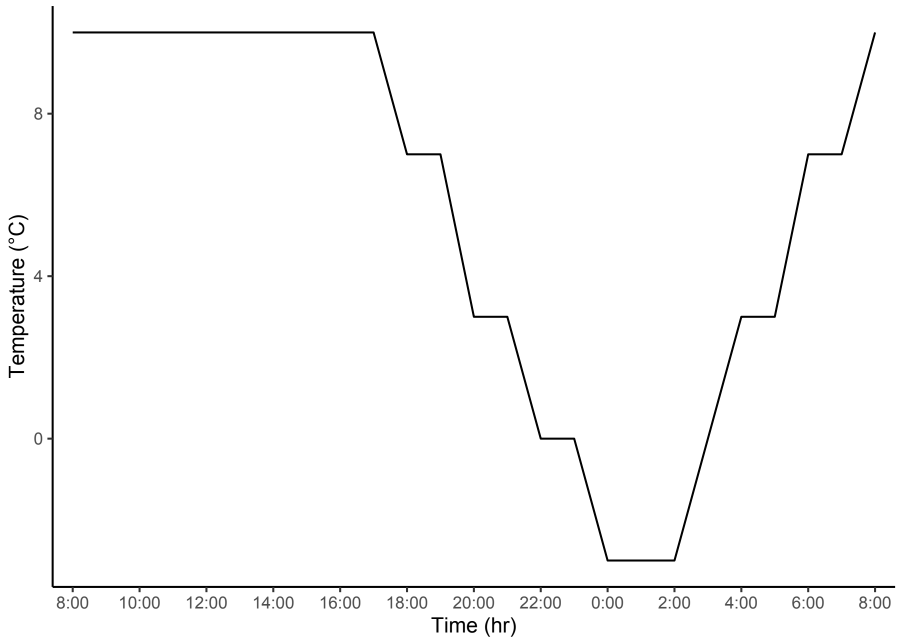

Figure 1: False spring treament temperature regime in the growth chamber

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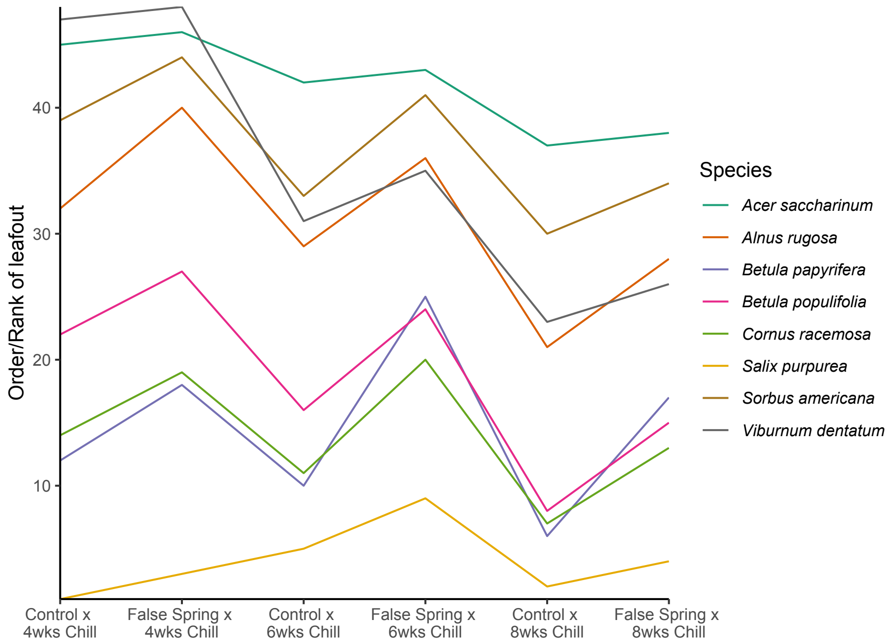

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Figure 2: Understanding rank order of leafout across all species using (a) mean trends and (b) raw estimates.

