

# Past freeze–thaw events on *Pinus* seeds increase seedling herbivory

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**Abstract.** Seed and seedling survival are key components of plant population persistence. Although freeze–thaw events are experienced by many dispersed seeds in temperate ecosystems, it is unclear whether freeze–thaw stresses experienced by seeds can alter seedling susceptibility to herbivores during the growing season. We evaluated how freezing stress (temperature at  $-6^{\circ}\text{C}$  for 6 h) experienced by seeds of two conifer species (*Pinus resinosa* and *Pinus strobus*) affects seedling herbivory by a generalist herbivore (*Spodoptera exigua*). For both *Pinus* species, herbivores consumed twofold more seedling biomass from freeze–thaw-treated plants than seedlings from the constant temperature treatment. Herbivores grew ~66% faster when feeding on freeze–thaw *P. resinosa* seedlings relative to controls, but herbivore growth rate did not differ between treatments for *P. strobus*. Our results show that the thermal environment experienced by a seed can have subsequent effects on plant–herbivore interactions, suggesting that (1) early ontogenetic stress could be a cryptic, yet unappreciated, determinant of future herbivory and (2) increasingly frequent cold events, such as those projected under winter climate change, may amplify seedling herbivory and reduce recruitment in managed and natural conifer forests.

**Key words:** cold tolerance; herbivore performance; seedling herbivory; soil temperature; tree recruitment; winter climate change.

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## INTRODUCTION

Predicting plant survival and reproduction often depends on understanding variation in abiotic conditions (Harper 1977, Kreyling 2010). However, these predictions may be complicated by the potential for climatic events in one season to have effects that carry over to subsequent seasons (Walter et al. 2013). For example, *Pinus nigra* saplings exposed to drought demonstrate greater frost hardiness in subsequent years (Kreyling 2012). While a growing number of studies attest to the potential for such “stress legacies” in seedlings and mature plants (Walter et al. 2013), plants also encounter abiotic stresses as seeds, particularly when seeds overwinter (Baskin and Baskin 1998). In temperate and boreal ecosystems, juvenile plants and plant propagules overwintering at the

soil–atmosphere interface may be injured or killed if exposed to soil freezing (de Chantal et al. 2007, Connolly and Orrock 2015). Although evidence suggests that, in mature plants, negative effects of cold stress can manifest up to a year after extreme cold events (Kreyling 2012, Lacoste et al. 2015), it remains unclear whether the sub-lethal effects of cold stress experienced by seeds can persist after germination. Understanding how cold stress experienced by seeds affects the performance of subsequent life stages (e.g., seedlings) may help explain the considerable intra- and interspecific variation often observed in seedling survival (e.g., Nicotra et al. 1999) and provide insight into drivers of plant population dynamics and community structure (Hulme 1996, Hanley and Sykes 2009).

Seedling herbivory can be an important regulator of survival and plant recruitment (Moles

and Westoby 2004, Barton and Hanley 2013) and can shape plant populations and communities (Hulme 1996, Strauss 2009). Despite the importance of herbivory, explaining the considerable variation in the strength of plant–herbivore interactions has proven challenging (Agrawal 2011), possibly because context-specific factors generate strong variation in plant susceptibility to attack (Hahn and Maron 2016). We suggest that shifts in seedling susceptibility to herbivores caused by stressful conditions experienced as a seed are a possible mechanism that could generate cryptic, but widespread, context-specific variation in seedling susceptibility to herbivores. Although abiotic conditions experienced by seeds are known to affect seedling performance and susceptibility to abiotic stresses (Lyons 1973, Bruce et al. 2007), whether abiotic stresses experienced by seeds lead to differences in seedling herbivory remains unclear (Walter et al. 2013).

