TESTING THE EFFECTS OF CLIMATE CHANGE ON THE EARLY DEVELOPMENTAL STAGES OF TREE SPECIES IN THE ACADIAN FOREST

by

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ABSTRACT

In this study, I experimentally simulated winter and summer climate change to determine the effects on seeds and seedlings of commercially and ecologically important tree species in the Acadian Forest Region. Objectives were to determine: 1) the effects of simulated winter warming on germination success of six Acadian Forest tree species with heated outdoor plots; and 2) the interactive effects of summer warming, elevated atmospheric carbon dioxide and reduced soil moisture on growth and survival of balsam fir (*Abies balsamea* (L.) Mill.), red spruce (*Picea rubens* Sarg.) and red maple (*Acer rubrum* L.) with chambers in a greenhouse. Winter warming did not significantly reduce germination success at the species level, but I observed high intraspecific variations in seedlot germination success and sensitivity to warming. Red maple displayed better survival and greater absolute growth under projected summer warming and drought, while red spruce exhibited some tolerance and balsam fir was the least adapted.

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Table of Contents

ABSTRACT ii
ACKNOWLEDGEMENTS iii
Table of Contents iv
List of Tables vi
List of Figures vii
List of Appendices ix
CHAPTER 1: GENERAL INTRODUCTION 1
1.1 Introduction 2
1.2 Objectives and hypotheses 4
1.3 Study description 5
1.4 Thesis structure 6
1.5 References 6
CHAPTER 2: SIMULATED WINTER WARMING HAS NEGLIGIBLE EFFECTS ON GERMINATION SUCCESS OF ACADIAN FOREST TREE SPECIES 11
2.1 Abstract 12
2.2 Introduction 13
2.3 Methods 16
2.3.1 Experimental design 16
2.3.2 Seed source and preparation 17
2.3.3 Plot design 18
2.3.4 Temperature manipulation 19
2.3.5 Seed germination 20
2.3.6 Statistical Analysis 21
2.4 Results 22
2.4.1 Site environmental conditions 22
2.4.2 Winter warming treatment effect and model comparison 24
2.5 Discussion 25
2.6 Conclusions 30
2.7 Acknowledgements 31
2.8 References 31
CHAPTER 3: EXPERIMENTAL WARMING OF ACADIAN FOREST TREE SEEDLINGS SUGGESTS DIVERGENT SPECIES RESPONSES TO 21st CENTURY CLIMATE CHANGE 46
3.1 Abstract 47
3.2 Introduction 49

3.3 Methods....51
3.3.1 Study species....51
3.3.2 Experimental design....52
3.3.3 Chamber design....53
3.3.4 Experimental factors....54
3.3.5 Seed source and seedling preparation....56
3.3.6 Seedling Growth and survival....57
3.3.7 Measurements of photosynthesis....57
3.3.8 Measurements of xylem water potential....58
3.3.9 Statistical analyses....58
3.4 Results....60
3.4.1 Seedling growth....60
3.4.2 Impacts of soil moisture on xylem water potential....63
3.4.3 Balsam fir photosynthesis and stomatal conductance....64
3.4.4 Seedling mortality....64
3.5 Discussion....65
3.5.1 Experimental effects of projected climate and CO_2 levels on growth....65
3.5.2 Effects of climate change on balsam fir photosynthesis and stomatal conductance....67
3.5.3 Seedling survival in a changing environment....68
3.6 Conclusions....69
3.7 Acknowledgements....70
3.8 References....70
CHAPTER 4: GENERAL DISCUSSION....91
4.1 Summary of results....92
4.2 Limitations and future research....95
4.3 Management implications....96
4.4 References....97
Appendix....100
Curriculum Vitae

List of Tables

Table 2.1. National Tree Seed Centre laboratory germination rates and the observed germination rates following control and heated treatments. Laboratory results were collected following the protocol for stratification (if necessary) and germination outlined in Bonner and Karrfalt (2008)……..…..38

Table 2.2. Comparisons between species' models with the random effect of plot $\pi plot$ and models with the additional random effect of seedlot ($\pi seedlot$). Small-sample-corrected Akaike's Information Criterion (AICc) were used to determine whether model fit significantly improved with the addition of the random effect of seedlot. The difference in criterion value with the best fit model is shown with ΔAICc (**0*** represents best fit model). Marginal and conditional R^2 values were calculated to quantify the variation explained by the fixed effect of treatment (T) and the additional variation accounted for by the random effects, respectively…….39

