

BRIEF COMMUNICATION

Extreme cold consistently reduces seedling growth but has species-specific effects on browse tolerance in summer

Peter W. Guiden^{1,3} , Brian M. Connolly^{1,2}, and John L. Orrock¹

Manuscript received 29 March 2018; revision accepted 17 September 2018.

¹ Department of Integrative Biology, University of Wisconsin-Madison, 250 N. Mills Street, Madison, WI 53706, USA

² Current address: Department of Biology, Eastern Michigan University, 441 Mark Jefferson, 15 Science Complex, Ypsilanti, MI 48198, USA

³ Author for correspondence (e-mail: guiden@wisc.edu)

Citation: Guiden, P. W., B. M. Connolly, and J. L. Orrock. 2018. Extreme cold consistently reduces seedling growth but has species-specific effects on browse tolerance in summer. *American Journal of Botany* 105(12): 2075–2080.

doi:10.1002/ajb2.1203

PREMISE OF THE STUDY: Extreme weather events can injure plants, causing decreased survival. However, we may underestimate the ecological importance of extreme events if they have strong sublethal effects that manifest after several months. We tested the hypothesis that late-winter extreme-cold events decrease the ability of woody plants to grow and tolerate stem removal in summer.

METHODS: Seedlings from four temperate tree species (*Abies balsamea*, *Pinus resinosa*, *P. strobus*, *Quercus rubra*) were acclimated to winter conditions in growth chambers, and experienced 1 week of warm temperatures before being exposed to one of three 24-h extreme-cold events (minimum temperature: 8°C control, –8°C, or –16°C). Seedlings were then transferred to a greenhouse where we monitored survival and growth. Three months after the extreme-cold event, we mimicked an herbivore attack by removing either 25% or 75% of new stem growth from seedlings of two species (*P. resinosa*, *Q. rubra*).

KEY RESULTS: While extreme cold had no immediate effect on seedling survival, the coldest temperature treatment reduced stem growth 51% relative to controls. Stem removal decreased *P. resinosa* survival in the –16°C treatment, but stem removal treatment had no effect on *P. resinosa* survival in the intermediate –8°C treatment or 8°C control. Stem removal did not alter *Q. rubra* survival.

CONCLUSIONS: Ephemeral late-winter cold temperatures can have unappreciated effects on growing-season seedling dynamics, including growth and herbivory. For predicting how extreme-cold events might alter large-scale patterns of tree distribution, seedlings should be monitored throughout the growing season following extreme late-winter frosts.

KEY WORDS *Abies balsamea*; cold hardiness; extreme event; false spring; *Pinus resinosa*; *Pinus strobus*; *Quercus rubra*; winter climate change.

Extreme weather events can influence plant survival, population persistence, and ecosystem function (Haddad et al., 2002; Royer et al., 2011). Climate change is expected to increase the frequency and intensity of many extreme events (Jentsch et al., 2007; Smith, 2011), highlighting the importance of understanding plant responses to extreme events. However, understanding plant responses to extreme events can be difficult, because negative effects may not manifest until long after extreme events occur (Walter et al., 2013;

Kong and Henry, 2016). For example, repeated freeze–thaw cycles can decrease biomass production, but in some communities this response may not become apparent until more than a year after the extreme event (Kreyling et al., 2010). Extreme events may also affect species interactions, such as herbivory. While most seedlings can withstand some herbivory (Canham et al., 1994), seedlings experiencing climate-mediated stress followed by herbivory may exhibit sharp decreases in survival (Niinemets, 2010).

In temperate ecosystems, the transition from winter to spring is becoming increasingly variable due to warming winter temperatures. Periods of elevated winter temperatures (“false springs”) can decrease cold hardiness in woody plants (Augsburger, 2013), and extreme-cold events after false springs may damage sensitive tissue (Gu et al., 2008; Augspurger, 2009; Vitasse et al., 2014a), causing decreased woody plant survival (Drescher and Thomas, 2013) and photosynthesis (Hufkens et al., 2012; Tessier, 2014) immediately following the frost event. However, whether temperate woody seedlings that survive extreme-cold events in late winter suffer reduced performance in the growing season is unknown. Experiments that test for delayed, sublethal responses of woody seedlings to extreme cold could therefore allow us to recognize cryptic abiotic limits on recruitment dynamics in scenarios of future climate change (Williams et al., 2014).