Projected changes in winter climate have the potential to radically alter plant population growth and community structure in temperate ecosystems (Kreyling 2010, Williams et al. 2014)—consequences that underscore the importance of understanding how cold temperature extremes alter biotic interactions that shape plant demography. We evaluated how exposing seeds to freezing temperatures in the soil influences subsequent seedling–herbivore interaction for two native conifers *Pinus strobus* (eastern white pine) and *Pinus resinosa* (red pine). Specifically, we examined how a single soil freeze–thaw event applied to the seed life stage influences patterns of herbivory (i.e., overall consumption, stem vs. leaf consumption) on *Pinus* tree seedlings by the generalist model herbivore *Spodoptera exigua*. *Pinus strobus* and *P. resinosa* are well suited for our experimental objectives as these species' native range includes most of eastern North America, a region that often experiences freezing stress in spring, and is likely to experience more severe cold stress as winter climates change (Augsburger 2013). Additionally, both *Pinus* spp. are widely cultivated, but herbivores may limit their recruitment and performance (Krugman and Jenkinson 2008). By examining whether freeze–thaw events experienced by seeds within the soil result in legacies that alter *Pinus* spp. seedling herbivory, we may gain particular insight into (1) how stress legacies “carry over” in plants to influence biotic interactions at

distinctly different life stages (Walter et al. 2013), (2) how cold stress may indirectly alter regeneration dynamics during periods of active growth (e.g., Inouye 2008, Augspurger 2013), and (3) how increases in cold stress via changing winter conditions may directly and indirectly influence plant fate (Henry 2008, Kreyling 2010, Williams et al. 2014).

## METHODS

We purchased *P. strobus* (eastern white pine) and *P. resinosa* (red pine) seeds through the Wisconsin Department of Natural Resources (DNR) Griffith State Nursery (Wisconsin Rapids, Wisconsin, USA). Prior to purchase, seeds were stored in dry plastic bags within cardboard canisters at  $\sim 0^{\circ}\text{C}$  at the Hayward State Nursery in Hayward, Wisconsin (J. Auer, Wisconsin DNR, *personal communication*). After purchase, seeds were stored at  $20\text{--}25^{\circ}\text{C}$  and ambient relative humidity. Seeds were collected in 2008 (*P. strobus*) and 1997 (*P. resinosa*) from northwestern Wisconsin populations (J. Auer, Wisconsin DNR, *personal communication*); evidence from descriptions of species natural history and management as well as supplementary experiments suggests this difference in seed age is unlikely to influence species response to cold stress (Rudolf 1990, Wendel and Smith 1990, see Appendix S1: Table S1, Appendix S2). Nevertheless, we minimize interspecific comparisons to limit the possible confounding issue of differing seed age. Three weeks before our experiment, seeds were presoaked for 48 h and then stratified on cold ( $1.0^{\circ}\text{C}$ ) damp sand.

### Soil collection and experimental methods

Field soil was collected from a central Wisconsin forest (Dexter County Park, Wood Co.; latitude  $44.39^{\circ}$ ; longitude  $-90.13^{\circ}$ ) dominated by *Acer saccharum*, *Acer rubrum*, and *Quercus rubra* with associated *P. resinosa* and *P. strobus*. We collected soil during late February to ensure that soil physical composition and microbial communities reflect the status of the region's soils prior to the onset of natural freeze–thaw cycles (Henry 2007, Connolly and Orrock 2015). Snow was removed and mineral soil was exhumed to a depth of 5 cm from three  $1 \times 1$  m plots located along a straight east–west transect; the center of each plot was 10 m apart. Soil was homogenized

and bulked by plot in order to standardize the effects of any pathogens within each plot. Soil was packed into a cooler to maintain field temperature, transported immediately back to the laboratory, and stored at 2°C until the initiation of our study.

Aliquots of 35 mL of soil were put into separate, sterile 50-mL centrifuge tubes. When soil was partitioned into centrifuge tubes, soil was handled in small batches (~200–300 mL) in multiple sessions to minimize the amount of time soil was handled outside refrigeration (Connolly and Orrock 2015). Eight cold-stratified *P. strobus* or *P. resinosa* seeds were added to each tube, which was capped and slowly inverted twice to distribute the seeds within the mineral soil. Soil tubes were then randomly positioned in 3.8-L plastic containers (diameter 20 cm, depth 12.7 cm; Airlite Plastics, Omaha, Nebraska, USA) filled with 200 g of medium coarse-grade vermiculite (Sunshine Medium Vermiculite; Sun Gro Horticulture, Agawam, Massachusetts, USA). Centrifuge tubes were positioned so that the soil in each tube aligned with the vermiculite surface in the surrounding container, ensuring natural top-down freezing processes in the soil during our freezing treatment (Henry 2007). Prior to the start of the experiment, all containers, tubes, and seeds were maintained at 2.0°C in a refrigerator for 1 week.