Table 3.1. Effects of temperature, CO_2, soil moisture treatments and their interactions on height growth, root-collar diameter growth, total biomass and root:shoot ratio of seedlings. Statistically significant terms ($p < 0.01$) are in bold. Three-way interaction terms that were not significant were removed from the model (NA)……82

List of Figures

Fig. 2.1. Seed plot layout and design: (a) - arrangement of the 24 seed bags within one seedbed and locations of the soil temperature and moisture sensors; (b) - the seedbed after the seed bags were covered with peat moss; (c) - the entire site where the eight plots were set up in two rows of four (the closest plot was not used) and the location of one of the ambient air temperature sensors; and (d) - location of the seedbed relative to the infrared heater and infrared temperature sensor. 40

Fig. 2.2. Winter temperature variation between ambient air temperature and (a) surface temperature of control and heated plots, and (b) seedbed temperature of control and heated plots. Technical difficulties with the network resulted in missing data on the following dates: December 28th to January 6th, January 8th, and January 24th to 26th. Red dotted line indicates the end of the programmed 0°C temperature limit. 41

Fig. 2.3. Mean (± 1 SE) snow depth of control and heated plots. Dashed and dotted lines indicate the 1st and 15th of each month, respectively. Measurements ceased after March 9 due to COVID-19 lockdown. 42

Fig. 2.4. Daily mean treatment maximum (a) and minimum (b) seedbed temperatures of control and heated plots. Technical difficulties with the network resulted in missing data on the following dates: December 28th to January 6th, January 8th, and January 24th to 26th. Red dotted line indicates the end of the programmed 0°C temperature limit..... 43

Fig. 2.5. Mean (± 1 SE) germination results for six species (BF = balsam fir, RS = red spruce, WP = white pine, RM = red maple, SM = sugar maple, YB = yellow birch) in control and heated plots. Each bar represents four replicate plots containing four seed bags (50 seeds per bag from one of four seedlots) totaling 800 seeds..... 44

Fig. 2.6. Mean (± 1 SE) germination results for four seedlots (different colors) of six species (BF = balsam fir, RS = red spruce, WP = white pine, RM = red maple, SM = sugar maple, YB = yellow birch) in control and heated plots. Each bar represents four replicate plots, with each plot containing 50 seeds of one seedlot, totaling 200 seeds. ... 45

Fig. 3.1. Map showing the Acadian Forest Region (Two Countries, One Forest 2014) and the distribution of balsam fir, red spruce and red maple in Canada and the United States (Little 1971)……...……83

Fig. 3.2. Average height growth, root-collar diameter growth, total biomass, and root:shoot ratio of all species in all treatment combinations. Each panel compares a response variable of one species to all combinations of chamber treatments. Colours represent CO_2 and soil moisture treatment combinations and error bars indicate standard error. Letter displays report the results of a Tukey test with a 99% confidence interval. Groups that share the same letters do not have a significant difference between means.

Pairwise comparisons were conducted between temperature treatments (red letters) and between all treatment combinations (black letters). 84

Fig. 3.3. Comparison of significant interactions between explanatory variables (temperature, CO_2 and soil moisture). Each panel demonstrates the effects of a significant two-way or three-way interaction on a response variable (average height growth, average diameter growth or average total biomass) for one species. Colours represent CO_2 and soil moisture treatments and error bars indicate standard error. Letter displays report the results of a Tukey test with a 99% confidence interval. Points that share the same letters do not have a significant difference between means. 86

Fig. 3.4. Boxplots showing the xylem water potential for seedlings growing in moderate (blue) and drought (red) soil moisture treatments. The horizontal lines within the boxes are the medians, and the box represents the upper and lower quartiles. Upper and lower whiskers extend to the most extreme observed points above and below the box that fall within 1.5 times the interquartile range, and all points that fall outside of the whiskers are outliers..... 88

Fig. 3.5. Mean ± 1 standard error net photosynthesis and stomatal conductance of three balsam fir seedlings from ambient and elevated CO_2 treatments for the baseline and +6°C temperature and drought and moderate soil moisture treatments. 89

Fig. 3.6. Mean ± 1 standard error mortality of balsam fir and red spruce seedlings for temperature, CO2, and soil moisture treatment combinations. Red maple seedlings did not exhibit mortality..... 90

List of Appendices

Fig. S1. Chamber ventilation design. Red arrows indicate the direction of airflow……100