Here, we examined how extreme-cold events shape tree recruitment, growth, and tolerance of stem removal in northern temperate forest communities by monitoring seedlings in a controlled environment for several months. Northern temperate forests, which are experiencing increasingly frequent and damaging extreme-cold events (Kreyling, 2010), represent an ecotone between boreal and temperate forest (Fisichelli et al., 2014). As temperatures warm, temperate species are expected to replace boreal species (Fisichelli et al., 2014), but herbivores may limit the rate of expansion in some temperate species (Fisichelli et al., 2012). However, the potential for extreme-cold events to limit range expansions of temperate species or increase the negative effects of herbivory in the growing season remains unexplored. We hypothesized that extreme cold would immediately reduce seedling survival and that the damage incurred by extreme cold would reduce the ability of seedlings to grow and tolerate stem removal in the following growing season.

MATERIALS AND METHODS

We investigated the effects of an experimental extreme-cold event on seedlings of four tree species (*Abies balsamea*, *Pinus resinosa*, *Pinus strobus*, and *Quercus rubra*), which are native to North American northern temperate forests but are expected to decline in importance in the next century (Iverson et al., 2008). We obtained seeds from the Wisconsin Department of Natural Resources Griffith State Nursery (Wisconsin Rapids, WI, USA). On 20 November 2015, seeds were placed in a growth chamber (model E-41L2, Percival Scientific, Perry, IA, USA) with a 12-h 25°/15°C, light/dark cycle to maximize germination (Burns and Honkala, 1990). When seedlings emerged, they were transferred to a greenhouse, where they received supplemental light and daily watering.

On 20 May 2016, 6 months after germinating, i.e., the approximate age of most seedlings in the field at the beginning of autumn, we measured stem height of all seedlings (*A. balsamea*: $n = 27$, *P. resinosa*: $n = 37$, *P. strobus*: $n = 31$, *Q. rubra*: $n = 28$) and transferred seedlings into one of three climate-controlled rooms with precise control of temperature and photoperiod. Each room was programmed to gradually decrease temperature and photoperiod, mimicking autumn and winter temperatures and photoperiod usually observed in northern temperate forests and inducing normal cold-hardening (dix S1, see the Supplemental Data with this article). To prevent seedling in pots from experiencing unrealistically deep soil-frost depths, we placed seedling pots in large bins of medium-grade vermiculite (Henry, 2007).

After 3 weeks of winter conditions, seedlings experienced a false spring for 1 week (daily max/min temperature: 10°C/8°C, 14-hr photoperiod, Appendix S1) that closely approximated recently observed late-winter temperature increases in northern temperate forests (Hufkens et al., 2012). We did not observe any new stem or leaf growth during the false spring. After the false spring, each room was assigned one of three 24-h extreme-cold event treatments, which only differed in minimum temperature: 8°C (control), –8°C, and –16°C. These temperatures represent late-winter air temperatures that seedlings could be exposed to in Great Lakes region northern temperate forests after an early snow retreat (Appendix S2). To understand how this temperature manipulation affected soil temperatures, we placed two iButton thermochrons (Maxim Integrated, San Jose CA) in each room approximately 2 cm below the soil surface. For each iButton, we determined the minimum soil temperature and the number of hours with temperatures <0°C. We subset these data to only include observations during our extreme-cold event, and constructed linear models describing minimum soil temperature and duration of freezing as a function of temperature treatment.

In response to a brief (8-h) equipment malfunction that led to a short rise in temperature in one room several weeks before the extreme-temperature treatment, we re-randomized the temperature treatment assigned to each seedling. This approach allowed us to appropriately control for the perturbation, as well as test for any subsequent effects of the perturbation. Our results demonstrate that this brief malfunction did not influence experimental outcomes (Appendix S3). Subsequent temperature profiles were precisely as desired: temperatures in each room were identical when rooms were meant to be identical and differed as planned when treatments were imposed (Appendix S1).

Following the extreme cold event, three seedlings per temperature treatment per species ($n = 36$) were destructively harvested to measure root, stem, and (for conifers) leaf percentage electrolyte leakage (PEL). Electrolyte leakage is a measure of cold damage at the cellular level, as cell membranes can rupture when the cell reaches freezing point (Burr et al., 1990; Kreyling et al., 2012). Because roots are less cold-tolerant than other tissue, and cold damage to tree seedling roots can directly influence tree seedling survival and growth (Schaberg et al., 2008), we focused our analysis on root PEL (see Appendix S4 for stem and leaf PEL responses to extreme cold). Before measuring root PEL, roots were washed in Millipore-filtered water for 30 s to remove electrolytes present on the root surface. No soil was visible on the root tissue before initial PEL estimation. We modeled the response of root PEL to temperature, species, and a temperature \times species interaction using a linear model with a Gaussian response. Seedling stem height, a proxy for root length ($r^2 = 0.71$, $P < 0.001$; P. W. Guiden, unpublished data), was added as a covariate, since seedlings with longer roots would be more likely to have root tissue unaffected by frost near the soil surface (Henry, 2007).