Our study was designed to independently test each *Pinus* spp. with one freezing treatment at two levels: (1) one freeze–thaw event and (2) a constant 2°C temperature control. With the soil tubes, we generated 17 replicates of each species × freezing treatment combination (2 species × 2 treatment levels × 17 replicates = 68 tubes). The freeze treatment was imposed using a Precision Low Temperature BOD incubator (ThermoFisher Scientific, Model 3733, Marietta, Ohio, USA). All centrifuge tube caps were slightly loosened to allow air pressures to equalize inside and outside of the tube during freezing treatment, but caps remained securely on the top of each tube to limit possible contamination by aerial dispersed spores of fungal pathogens. All containers were removed from the refrigerator and immediately placed in the incubator for 2 h at 2°C in order to expose both control and freeze–thaw treatments to any aerially dispersed spores of potentially pathogenic fungi associated with the incubator. After two hours, the control containers were

removed from the incubator and were immediately returned to the refrigerator maintained at 2°C. Incubator air temperature was cooled from 2°C to –6°C (rate: –3°C/h), remained at –6°C for 6 h, and then warmed to 2°C (rate: 3°C/h); see Appendix S2: Fig. S1 for details and field data supporting temperature selection criteria. Air temperatures within the single low-temperature incubator indicate that freezing treatment accurately reflects programmed treatment conditions (Appendix S3: Fig. S1). At the completion of the freezing trial, all containers were immediately placed back in the refrigerator to thaw for 48 h at 2°C.

Greenhouse trays lined with cell inserts (4.9 × 5.7 × 5.7 cm) were filled three-quarters full with potting media (Redi-Earth mix). Potting mix was homogenized prior to addition to trays. Each seed–soil mixture was transferred from its individual centrifuge tube to an individual greenhouse tray cell and covered with approximately 1.0 cm of the potting media. Trays were watered as needed and allowed to drain freely to mimic water infiltration through the soil profile. Trays were incubated at 20–24°C with a 12-h light/12-h dark photoperiod for 21 d.

#### Seedling emergence and feeding trials

We monitored seedling emergence daily. The initial *Pinus* seedling in each cell was marked with a piece of wire stuck in the soil adjacent (~6 mm) to the seedling stem; at least one seedling emerged in each cell. After 21 d, we counted the number of seedlings and harvested the aboveground biomass of the initial germinant in each cell. Harvested seedlings were immediately transferred to a plastic petri dish (100 × 15 mm) with a saturated germination blotter (Anchor Paper Co., St. Paul, Minnesota, USA) to prevent desiccation. We weighed the total aboveground biomass for each seedling, and then cut the stem of each seedling just below the needle whorl to separate needle and stem biomass.

To evaluate seedling herbivory, we used second-instar larvae of a generalist lepidopteran herbivore, the beet armyworm (*S. exigua*; Benzon Research Inc., Carlisle, Pennsylvania, USA). To the best of our knowledge, *S. exigua* are not a natural pest on pine seedlings. However, larval *S. exigua* are a model generalist herbivore (occasionally referred to as a “supergeneralist,” e.g., Langenheim et al. 1980) and are well suited for

use in this study because (1) *S. exigua* has been successfully used to estimate woody plant material palatability to generalist herbivores (e.g., Stubblebine and Langenheim 1977, Langenheim et al. 1980, Mooney et al. 2009), (2) *S. exigua* are readily available from insect-rearing laboratories, thereby permitting researchers tight control of rearing conditions, and (3) methods for conducting feeding trials with *S. exigua* are well established (Thaler et al. 1999, 2010).

*Spodoptera exigua* larvae were starved for 1.5 h prior to the start of the feeding trial, weighed, and randomly introduced to a petri dish with one *Pinus* seedling. Petri dishes were sealed with Parafilm "M" to limit desiccation and to prevent escape of larvae. Sealed dishes were spaced evenly on a laboratory counter at 23°C. After 24 h, larvae were isolated, starved for another 1.5 h, and then reweighed to determine larvae post-feeding trial weight. We weighed the remaining *Pinus* seedling stem and needle tissue to determine the biomass consumed. Three replicates each of *P. resinosa* and *P. strobus* did not receive *Spodoptera* larvae in their corresponding feeding trial and were excluded from the feeding trial analysis.