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The early life stages of plant species are the most sensitive to fluctuations in environmental conditions and may greatly influence plant community dynamics under a changing climate (Johnstone et al. 2010, Kroel-Dulay et al. 2015). Climate change may affect key life history events of tree seedlings, including seed germination (Walck et al. 2011, Drescher and Thomas 2013, Solarik et al. 2016), early growth and establishment (McDowell et al. 2008, Will et al. 2013, Fisichelli et al. 2014). By the end of this century, the mean annual temperature of northeastern North America's Acadian Forest Region (Rowe 1972) is projected to increase by as much as 5.4°C, relative to the 1986 to 2005 average, under the high greenhouse gas emission scenario (Environment Canada 2019), which we are currently following (UNEP 2019, Voosen 2019). The Acadian Forest Region is an ecological transition zone between the boreal and temperate forests wherein lie the northern and southern climatic range limits of many local tree species. Over the course of the 21st century, some currently abundant boreal tree species in this region are projected to become less suited under projected climate change, while warm-adapted temperate tree species are expected to thrive (Bourque and Hassan 2008, Ashraf et al. 2015, Taylor et al. 2017). However, further research pertaining to the effects of climate change on the early ontogeny of these species is needed to address critical knowledge gaps (Kreyling 2010, Walck et al. 2011) and inform adaptive management strategies (Gauthier et al. 2014).

The early life stages of trees can be affected by warming during both the winter (Kreyling 2010) and summer (Will et al. 2013) seasons. With winter warming, snow cover period

and depth are projected to decrease (Houle et al. 2012). This may cause significant reductions in germination success of species, like sugar maple (*Acer saccharum* Marsh.), that produce dormant seeds with extensive cold stratification requirements (Drescher and Thomas 2013, Solarik et al. 2016), in comparison with tree species, like red spruce (*Picea rubens* Sarg.), that produce non-dormant seeds. Thus, fluctuations in winter conditions may preclude, delay or enhance regeneration from seed and, ultimately, affect seedling recruitment (Fay and Schultz 2009, Walck et al. 2011). Once germinated, seedlings will face a myriad of other challenges due to climate warming. While both boreal and temperate species have shown a positive growth response to warming (Fisichelli et al. 2014, Carles et al. 2015), rising temperature will also increase plant water use and mortality risk during drought (Will et al. 2013). However, given the projected two-fold increase in atmospheric carbon dioxide (CO_2; IPCC 2013), these effects may be counteracted to some degree by a CO_2-induced increase in water use efficiency (Brodribb et al. 2020). It is unclear how the interaction between warming and rising atmospheric CO_2 will affect seedling growth and survival of species in the Acadian Forest over the long term. While recent modelling studies (Bourque and Hassan 2008, Ashraf et al. 2015, Taylor et al. 2017) project an increase in the abundance of warm-adapted tree species, like red maple (*Acer rubrum* L.), and a reduction in cold-adapted boreal species, like balsam fir (*Abies balsamea* (L.) Mill.), these models lack empirical data on seedling growth and survival, which may undermine the accuracy of their predictions (Bugmann et al. 2001, Urban et al. 2016).

1.2 Objectives and hypotheses

The main purpose of this study was to determine the effects of projected climate change on early developmental stages of tree species in the Acadian Forest Region under the Representative Concentration Pathway (RCP; Van Vuuren et al. 2011) 8.5 radiative forcing scenario. The specific objectives were to:

1. Determine winter warming effects on germination success of species with varying levels of seed dormancy (Chapter 2); and
2. Determine the interactive effects of summer warming, elevated CO_2 and reduced soil moisture on growth and survival of balsam fir, red spruce and red maple seedlings (Chapter 3).

The hypotheses were:

1. Germination success of seeds that require long durations of cold stratification to break dormancy (balsam fir and sugar maple) will decrease with warming due to insufficient stratification period length (Chapter 2);
2. Seeds of species that lack dormancy or require only short periods of cold stratification will not be affected by warming because the conditions of warmer winters will remain sufficient for their release from dormancy and/or seed imbibition (Chapter 2);
3. Warming will increase growth of species due to increased photosynthetic activity (Chapter 3);

4. Low soil moisture will reduce growth because of hydraulic stress-induced decreases in stomatal conductance (Chapter 3);
5. Elevated CO_2 will increase growth and counteract the drought effect through enhanced photosynthetic activity and improved water use efficiency (Chapter 3); and
6. Red maple will respond more positively than balsam fir and red spruce to increasing temperature due to its more southerly range limit (Chapter 3).