After exposing seedlings to cold temperatures, each room returned to warm temperatures (analogous to spring) for 10 d before seedlings were transferred to a greenhouse (Appendix S1). We monitored seedlings for approximately 8 wk (29 June 2016 to 24 August 2016), recording survival and growth. Seedlings were considered alive if green leaf tissue was present after 8 wk in the greenhouse. Seedling survival was modeled as a binomial response to temperature (treated as a categorical variable), species, and a temperature \times species interaction. Because quasi-complete separation was present

in our data set (i.e., temperature treatment perfectly predicted survival in some species), we used Firth bias correction with penalized likelihood estimation in all models of seedling survival (Firth, 1993), using the *brglm2* package in R (Kosmidis, 2017).

We also examined the effect of the extreme-cold event on seedling stem growth. To account for differences in initial stem height between and within species, we calculated seedling relative stem growth rate. Relative stem growth rate was defined as the difference of natural-log transformed stem height at the conclusion of the study and natural-log transformed stem height when seedlings were placed in climate-controlled rooms, divided by the time elapsed between measurements ($t = 0.4$ years, Gibson, 2002). Relative stem growth rate was modeled as a function of temperature, species, and a temperature \times species interaction using a Gaussian error model.

Additionally, we tested the hypothesis that an extreme-cold event could also increase the negative effects of stem removal, mimicking seedling herbivory by mammalian herbivores. Three months following the extreme-cold event, we selected a random subset of seedlings from two species ($n = 31$, *P. resinosa*; $n = 22$, *Q. rubra*), measured stem height, and applied one of two experimental stem removal treatments (removing either 25% or 75% of new stem growth; Canham et al., 1994) with pruning shears. Experimental stem removal provides a good approximation of ungulate herbivory (Juenger and Bergelson, 2000) that could occur several months after the extreme-cold event. We tracked seedling survival for 8 wk following the application of our stem removal treatment (17 October 2016 to 12 December 2016). We modeled the response of seedling survival as a function of temperature, stem removal, and a temperature \times stem removal interaction using a binomial distribution. Due to large differences in the variance of survival among species, each species was modelled separately, but pooling species for this analysis did not qualitatively change our conclusions. Because *P. resinosa* survival was affected by a significant temperature \times stem removal interaction (see Results), we report pairwise comparisons of seedling survival least-square means between stem removal treatment

levels within each temperature treatment, computed from the contrasts calculated in the *lsmeans* package in R (Lenth, 2016). All test statistics and *P* values represent ANOVA results calculated in R (R Core Team, 2017).

RESULTS

Our late-winter extreme-cold treatment resulted in lower minimum soil temperatures that were frozen for a longer duration of time. Minimum soil temperatures during the simulated extreme-cold event were below freezing in the -16°C treatment ($-0.12 \pm 0.09^{\circ}\text{C}$, least-squares mean \pm SE), not significantly different from freezing in the -8°C treatment ($0.06 \pm 0.09^{\circ}\text{C}$), and well above freezing in the 8°C control ($7.01 \pm 0.09^{\circ}\text{C}$; $F_{2,3} = 1922.4$, $P < 0.001$). In the -16°C treatment, soils were frozen four times longer (20.0 ± 2.9 h) compared to the -8°C treatment (5.0 ± 2.9 h; $F_{2,3} = 12.5$, $P = 0.03$), while soil in the 8°C treatment never froze.

Extreme cold increased root damage in all four species. Averaged across all species, root PEL was 17% greater in seedlings exposed to the -16°C treatment (0.41 ± 0.02), compared to the intermediate -8°C treatment (0.34 ± 0.02) and 8°C control (0.35 ± 0.03), representing a weak but marginally significant difference ($F_{2,23} = 2.51$, $P = 0.09$). Root PEL also differed among species ($F_{3,23} = 6.96$, $P < 0.001$, Appendix S4) and decreased with stem height ($F_{1,23} = 5.54$, $P = 0.03$), but the response of root PEL to temperature was consistent across all species (temperature \times species interaction: $F_{6,23} = 1.08$, $P = 0.40$). Stem and leaf tissue were not injured by extreme cold (Appendix S4). Seedling survival differed across species ($F_{3,111} = 5.59$, $P = 0.001$, Appendix S4), with survival ranging from 0.70 ± 0.09 (*A. balsamea*) to 0.96 ± 0.03 (*P. strobus*), but there was no effect of extreme cold on seedling survival ($F_{2,111} = 1.88$, $P = 0.16$, Fig. 1A), regardless of species (temperature \times species interaction: $F_{3,111} = 1.07$, $P = 0.38$).