We conducted ancillary trials with four seedlings (one *P. resinosa* and three *P. strobus*) that did not receive herbivores to evaluate the role of autogenic mass loss in our experiment (Appendix S4); these losses were negligible and did not affect the interpretation of our results. For a random subset of all seedlings ( $n = 48$ , 54% of all samples), we separately weighed the needle and stem biomass for each seedling using an analytical balance (Model: XP26; Mettler-Toledo International, Columbus, Ohio, USA) before the herbivory trial in order to quantify differences in seedling stem and needle consumption (Appendix S5).

We used generalized linear mixed models with a binomial response distribution to analyze whether freeze–thaw treatment influenced the proportion emergence for each *Pinus* species 21 d after seeds were sown. In all models, freeze–thaw treatment was treated as a fixed effect. The refrigerator shelf holding each centrifuge tube was treated as a random effect to accommodate spatial variation within the refrigerator during seed exposure to constant temperatures. Seedling consumption was analyzed as the proportion of initial seedling biomass consumed during the 24-h feeding trial. *Spodoptera exigua* growth rate was calculated as the

amount of mass gained by an individual divided by the product of trial duration (1 d) and the average mass of the larva during the feeding trial (Waldbauer 1968). We calculated average *S. exigua* mass by taking the mean of pre-trial and post-trial larval weights. We used general linear mixed models to analyze the effect of the freeze–thaw treatment on pre-trial seedling biomass, proportion of total seedling consumed, proportion of needle tissue consumed, proportion of stem tissue consumed, and *S. exigua* growth rate; see Appendix S5 for model parameters specific to stem and needle consumption. In all models, freeze–thaw treatment served as the main fixed factor and the identity of the refrigerator shelf holding each bucket was treated as a random intercept. All analyses were conducted in SAS (SAS 9.3; SAS Institute, Cary, North Carolina, USA), and maximum-likelihood estimates in all models used Laplace approximation (Littell et al. 2006).

## RESULTS

Both populations of *Pinus* species demonstrated high seed viability, rapid germination speed, and comparable seedling cold tolerance prior to the start of this experiment (Appendix S1: Table S1). The proportion of seedlings emerging did not differ between our freeze–thaw treatment and control for *P. resinosa* (control:  $0.76 \pm 0.04$  vs. freeze–thaw:  $0.79 \pm 0.04$  [least-squares mean  $\pm$  standard error (SE)];  $F_{1,28} = 0.34$ ,  $P = 0.565$ ) or *P. strobus* (control:  $0.66 \pm 0.04$  vs. freeze–thaw:  $0.60 \pm 0.05$ ;  $F_{1,28} = 1.09$ ,  $P = 0.306$ ); see Appendix S6: Fig. S1 for a summary of seedling demography and performance. There was a marginally significant difference between the aboveground biomass of *P. resinosa* seedlings collected from the freeze–thaw treatment and those from the control (control:  $41.27 \pm 1.70$  mg vs. freeze–thaw:  $37.22 \pm 1.64$  mg;  $F_{1,28} = 2.94$ ,  $P = 0.097$ ; Appendix S6: Fig. S1). The freeze–thaw treatment did not influence *P. strobus* aboveground biomass prior to the herbivory trial (control:  $50.02 \pm 3.37$  mg vs. freeze–thaw:  $41.98 \pm 3.72$  mg;  $F_{1,28} = 2.57$ ,  $P = 0.120$ ). There was no difference in pre-trial needle or stem biomass between freezing treatment levels for either *Pinus* species (Appendix S6: Fig. S1).

Herbivores consumed approximately twice as much total proportion biomass from freeze–thaw-treated *P. resinosa* and *P. strobus* seedlings