1.3 Study description

In this project, I used two experimental approaches to determine the effects of winter warming on seed germination success and effects of summer warming on seedling growth and survival. In the first experiment, I used infrared heat to artificially warm outdoor seedbeds during the winter to 6.0°C above ambient temperature. Seeds of four parent trees (seedlots) of balsam fir, red spruce, white pine (*Pinus strobus* L.), red maple, sugar maple and yellow birch (*Betula alleghaniensis* Britton) were randomly placed in eight outdoor seedbeds during the winter of 2019-2020 from December 17th to April 15th, and germinants were counted after trial completion. In the second experiment, I constructed six fully automated chambers that controlled for temperature, CO_2 and soil moisture. Each chamber was split into two soil moisture treatments (moderate and drought) and randomly assigned one of three temperature treatments (baseline, +3°C, and +6°C) and one of two CO_2 treatments (ambient and 900 ppm). Historical temperature data for Fredericton, New Brunswick, Canada were used to create the baseline temperature treatment, and the +3°C and +6°C treatments were consistently 3.0°C and

6.0°C above baseline temperature. Seedlings of balsam fir, red spruce and red maple were randomly placed within the chambers on June 4th, and the experiment ran until October 1st (August 21st for red maple). During the trial, balsam fir photosynthesis and stomatal conductance were measured, and xylem water potentials were measured for all species. Additionally, height growth, diameter growth, total biomass, root:shoot ratio and mortality rates were recorded after trial completion.

1.4 Thesis structure

This thesis comprises four chapters. Following this Introduction, Chapter 2, *Simulated winter warming has negligible effects on germination success of Acadian Forest tree species*, analyzes the effects of winter warming, under the RCP 8.5 forcing scenario, on the germination success of six Acadian Forest species with varying levels of seed dormancy. This chapter has been submitted to the *Canadian Journal of Forest Research*. Chapter 3, *Experimental warming of Acadian Forest tree seedlings suggests divergent species responses to 21st century climate change*, examines the interactive effects of warming, elevated CO_2 and reduced soil moisture on the growth and survival of balsam fir, red spruce and red maple in the Acadian Forest. This chapter has been submitted to the *Canadian Journal of Forest Research*. Chapter 4 summarizes the results of each study and discusses limitations, possible future work and management implications.

1.5 References

Ashraf, M.I., Bourque, C.P-A., MacLean, D.A., Erdle, T., and Meng, F-R. 2015. Estimation of potential impacts of climate change on growth and yield of temperate tree species. Mitig. Adapt. Strat. Glob. Change 20:159-178.

Bourque, C.P-A., and Hassan, Q.K. 2008. Projected impacts of climate change on species distribution in the Acadian Forest region of eastern Nova Scotia. For. Chron. 84:553-557.

Brodribb, T.J., Powers, J., Cochard, H., and Choat, B. 2020. Hanging by a thread? Forests and drought. Science 368:261-266.

Bugmann, H.K.M., Wullschleger, S.D., Price, D.T., Ogle, K., Clark, D.F., and Solomon, A.M. 2001. Comparing the performance of forest gap models in North America. Clim. Change 51:349-388.

Carles, S., Groulx, D.B., Lamhamedi, M.S., Rainville, A., Beaulieu, J., Bernier, P., Bousquet, J., Deblois, J., and Margolis, H.A. 2015. Family variation in the morphology and physiology of white spruce (*Picea glauca*) seedlings in response to elevated CO_2 and temperature. J. Sustain. Forest. 34:169-198.

Drescher, M., and Thomas, S.C. 2013. Snow cover manipulations alter survival of early life stages of cold-temperate tree species. Oikos 122:541-554.

Environment Canada 2019. Canadian Climate Data and Scenarios [online]. Available from http://climate-scenarios.canada.ca/index.php?page=main [accessed 28 May 2020].

Fay, P.A., and Schultz, M.J. 2009. Germination, survival, and growth of grass and forb seedlings: Effects of soil moisture variability. Acta Oecol. 35:679-684.

Fisichelli, N., Wright, A., Rice, K., Mau, A., Buschena, C., and Reich, P.B. 2014. First-year seedlings and climate change: species-specific responses of 15 North American tree species. Oikos 123:1331-1340.

Gauthier, S., Bernier, P., Burton, P.J., Edwards, J., Isaac, K., Isabel, N., Jayen, K., Le Goff, H. and Nelson, E.A. 2014. Climate change vulnerability and adaptation in the managed Canadian boreal forest. Environ. Rev. 22:256-285.

Houle, D., Bouffard, A., Duchesne, L., Logan, T., and Harvey, R. 2012. Projections of future soil temperature and water content for three southern Quebec forested sites. J. Clim. 25:7690-7701.