While extreme-cold temperatures had little direct effect on seedling survival, extreme-cold temperatures decreased relative stem growth rate ($F_{2,98} = 8.25$, $P < 0.001$, Fig. 1B). Averaged across

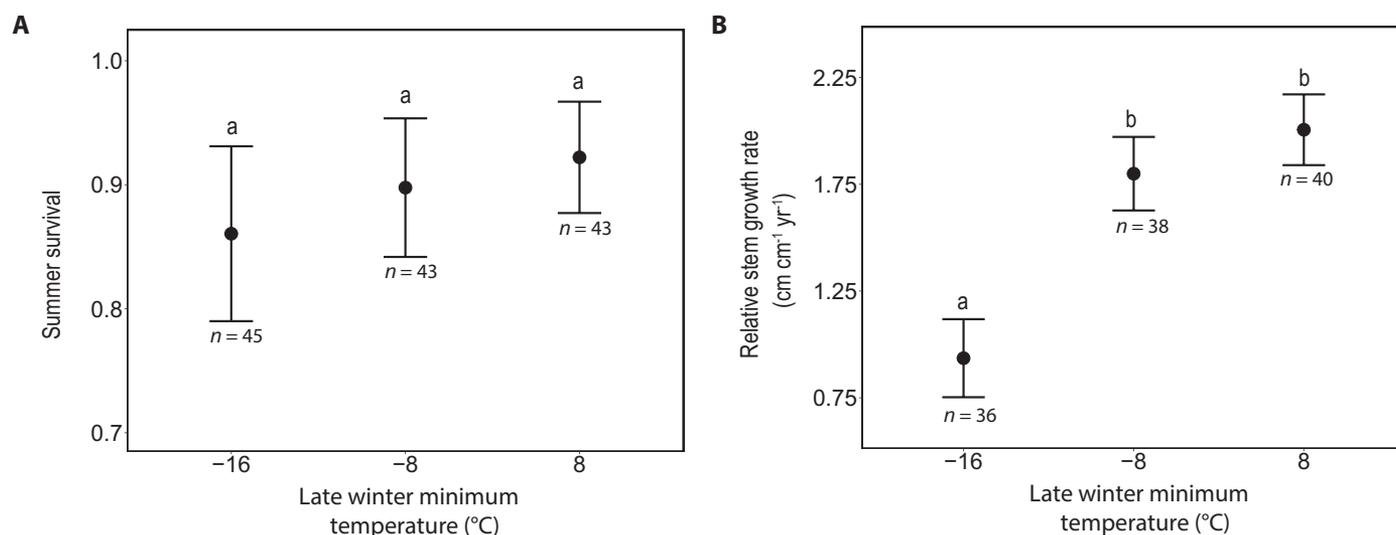


FIGURE 1. (A) Seedling survival averaged across four species (*Abies balsamea*, *Pinus resinosa*, *Pinus strobus*, *Quercus rubra*) showed no consistent response to temperature treatment ($F_{2,111} = 1.88$, $P = 0.16$). (B) Stem relative growth rate averaged across the same species decreased in the coldest temperature treatment in surviving seedlings ($F_{2,98} = 8.25$, $P < 0.001$). Points represent least-squares means and error bars represent one standard error. Treatment groups with different letters are significantly different ($P < 0.05$).

all species, relative stem growth rate in the coldest treatment (-16°C , $0.94 \pm 0.18 \text{ cm cm}^{-1} \text{ yr}^{-1}$) was approximately half of the relative growth rate observed in the intermediate cold treatment (-8°C , $1.80 \pm 0.17 \text{ cm cm}^{-1} \text{ yr}^{-1}$) and the 8°C control ($2.01 \pm 0.18 \text{ cm cm}^{-1} \text{ yr}^{-1}$). Species also differed in their mean relative stem growth rate ($F_{3,98} = 12.22$, $P < 0.001$, Appendix S4), but there was no significant species \times temperature interaction ($F_{6,98} = 1.11$, $P = 0.36$).