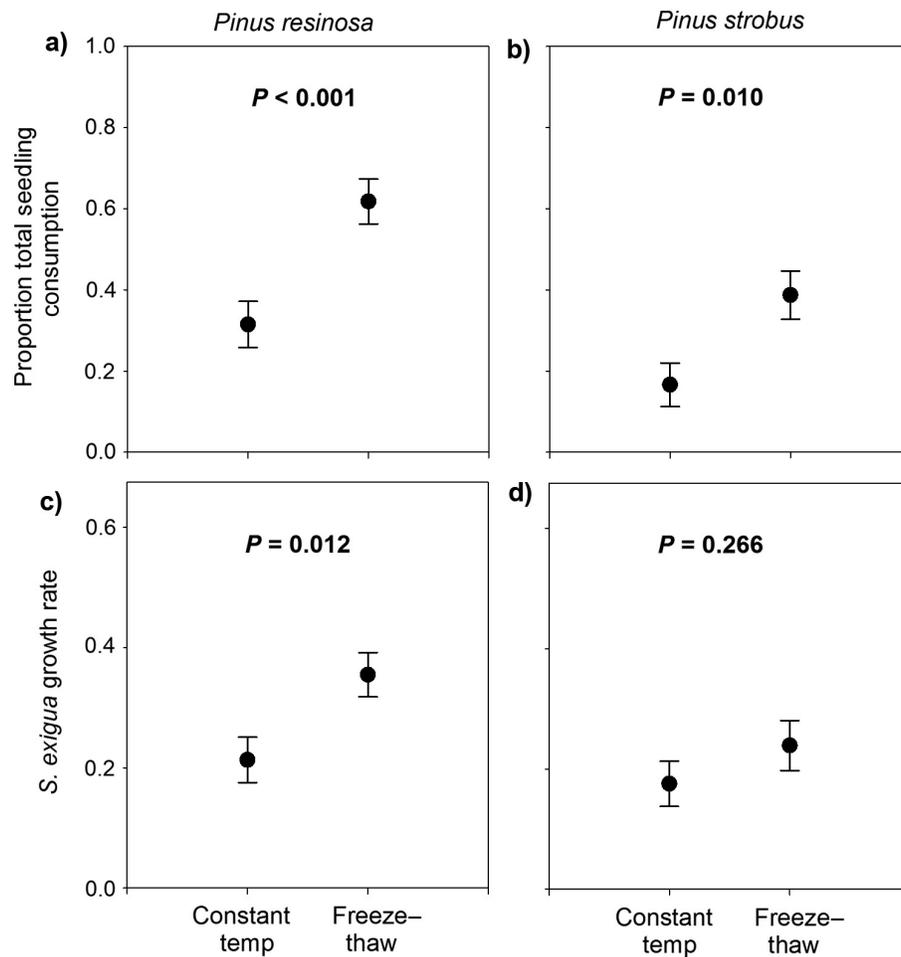


Fig. 1. The effect of freeze–thaw treatment on the proportion of total seedling consumed (*Pinus resinosa* [a], *Pinus strobus* [b]) and herbivore growth rate (*P. resinosa* diet [c], *P. strobus* diet [d]). Definitions and calculations of insect growth rate follow Waldbauer (1968). Reported values represent calculated least-squares means  $\pm$  1 standard error.

than conspecific seedlings from the constant temperature treatment (*P. resinosa* [Fig. 1a], control:  $31.51\% \pm 5.68\%$  vs. freeze–thaw:  $61.79\% \pm 5.50\%$  [least-squares mean total proportion consumed  $\pm$  SE];  $F_{1,28} = 14.67$ ,  $P < 0.001$ ; *P. strobus* [Fig. 1b], control:  $16.65\% \pm 5.39\%$  vs. freeze–thaw:  $38.73\% \pm 5.93\%$ ;  $F_{1,28} = 7.59$ ,  $P = 0.010$ ). Patterns of absolute seedling biomass consumption were comparable to proportion biomass consumed (Fig. 2); herbivores consumed approximately twice as much absolute seedling biomass from freeze–thaw-treated *P. resinosa* and *P. strobus* seedlings than conspecific seedlings from the constant temperature treatment (*P. resinosa*, control:  $13.058 \pm 2.347$  mg consumed vs. freeze–thaw:

$23.204 \pm 2.273$  mg consumed [least-squares mean total seedling mass consumed  $\pm$  SE];  $F_{1,28} = 9.64$ ,  $P = 0.004$ ; *P. strobus*, control:  $7.489 \pm 1.704$  mg consumed vs. freeze–thaw:  $14.656 \pm 1.878$  mg consumed;  $F_{1,28} = 7.98$ ,  $P = 0.009$ ).