IPCC 2013. Climate change 2013: the physical science basis. In: Stocker, T.F., Qin, D., Plattner, G-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgle, P.M. (Editors) *Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Johnstone, J.F., McIntire, E.J.B., Pedersen, E.J., King, G., and Pisaric, M.J.F. 2010. A sensitive slope: estimating landscape patterns of forest resilience in a changing climate. Ecosphere 1:14.

Kreyling, J. 2010. Winter climate change: a critical factor for temperate vegetation performance. Ecology 91:1939-1948.

Kroel-Dulay, G., Ransijn, J., Schmidt, I.K., Beier, C., De Angelis, P., de Dato, G., Dukes, J.S., Emmett, B., Estiarte, M., Garadnai, J., Kongstad, J., Kovacs-Lang, E., Larsen, K.S., Liberati, D., Ogaya, R., Riis-Nielsen, T., Smith, A.R., Sowerby, A., Tietema, A., and Penuelas, J. 2015. Increased sensitivity to climate change in disturbed ecosystems. Nat. Commun. 6:6682.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., and Yepez, E.A. 2008. Mechanisms of

plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol. 178:719-739.

Rowe, J.S. 1972. Forest regions of Canada. Natural Resources Canada, Canadian Forest Service, Ottawa, Canada.

Solarik, K.A., Gravel, D., Ameztegui, A., Bergeron, Y., and Messier, C. 2016. Assessing tree germination resilience to global warming: a manipulative experiment using sugar maple (*Acer saccharum*). Seed Sci. Res. 26:153-164.

Taylor, A.R., Boulanger, Y., Price, D.T., Cyr, D., McGarrigle, E., Rammer, W., and Kershaw, J.A. 2017. Rapid 21st century climate change projected to shift composition and growth of Canada's Acadian Forest Region. For. Ecol. Manag. 405:284-294.

UNEP 2019. Emissions Gap Report 2019. United Nations Environment Programme, Nairobi.

Urban, M.C., Bocedi, G., Hendry, A.P., Mihoub, J.B., Pe'er, G., Singer, A., Bridle, J.R., Crozier, L.G., De Meester, L., Godsoe, W., Gonzalez, A., Hellmann, J.J., Holt, R.D., Huth, A., Johst, K., Krug, C.B., Leadley, P.W., Palmer, S.C., Pantel, J.H., Schmitz, A., Zollner, P.A., and Travis, J.M. 2016. Improving the forecast for biodiversity under climate change. Science 353:aad8466.

van Vuuren, D.P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G., Kram, T., Krey, V., Lamarque, J-F., Masui, T., Meinshausen, M., Nakicenovic, N., Smith, S., and Rose, S. 2011. The representative concentration pathways: an overview. Clim. Change 109:5-31.

Voosen, P. 2019. New climate models forecast a warming surge. Science 364:222-223.

Walck, J.L., Hidayati, S.N., Dixon, K.W., Thompson, K., and Poschlod, P. 2011. Climate change and plant regeneration from seed. Glob. Change Biol. 17:2145-2161.

Will, R.E., Wilson, S.M., Zou, C.B., and Hennessey, T.C. 2013. Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest-grassland ecotone. New Phytol. 200:366-374.

CHAPTER 2: SIMULATED WINTER WARMING HAS NEGLIGIBLE EFFECTS ON GERMINATION SUCCESS OF ACADIAN FOREST TREE SPECIES

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Authors: Vaughn, W.R., Taylor, A.R., MacLean, D.A., and D'Orangeville, L.

2.1 Abstract

Dormant seeds that require long periods of cold stratification to become germinable may be most sensitive to increasing winter temperature caused by anthropogenic climate change. In this study, we used outdoor plots with infrared heaters to simulate the effects of projected winter warming (+6°C) for Canada's Acadian Forest Region and compared seed germination success of tree species with varying stratification requirements. We evaluated four seedlots per species of balsam fir (*Abies balsamea* (L.) Mill.), red spruce (*Picea rubens* Sarg.), white pine (*Pinus strobus* L.), red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marshall) and yellow birch (*Betula alleghaniensis* Britton). Three central findings emerged from this study: (1) none of the tested species were significantly affected by warming; (2) the random effect of seedlot explained more variation in germination success of deciduous species than it did for conifers; and (3) balsam fir seedlots exhibited considerable differences in their response to warming, implying intraspecific variation in depth of dormancy. These results suggest seed germination success of the tested tree species may not be impeded by their individual seed characteristics under the magnitude of winter warming projected over the coming century in our study area.