Extreme cold also influenced the effect of growing-season tissue loss on *P. resinosa*, but not *Q. rubra* seedlings. *Pinus resinosa* survival was lowest in seedlings that experienced both extreme-cold temperatures and heavy stem removal (temperature \times stem loss: $F_{2,25} = 3.26$, $P = 0.05$, Fig. 2A). *Pinus resinosa* survival was more than 10 times greater in the 25% stem removal treatment (0.92 ± 0.12) relative to the 75% stem removal treatment (0.08 ± 0.12) in seedlings that experienced the -16°C extreme-cold event (least-square means pairwise comparison: $z = 2.10$, $P = 0.03$). *Pinus resinosa* survival did not differ between herbivory treatments in either the -8°C treatment ($z = 0.80$, $P = 0.43$) or 8°C control ($z = 1.46$, $P = 0.15$). There was also a strong main effect of stem removal on *P. resinosa* survival ($F_{1,25} = 28.39$, $P < 0.0001$), as mean seedling survival in the 25% stem removal treatment (0.91 ± 0.08) was more than twice as great compared to survival in the 75% stem removal treatment (0.34 ± 0.16). Temperature also influenced *P. resinosa* survival (temperature: $F_{2,25} = 3.72$, $P = 0.04$) because mean *P. resinosa* survival was slightly lower in the -16°C treatment (0.72 ± 0.17) than the -8°C treatment (0.87 ± 0.09 , pairwise comparison: $z = 2.51$, $P = 0.05$), but not significantly different from the 8°C control (0.76 ± 0.12 , pairwise comparison: $z = 1.32$, $P = 0.40$). There was no significant difference between *P. resinosa* survival in the -8°C treatment and 8°C control (pairwise comparison: $z = 1.27$, $P = 0.42$). *Quercus rubra* seedlings showed no response to temperature ($F_{2,16} = 0.96$, $P = 0.41$), stem removal ($F_{1,16} = 1.79$, $P = 0.20$), or a temperature \times stem removal interaction ($F_{2,16} = 1.61$, $P = 0.23$).

DISCUSSION

Winter climate change has the potential to strongly affect northern temperate plant communities (Kreyling, 2010) and may increase the frequency of extreme cold damage (Gu et al., 2008; Augspurger, 2013). Although the effects of late-winter extreme-cold events remain relatively unexplored (Jentsch et al., 2007), our experiment suggests that transient periods of extreme cold can have important delayed effects on seedling growth and responses to herbivory long after the initial injury occurs. We found evidence that extreme-cold events can decrease seedling growth (Fig. 1B), even in the absence of direct lethal effects. Our study also showed that extreme-cold events can amplify the negative effect of stem removal on seedling survival, but this response was only observed with one species in the coldest temperature treatment (Fig. 2A). These results suggest that extreme-cold temperatures might directly alter patterns of tree growth and increase the negative effects of herbivory in some species, but further research will be needed to understand the generality of these effects.

Extreme-cold temperatures reduce seedling growth, but not survival

Extreme events may limit plant recruitment by decreasing survival (Royer et al., 2011), but extreme cold did not decrease short-term seedling survival in this study (Fig. 1A). Although our data suggest that seedlings of these temperate woody species may have the capacity to withstand at least a single extreme-cold event, our results also reveal that a single extreme-cold event was sufficient to decrease seedling growth. A possible interpretation of these findings is that failing to consider the delayed, yet significant, effects of previous extreme events could result in overly optimistic estimates of seedling recruitment in habitats where late-winter temperature variation is common.

Decreased growth could be caused by injuries to root, stem, or bud tissue (Burr et al., 1990; Kreyling et al., 2012; Basler and