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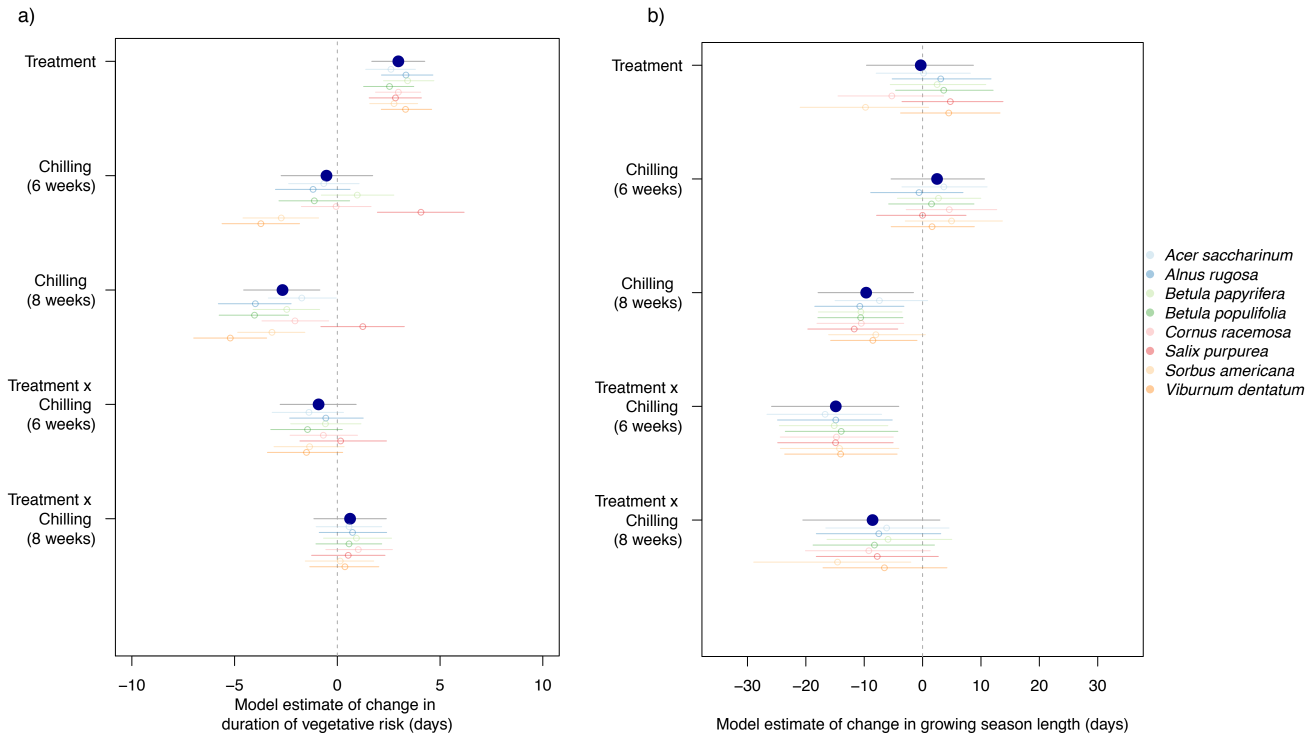

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Figure 3: Effects of false spring treatment, six weeks of chilling and eight weeks of chilling on a) duration of vegetative risk and b) growing season length. Dots and lines show means and 90% uncertainty intervals.

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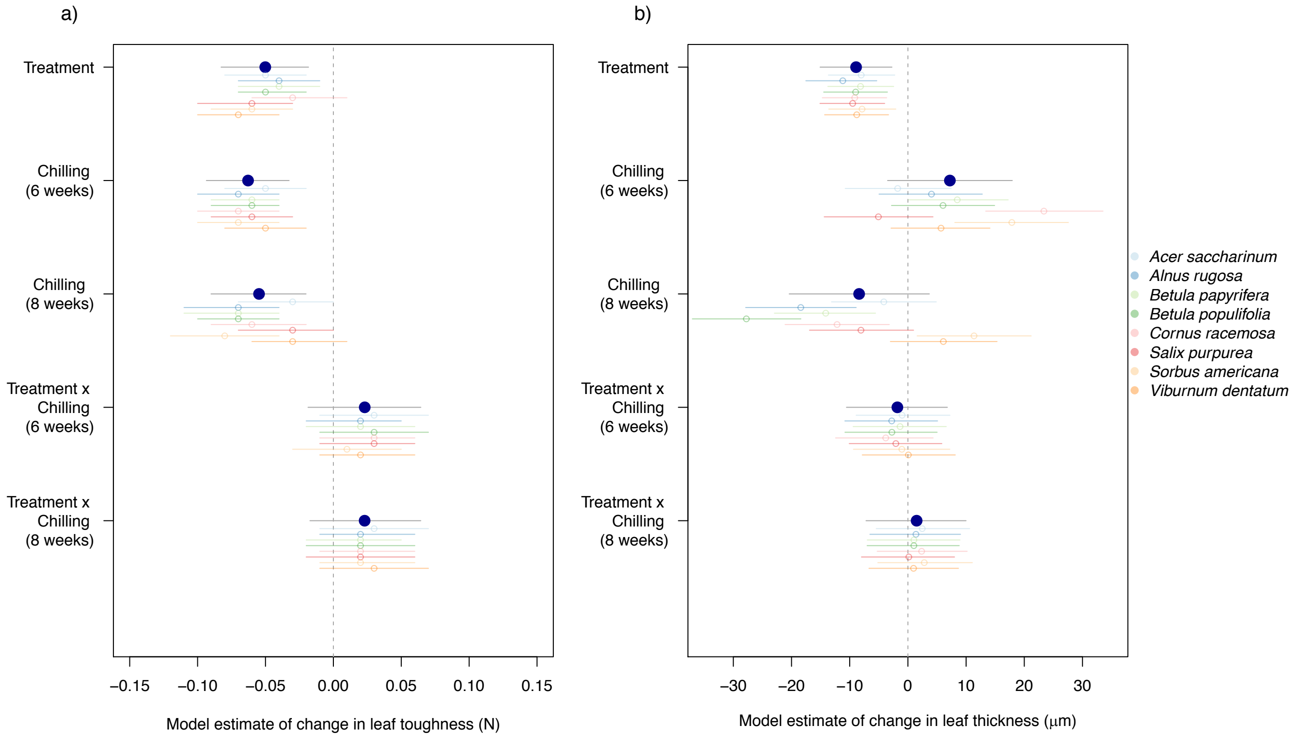