Keywords: seed dormancy, climate change, winter warming, germination, stratification, Acadian Forest, balsam fir, sugar maple

2.2 Introduction

Evaluating the effects of climate change on forest stand regeneration is of increasing importance to forest managers and stakeholders. In particular, the role of winter warming on successful tree seedling establishment has been suggested as an important potential mechanism by which climate change may influence stand succession (Campbell et al. 2005, Kreyling 2010, Taylor et al. 2020). In the Acadian Forest Region (Rowe 1972), part of North America's boreal-temperate ecotone, many constituent tree species lie at either the northern or southern limit of their geographical range. Relative to the 1986 to 2005 average, mean annual temperature of the Acadian Forest Region is predicted to rise by 4.2 to 5.4°C by the end of this century under Representative Concentration Pathway (RCP; van Vuuren et al. 2011) 8.5 (Environment Canada 2019a). Warming during the winter will reduce snow cover period and depth, and spring soil moisture (Houle et al. 2012), which could have significant impacts on ecological processes that control stand regeneration (Campbell et al. 2005). Specifically, the successful germination of some tree species requires a particular range of environmental conditions to relieve seed dormancy and become germinable (Walck et al. 2011). Thus, variations in these conditions may affect regeneration and establishment of these species.

The mechanisms that control seed dormancy enable a mature, viable seed to avoid germination at times when temperature and moisture conditions are not conducive to seedling survival (Baskin and Baskin 2004, Tielborger et al. 2012). Seeds are either non-

dormant (capable of germinating under favourable environmental conditions immediately after dispersal) or dormant due to embryonic or structural characteristics (Baskin and Baskin 1998, Walck et al. 2011). Physiological dormancy is a common type of embryonic dormancy (Baskin and Baskin 1998) that requires a specific combination of temperature and moisture over a period of time in order for seeds to become germinable: warm and moist conditions (warm stratification) or cool (0 to 10°C) and moist conditions (cold stratification) (Walck et al. 2011). Physiological dormancy can be described as deep (requires three to four months of cold stratification), intermediate (requires two to three months of cold stratification), or non-deep (requires short periods of either warm or cold stratification) (Baskin and Baskin 2004). Once seeds have been released from dormancy, they enter a quiescent state, in which they remain until exposed to specific environmental conditions that trigger germination (Baskin and Baskin 1998, Bonner and Karrfalt 2008). Dormancy and germination are distinctly different physiological processes (Fenner and Thompson 2005); therefore, climate change may affect them independently (Walck et al. 2011).

Warmer winters could significantly lower the germination success of species that require long periods of cold stratification for seeds to become non-dormant. For example, sugar maple (*Acer saccharum* Marshall), a common shade-tolerant species in the Acadian Forest Region and often found in remnant old growth stands (Mosseler et al. 2003), requires cold stratification for 40 to 90 days (Bonner and Karrfalt 2008) at temperatures slightly above freezing to release the seeds from dormancy (Godman et al. 1990). Solarik et al. (2016) observed substantial declines (up to 75%) in germination rates of sugar

maple under moderate (+5°C) and upper threshold (+7°C) warming scenarios. Similarly, balsam fir (*Abies balsamea* (L.) Mill.) requires 28 to 60 days of cold stratification to break dormancy and become germinable (Bonner and Karrfalt 2008). Conversely, species that produce seeds that lack dormancy, like red spruce (*Picea rubens* Sarg.) (Sullivan 1993), may be unaffected by winter warming. If winter temperatures in North America continue to rise as predicted by current climate change projections, tree species that require specific environmental conditions to release their seeds from dormancy may experience reduced germination success (Solarik et al. 2016). However, should these species possess a high degree of intraspecific variation in their dormancy requirements, the range of suitable winter conditions will increase, creating a variable pattern in the individual response to winter warming (Bolnick et al. 2011).

In addition to winter temperature, snow depth plays a key role in insulating soil from cold air during winter months. Sharratt et al. (1992) found snow depths of 25 cm or more resulted in a disconnect between air and soil temperatures. Consequently, winter soil temperature may decrease with less snow cover due to loss of insulation (Venalainen et al. 2001, Decker et al. 2003). Drescher and Thomas (2013) found that germination success declined with decreasing snow depth for balsam fir, red maple and sugar maple, while germination success of white pine (*Pinus strobus* L.) showed no significant change. Variations in snow regime will have other effects, including an increased frequency of freeze-thaw cycles of the upper soil layers (Decker et al. 2003, Mellander et al. 2007). This could cause some seeds to germinate early (if cold stratification requirement has

already been satisfied) during a period of warm weather and subsequently die once cold temperatures resume (Walck et al. 2011).