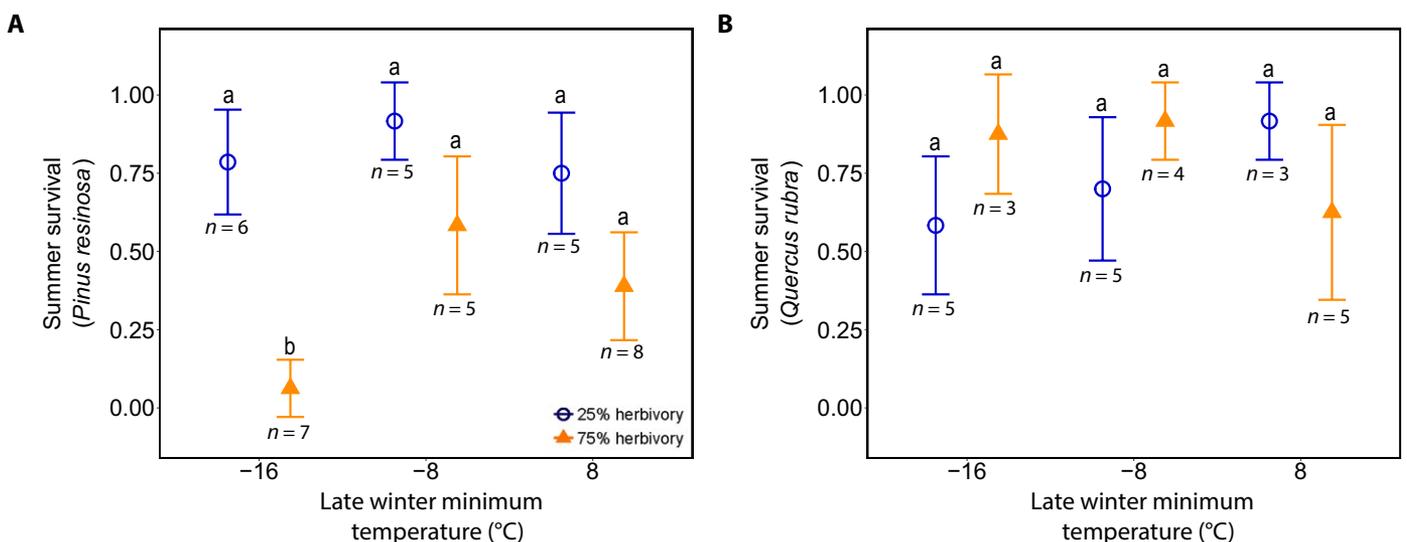


FIGURE 2. Seedling survival as a function of an extreme-cold event (minimum temperature: 8°C control, -8°C , and -16°C) and herbivory (blue circles: 25% of new growth removed, orange triangles: 75% of new growth removed). The response of two species, (A) *Pinus resinosa* and (B) *Quercus rubra*, were modeled separately. Points represent least-squares means and error bars represent one standard error. Stem removal treatment groups with different letters within a temperature treatment are significantly different ($P < 0.05$).

Körner, 2014; Vitasse et al., 2014b). While our data suggest that root damage was also greatest in the -16°C treatment, soil freezing was relatively mild in this treatment (mean temperature: $-0.12 \pm 0.09^{\circ}\text{C}$), which is likely not cold enough to explain this pattern in root damage (Schaberg et al., 2008). It is similarly unlikely that stem damage caused patterns of stem growth rate, given the lack of stem PEL response to extreme-cold temperatures (Appendix S4) and the typically high cold-hardiness of seedling stems (Burr et al., 1990). Therefore, it seems plausible that the effect of extreme-cold on growth may have resulted from bud damage. While we did not measure the response of buds in this experiment, seedling bud tissue experiences much colder air temperatures than roots, and bud damage can delay or inhibit seedling growth during summer (Basler and Körner, 2014; Vitasse et al., 2014b). However, because seedlings only exhibited decreased growth in the most extreme cold treatment, bud damage may exhibit nonlinear responses to extreme cold (Fig. 1C; Smith, 2011). Our ability to identify species-specific threshold temperatures that may inhibit growth is limited by our experimental design, but future experiments could determine these critical temperatures by using more than three treatment levels as well as replicate climate-controlled rooms.

Extreme-cold events amplify negative effects seedling herbivory

Winter climate can have a strong influence on the ability of plants to withstand tissue loss in summer, including tissue lost to herbivores (Roy et al., 2004; Connolly et al., 2017). While stem removal had a strong negative effect on *P. resinosa* seedlings, this was driven by decreased survival in the -16°C treatment. As a result, seedlings experiencing both extreme cold and heavy stem loss had lower survival than expected from extreme cold alone (Fig. 2A; Niinemets, 2010). However, *Q. rubra* survival was not affected by extreme cold, stem removal, or their interaction (Fig. 2B). While both *P. resinosa* and *Q. rubra* are known to resprout following stem damage (Del Tredici, 2001), it is unclear from our results how many species may be affected by interactions between extreme cold and aboveground stem loss. Conducting similar experiments on a wider range of functional traits (e.g., resprouting ability; Del Tredici, 2001) and in the field across multiple forest habitats (e.g., closed versus open canopies; Canham et al., 1994) will help us identify scenarios in which extreme cold increases seedling tolerance of herbivory.

Extreme-cold events may also indirectly increase the negative effects of seedling herbivory by mediating seedling growth (Fig. 1B). Reduced growth rate can lead to reduced synthesis of defensive compounds such as tannins in temperate woody seedlings (Albrechtsen et al., 2004). Additionally, slower-growing seedlings may take longer to reach a size refuge from mammalian herbivores, lengthening the time in which herbivory may result in seedling mortality (Boege and Marquis, 2005). While we demonstrate that extreme-cold temperatures and herbivory can interact to shape seedling survival in a controlled environment, it will be critical to understand how interactions between extreme winter climatic events and herbivory vary in the field (Agrawal, 2011).