*Spodoptera exigua* consumed both stem and needle biomass of each *Pinus* species, but consumed more needle biomass than stem biomass (Appendix S5: Fig. S1). More needle tissue was consumed from freeze–thaw-treated *P. resinosa* than control *P. resinosa* seedlings (Appendix S5: Fig. S1). Seedling tissue was never completely consumed, indicating herbivores were never food-limited during feeding trials. Larval growth rate was 66% faster on a diet consisting of

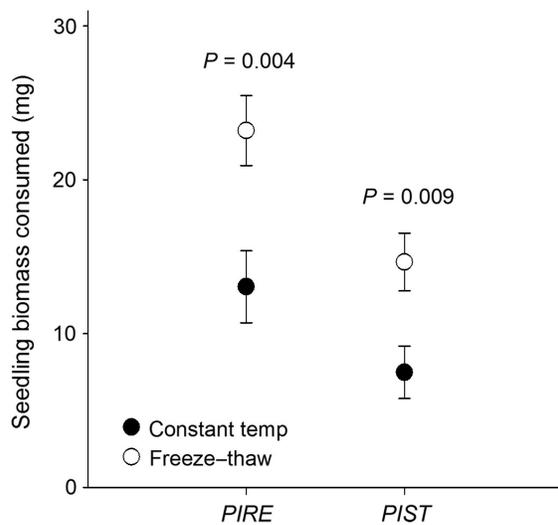


Fig. 2. The effect of freeze-thaw treatment on the absolute seedling tissue mass consumed (*Pinus resinosa* [PIRE], *Pinus strobus* [PIST]). Reported values represent calculated least-squares means  $\pm$  1 standard error.

freeze-thaw-treated *P. resinosa* plants than on a diet consisting of control *P. resinosa* seedlings (Fig. 1c;  $F_{1,28} = 7.23$ ,  $P = 0.012$ ), but larval growth rate on *P. strobus* seedlings did not differ due to seed freeze-thaw treatment (Fig. 1d;  $F_{1,28} = 1.29$ ,  $P = 0.266$ ).

## DISCUSSION

Extreme cold events can structure plant populations (Kreyling 2010, Williams et al. 2014), but the potential for winter climate stress experienced by seeds to shape plant-herbivore interactions during the growing season is less appreciated. Our results demonstrate that seeds that experience a single freeze-thaw cycle within the soil are more susceptible to generalist herbivores as seedlings relative to conspecific seedlings that experience a stable thermal environment as seeds. The effects of thermal variability experienced by seeds on seedling herbivory may vary by species (Fig. 1), but future studies controlling for seed age between species will be important to directly address that hypothesis. Our work suggests that freeze-thaw events may (1) increase seedling susceptibility to herbivores, which could limit seedling recruitment, and (2) alter the spatial distribution of tree recruitment as a function of topography (e.g., frost pockets),

highlighting the importance of understanding past stress when predicting plant-herbivore interactions or developing forest management strategies.

### *The thermal variability experienced by seeds influences future herbivory on seedlings*

Environmental context plays a strong role in determining the severity of plant-herbivore interactions (Hahn and Maron 2016) but there is growing recognition that past abiotic and biotic stresses endured by plants may also shape individual plant response to herbivory (Walter et al. 2013, Lacoste et al. 2015). Some evidence suggests that legacies within plants can alter the outcome of future herbivory events: Orrock (2013), for example, demonstrated that plants exposed to non-consumptive cues of herbivory risk (i.e., snail mucus) as seeds were less palatable to snails than plants that grew from seeds that were not exposed to cues associated with herbivory. In this study, we demonstrate that sub-lethal thermal stress experienced at an early plant life stage (i.e., the seed) caused greater amounts of seedling herbivory and resulted in double the total tissue consumption, relative to unstressed conspecifics, in subsequent life stages (Fig. 1). Ecological legacies resulting from early-life climatic events might be particularly relevant for ecologists attempting to understand how herbivory will shape (1) plant performance during the active growing season and (2) recruitment of seed-limited plant species (Walck et al. 2011). However, unlike predictions that exposure to abiotic stress may heighten plant resistance to natural enemies (Walter et al. 2013), our work suggests that abiotic stresses applied to early life stages are likely to exacerbate herbivory on afflicted plants. If early-life stress is one of the causal factors dictating ecological interactions in later life, it may be necessary for ecologists to consider site-specific stress history (e.g., extreme cold events, early-season droughts) in order to accurately detect and predict factors driving herbivory during the growing season.