In this study, we conducted an outdoor experiment to test the effects of projected winter warming on the germination success of balsam fir, red spruce, white pine, red maple (*Acer rubrum* L.), sugar maple and yellow birch (*Betula alleghaniensis* Britton), all commercially and ecologically important tree species in the Acadian Forest Region that vary in their level of dormancy requirements. We hypothesized that (1) germination success of seeds that require long durations of cold stratification (balsam fir and sugar maple) will decrease with warming due to an insufficient duration of stratification; and (2) seeds of species that lack dormancy or require only short periods of cold stratification will not be affected by warming because the conditions of warmer winters will remain sufficient for their release from dormancy and/or seed imbibition.

2.3 Methods

2.3.1 Experimental design

Our study was located at the Canadian Forest Service's Atlantic Forestry Centre in Fredericton, New Brunswick, approximately in the middle of the Acadian Forest Region (45.96° N, 66.64° W). Eight outdoor seedbeds were constructed to compare seed germination success under current Fredericton climate conditions with RCP 8.5 winter climate projections for the end of the 21st century using climate data from the Coupled Model Intercomparison Project phase 5 (Environment Canada 2019a). Unless further mitigation efforts are initiated to reduce greenhouse gas emissions beyond those currently

in place, the RCP 8.5 high emission scenario is becoming increasingly likely (Pachauri et al. 2014, Foster et al. 2017, United Nations Environment Programme 2019, Voosen 2019). Mean annual temperature at Fredericton is 5.6°C and mean January and July temperatures are -9.4°C and 19.3°C, respectively. Mean annual snowfall is 252 cm and the average annual frost-free period is 130 days (Environment Canada 2019b).

Two treatment factors were tested in a fully crossed design: temperature (control and heated) and species (balsam fir, red spruce, white pine, red maple, sugar maple and yellow birch). Temperature was manipulated with infrared heaters to mimic the projected 6.0°C increase under RCP 8.5 (Environment Canada 2019a) for the Acadian Forest Region. The experiment had four replicates each of the control and heated plots and ran from 17 December 2019 to 15 April 2020. To help account for intraspecific variation, four seedlots of each of the six tree species were selected for the trial, with all seedlot locations within 75 km of the City of Fredericton to minimize variation in seedlot origin climate.

2.3.2 Seed source and preparation

Tree seeds from Canada's National Tree Seed Centre, which were dried and stored at a temperature of -20.0°C, were used for the experiment. Both Greenwood et al. (2008) and Liu and El-Kassaby (2015) found that dormancy and germination timing were not affected by cold storage, so for the purpose of this study, seeds from the National Tree Seed Centre were considered suitable. For all six species, eight replicates (four control and four heated) of 50 seeds of each of the four seedlots were counted and placed in mesh

bags (192 bags total; 24 for each plot). The bags were then placed into seedbeds measuring 60 cm wide by 60 cm long x 9 cm deep and buried in peat moss (Fig. 2.1a, b). Wire mesh covered the top and bottom of the seedbeds to prevent rodent predation. Results of previous, standardized germination tests conducted by the National Tree Seed Centre for the selected seedlots are displayed in Table 2.1. These germination rates ranged from 51.5 to 67.5% (balsam fir), 72.0 to 98.5% (red spruce), 90.0 to 100% (white pine), 74.0 to 96.0% (red maple), 56.0 to 65.0% (sugar maple) and 60.5 to 91.0% (yellow birch).

2.3.3 Plot design

The eight seedbeds were randomly placed in two rows of four plots in an outdoor space adjacent to the Atlantic Forestry Centre greenhouse (Fig. 2.1c). Each plot consisted of a seedbed placed on the ground under a 150 cm tall wooden stand that was used to mount the infrared heaters (heated plots only) and infrared temperature sensors (Fig. 2.1d). The infrared temperature sensors (MLX90614ESF-BCF-000-SP, Melexis, Ypres, Belgium) were mounted on the side of each stand, and ambient air temperature sensors (SEN0227, DFRobot, Shanghai, P. R. China) were mounted 180 cm above the ground on each heated plot stand (Fig. 2.1c). Seedbed temperature and moisture were measured throughout the trial with soil temperature (DS18B20, DFRobot, Shanghai, P. R. China) and moisture (SEN-13637, Sparkfun Electronics, Niwot, Colorado, USA) sensors that were located under the peat moss among the seed bags (Fig. 2.1a). Seedbed moisture was measured throughout the trial to ensure adequate levels were maintained for seed imbibition. Snow

was allowed to accumulate during the trial and the depth was measured throughout the trial with a measuring tape installed on the side of each stand.