CONCLUSIONS

We showed that the effects of late-winter extreme events may not manifest until several months into the growing season. Specifically,

an experimental extreme-cold event reduced seedling growth in four northern-temperate tree species, despite not directly reducing survival. These results raise several interesting lines of future inquiry for plant–animal interactions. For example, while our work suggests that extreme events could play an important role the response of some species to seedling herbivory (Fig. 2A), future studies could also explore the response of herbivores to extreme-cold events. Arthropod herbivores often prefer to consume plants that recently experienced extreme drought (Gutbrodt et al., 2011), but it remains unknown whether extreme-cold events have similar effects on herbivore preference. Additionally, little is known about how mutualisms (e.g., plant–fungal interactions) might influence seedling responses to extreme cold. Laboratory studies demonstrate that arbuscular mycorrhizal fungi can increase plant tolerance of cold temperatures (Zhu et al., 2010), but this idea has not been tested extensively in the field. Developing a better appreciation for how extreme-cold temperatures alter seedling performance and interactions with other stressors during the growing season will likely improve our ability to anticipate future changes in the composition of northern forests.

ACKNOWLEDGEMENTS

We thank Trout Lake Field Station for exceptional field assistance during preliminary trials. This work was funded by an AFRI-NIFA Fellowship (Grant # 2014-02074) awarded to J.L.O. and B.M.C., and a Vilas Associates Fellowship awarded to J.L.O. P.W.G. was supported by a National Science Foundation IGERT award (grant no. DGE-1144752) and Doctoral Dissertation Improvement Grant (grant no. DEB-1701506). We also thank three anonymous reviewers who provided feedback on this manuscript.

AUTHOR CONTRIBUTIONS

P.W.G., B.M.C., and J.L.O. contributed to experimental design; P.W.G. and B.M.C. collected data; P.W.G. performed all analyses and led preparation of the manuscript; B.M.C. and J.L.O. helped revise the manuscript.

DATA ACCESSIBILITY

Data for seedling survival, growth, and browse tolerance (Guiden, 2018) are available on FigShare (<https://doi.org/10.6084/m9.figshare.7078232.v2>).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

LITERATURE CITED

- Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence. *Functional Ecology* 25: 420–432.
- Albrechtsen, B., R. H. Gardfjell, C. M. Orians, B. Murray, and R. S. Fritz. 2004. Slugs, willow seedlings and nutrient fertilization: intrinsic vigor inversely affects palatability. *Oikos* 105: 268–278.