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Figure 4: Effects of false spring treatment, six weeks of chilling and eight weeks of chilling on a) leaf toughness and b) leaf thickness. Dots and lines show means and 90% uncertainty intervals.

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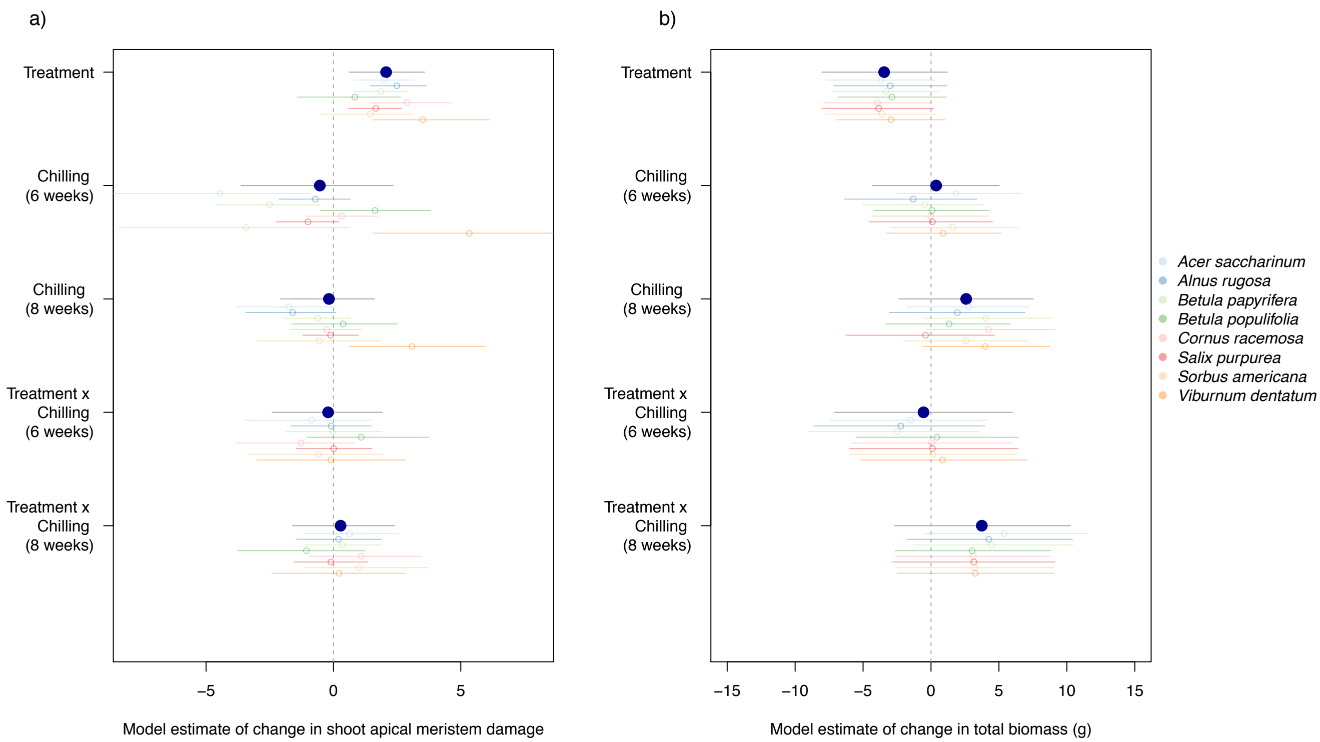

Figure 5: Effects of false spring treatment, six weeks of chilling and eight weeks of chilling on a) shoot apical meristem damage and b) total biomass. Dots and lines show means and 90% uncertainty intervals.