Exposure to extreme physical conditions during early life stages may differentially influence tree species seedling performance via differential herbivory and survival. Seedling herbivory can directly alter individual plant survival and resource acquisition (e.g., light) with long-term

effects on plant growth and reproductive capacity (Hanley and May 2006, Barton and Hanley 2013) and the outcome of interspecific competition (i.e., species co-existence, competitive exclusion, Hanley and Sykes 2009). For example, *P. resinosa* is an early-successional species in northern temperate forests and disproportionate loss of *P. resinosa* seedlings to herbivores may accelerate successional dynamics in northern forests by reducing *P. resinosa*'s presence in the understory and canopy and increasing light availability for later-successional species (e.g., *P. strobus*, *Tsuga canadensis*, *Abies balsamea*). Congeneric differences in seedling herbivory observed in our study must be interpreted cautiously given differences in seed accession age. Both *Pinus* species demonstrate strong cumulative germination and rapid germination rate (confirmed via ancillary trials with the seeds we used; Appendix S1: Table S1), suggesting that seedlings of these species did not differ in health or vigor (Maguire 1962, Barnett and McLemore 1984). Nevertheless, for some herbaceous species (e.g., *Bromus tectorum*), competitive ability declines with seed age (Rice and Dyer 2001) and we cannot completely disentangle the effects of species and seed age in this work. Given that tree nursery seed stock can retain high germination performance after prolonged cold storage (Simpson et al. 2004) and that forest land managers may have to make decisions between using seed stocks that can vary considerably in age (Bonner 2008), future work considering the role of age on seed response to abiotic stresses and seedling herbivory will directly inform the likelihood of aged tree seeds resisting physical and biotic barriers to establishment.

#### **Freeze–thaw events and predictions of tree survival and recruitment**

Freeze–thaw cycling may damage seed tissue, reduce growth, and physiologically compromise developing seedlings (e.g., Schaberg et al. 2008) and, in adult plants, cold stress can alter plant defense against herbivores (Lacoste et al. 2015). Understanding plant responses to cold stress is particularly relevant given projections of colder soil temperatures as snow depth declines in the Northern Hemisphere (Kreyling 2010, Williams et al. 2014). Cold stress can shape the demography of temperate terrestrial vegetation (Kreyling 2010), with recent studies highlighting how

untimely cold stress can strongly affect seed viability (Connolly and Orrock 2015), juvenile survival (Augsburger 2011), and adult performance and reproduction (Gu et al. 2008, Inouye 2008). Our work builds on these studies by indicating that, in addition to direct negative effects on plant survival and growth, freezing experienced within the soil during an early life stage intensifies the effect of biotic interactions in later life stages. Accurate predictions of plant population demography, particularly with respect to winter climate change, will likely be contingent on our ability to anticipate how climate stressors generate legacies within individuals that might modify important species interactions governing seed and seedling survival (Walck et al. 2011).

#### **Future directions**

Given that winter climate may have an underappreciated role in shaping plant–herbivore interactions in the growing season, our work suggests several future research directions. The mechanistic drivers of these patterns of herbivory are still unclear and future studies quantifying the magnitude of freezing damage on seeds (e.g., relative electrolyte leakage, Cao et al. 2007) or evaluating how tree seedling nutrient content (e.g., macronutrient availability) shifts as a function of seed freezing history will help shape possible management solutions to minimize deleterious effects of cold extremes on seeds. Importantly, our work may advance questions addressing the role of thermal variability in influencing spatial patterns in tree seedling mortality, the performance of lepidopteran herbivores, possible forest land management strategies to mitigate the effects of climate extremes, and latitudinal patterns of herbivory. For example, differences in thermal regime resulting from the physical characteristics of a habitat (e.g., deciduous vs. conifer canopy structure) may alter the history of thermal conditions experienced by tree seedlings, producing disparate patterns of consumer pressure on juvenile trees (e.g., Kotanen 2007). Cold stress in the soil may also indirectly facilitate the growth of generalist lepidopteran herbivores by modifying seedling palatability (Fig. 1c, d), suggesting that shifts in the quality of food resources might be an underrepresented component of predictive models estimating the performance of lepidopteran forest pests (Netherer and Schopf 2010). Forest land managers may be

able to mitigate the influence of climate extremes on seed and seedling stress by altering the density of coarse woody debris in post-harvest landscapes (Harmon 1986). At larger spatial scales, variation in cold stress may in part generate latitudinal patterns in seedling herbivory, and also suggests that studies evaluating drivers of latitudinal variation in herbivory (Moles et al. 2011, Anstett et al. 2016) may need to consider physical conditions experienced by seeds when interpreting patterns of herbivory on seedlings and adult plants.

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