- Augsburger, C. K. 2009. Spring 2007 warmth and frost: phenology, damage and refoliation in a temperate deciduous forest. *Functional Ecology* 23: 1031–1039.
- Augsburger, C. K. 2013. Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. *Ecology* 94: 41–50.
- Basler, D., and C. Körner. 2014. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiology* 34: 377–388.
- Boege, K., and R. J. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution* 20: 441–448.
- Burns, R. M., and B. H. Honkala. 1990. *Silvics of North America*, 2nd ed. U.S. Department of Agriculture, Forest Service, Washington, D.C., USA.
- Burr, K. E., R. W. Tinus, S. J. Wallner, and R. M. King. 1990. Comparison of three cold hardiness tests for conifer seedlings. *Tree Physiology* 6: 351–369.
- Canham, C. D., J. B. McAninch, and D. M. Wood. 1994. Effects of the frequency, timing, and intensity of simulated browsing on growth and mortality of tree seedlings. *Canadian Journal of Forest Research* 24: 817–825.
- Connolly, B. M., P. W. Guiden, and J. L. Orrock. 2017. Past freeze–thaw events on *Pinus* seeds increase seedling herbivory. *Ecosphere* 8: e01748.
- Del Tredici, P. 2001. Sprouting in temperate trees. *Botanical Review* 67: 121–140.
- Drescher, M., and S. C. Thomas. 2013. Snow cover manipulations alter survival of early life stages of cold-temperate tree species. *Oikos* 122: 541–554.
- Firth, D. 1993. Bias reduction of maximum likelihood estimates. *Biometrika* 80: 27–38.
- Fischelli, N., L. E. Frelich, and P. B. Reich. 2012. Sapling growth responses to warmer temperatures “cooled” by browse pressure. *Global Change Biology* 18: 3455–3463.
- Fischelli, N., A. Wright, K. Rice, A. Mau, C. Buschena, and P. B. Reich. 2014. First-year seedlings and climate change: species-specific responses of 15 North American tree species. *Oikos* 123: 1331–1340.
- Gibson, D. 2002. *Methods in comparative plant population ecology*. Oxford University Press, Oxford, UK.
- Gu, L., P. J. Hanson, W. MacPost, D. P. Kaiser, B. Yang, R. Nemani, S. G. Pallardy, et al. 2008. The 2007 eastern US spring freeze: increased cold damage in a warming world. *BioScience* 58: 253–262.
- Guiden, P. 2018. Seedling survival and growth. Figshare. Dataset. <https://doi.org/10.6084/m9.figshare.7078232.v2>
- Gutbrodt, B., K. Mody, and S. Dorn. 2011. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos* 120: 1732–1740.
- Haddad, N. M., D. Tilman, and J. M. H. Knops. 2002. Long-term oscillations in grassland productivity induced by drought. *Ecology Letters* 5: 110–120.
- Henry, H. A. L. 2007. Soil freeze–thaw cycle experiments: trends, methodological weaknesses and suggested improvements. *Soil Biology and Biochemistry* 39: 977–986.
- Hufkens, K., M. A. Friedl, T. F. Keenan, O. Sonnentag, A. Bailey, J. O’Keefe, and A. D. Richardson. 2012. Ecological impacts of a widespread frost event following early spring leaf-out. *Global Change Biology* 18: 2365–2377.
- Iverson, L. R., A. M. Prasad, S. N. Matthews, and M. Peters. 2008. Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *Forest Ecology Management* 254: 390–406.
- Jentsch, A., J. Kreyling, and C. Beierkuhnlein. 2007. A new generation of climate-change experiments: events, not trends. *Frontiers in Ecology and the Environment* 5: 365–374.
- Juenger, T., and J. Bergelson. 2000. The evolution of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*: herbivore-imposed natural selection and the quantitative genetics of tolerance. *Evolution* 54: 764–777.
- Kong, R. S., and H. A. L. Henry. 2016. Prior exposure to freezing stress enhances the survival and recovery of *Poa pratensis* exposed to severe drought. *American Journal of Botany* 103: 1890–1896.
- Kosmidis, I. 2017. brglm: Bias reduction in binary-response generalized linear models. Website <https://CRAN.R-project.org/package=brglm2>.
- Kreyling, J. 2010. Winter climate change: a critical factor for temperate vegetation performance. *Ecology* 91: 1939–1948.
- Kreyling, J., C. Beierkuhnlein, and A. Jentsch. 2010. Effects of soil freeze–thaw cycles differ between experimental plant communities. *Basic and Applied Ecology* 11: 65–75.
- Kreyling, J., D. Peršoh, S. Werner, M. Benzenberg, and J. Wöllecke. 2012. Short-term impacts of soil freeze–thaw cycles on roots and root-associated fungi of *Holcus lanatus* and *Calluna vulgaris*. *Plant and Soil* 353: 19–31.
- Lenth, R. V. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software* 69: 1–33.
- Niinemets, Ü. 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management* 260: 1623–1639.
- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website <https://www.R-project.org/>.
- Roy, B. A., S. Gusewell, and J. Harte. 2004. Response of plant pathogens and herbivores to a warming experiment. *Ecology* 85: 2570–2581.
- Royer, P. D., N. S. Cobb, M. J. Clifford, C.-Y. Huang, D. D. Breshears, H. D. Adams, and J. C. Villegas. 2011. Extreme climatic event-triggered overstorey vegetation loss increases understorey solar input regionally: primary and secondary ecological implications. *Journal of Ecology* 99: 714–723.
- Schaberg, P. G., P. E. Hennon, D. V. D’Amore, and G. J. Hawley. 2008. Influence of simulated snow cover on the cold tolerance and freezing injury of yellow-cedar seedlings. *Global Change Biology* 14: 1282–1293.
- Smith, M. D. 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *Journal of Ecology* 99: 656–663.
- Tessier, J. T. 2014. Reduced winter snowfall damages the structure and function of wintergreen ferns. *American Journal of Botany* 101: 965–969.
- Vitasse, Y., A. Lenz, G. Hoch, and C. Körner. 2014a. Earlier leaf-out rather than difference in freezing resistance puts juvenile trees at greater risk of damage than adult trees. *Journal of Ecology* 102: 981–988.
- Vitasse, Y., A. Lenz, and C. Körner. 2014b. The interaction between freezing tolerance and phenology in temperate deciduous trees. *Frontiers in Plant Science* 5: 1–12.
- Walter, J., A. Jentsch, C. Beierkuhnlein, and J. Kreyling. 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environmental and Experimental Botany* 94: 3–8.
- Williams, C. M., H. Henry, and B. Sinclair. 2014. Cold truths: how winter drives responses of terrestrial organisms to climate change. *Biological Reviews* 90: 214–235.
- Zhu, X. C., F. Bin Song, and H. W. Xu. 2010. Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. *Plant and Soil* 331: 129–137.