2.3.4 Temperature manipulation

The heated treatment maintained a 6.0°C difference between control and heated plot surface temperatures. The temperature in each of the heated plots was maintained by an infrared heater that was cycled on and off with a relay. The relay was controlled by an Adafruit Feather Huzzah ESP 8266 microcontroller (Adafruit, New York, New York, USA), which measured the temperature difference between the heated plot surface temperature and the ambient air temperature every four seconds. Ambient air temperature was used as an estimate of control plot surface temperature as it was observed to be ±1°C of the actual surface temperature and was less sensitive to fluctuations in solar radiation.

The physical properties of snow posed a challenge when attempting to warm plot surfaces to temperatures above 0°C. For example, if the air temperature was -3°C, the heated plot infrared lamps would attempt to warm the plot surface to +3°C; however, if the plot was covered with snow, which has a maximum temperature of 0°C, the target temperature would not be reached until the snow melted enough to expose the seedbed. To prevent an unnatural rate of snowmelt during the coldest winter months, the microcontrollers were programmed to warm the heated plots to a maximum of 0°C from December 17th to March 15th. Climate data for the years 2075 to 2100 under RCP 8.5 indicates average winter low temperature during this period will be below 0°C, so we assumed that significant, prolonged snowmelt should not frequently occur during this

time. After March 15th, the microcontrollers were programmed to maintain a constant 6°C difference between the control and heated plots with no restrictions. This was done to mimic an early spring onset, which is predicted to occur under the RCP 8.5 scenario (Bush and Lemmen 2019).

2.3.5 Seed germination

The seed bags were extracted from all plots on April 15th when the plot areas were completely free of snow cover. The seeds were then removed from the bags and thoroughly rinsed with water. At this point, any seeds that had germinated during the stratification trial were counted. Radical protrusion through the seed coat was used as the indicator of successful germination (Baskin and Baskin 2004). The remaining seeds from each plot were then divided by species and placed in germination trays (six trays per plot) on Kimpacks moistened with 125 mL of distilled water. Each tray bottom received an additional 125 mL of distilled water to maintain high humidity during the germination trial. The filled trays were loaded into a germinator that maintained a temperature schedule of 20°C for 16 hours and 30°C for 8 hours over a period of 28 days (Bonner and Karrfalt 2008). The germinator consisted of a wood frame equipped with eight open shelves and covered with thin, translucent poly plastic. Temperature was maintained with a space heater controlled by an ITC-308 temperature controller (Inkbird, Shenzhen, P. R. China) and the air was constantly circulated with a fan. Each shelf contained all six trays of one plot, and trays were rotated every two days. Germinants were counted every two to four days for the first two weeks and once every seven days for last two weeks.

2.3.6 Statistical Analysis

To test our hypotheses regarding the effects of winter warming projected under RCP 8.5 on germination success, mixed effect analysis of variance (ANOVA) was conducted using the "lme4" package (Bates et al. 2017) of R (R Core Team 2020). Although tree species was found to be a highly significant factor, we instead chose to conduct separate, species-specific ANOVA models to help simplify interpretation using the following model structure:

$$Y = \beta_0 + \beta_1 T + \pi_{plot} + \pi_{seedlot} + \varepsilon$$

where Y is the proportion of germinated seeds per seed bag; T is the treatment (control or heated); π_{plot} is the random effect of plot; $\pi_{seedlot}$ is the random effect of seedlot; and ε is random error. All germination proportions were arcsine square-root transformed to stabilize variance (Sokal and Rohlf 1995). The "AICcmodavg" package (Mazerolle 2020) was used to calculate the small-sample-corrected Akaike's Information Criterion (AICc) of models with the random effect of plot, and models with the additional random effect of seedlot, using the restricted maximum likelihood estimation procedure. Marginal and conditional R^2 values were also calculated for each model using the "MuMIn" package (Barton 2020) to further evaluate model fit and the contribution of the random effect factors using the methods of Nakagawa and Schielzeth (2013). The marginal R^2 estimates the variation explained by the fixed effects only, while conditional R^2 includes the additional variation contributed by the random effects, which approximates the conventional R^2. Heteroscedasticity and normality of model residuals were visually

assessed by inspecting the model residuals versus fitted values and Normal Q-Q plots using the "ggResidPanel" package (Goode and Rey 2019), and statistically analyzed with the Shapiro-Wilk test.

2.4 Results

2.4.1 Site environmental conditions

The heat treatment resulted in plot surface and seedbed temperature differences between the control and heated plots (Fig. 2.2a, b). Between December 17th and April 15th, mean ambient air temperature was -3.4±6.4°C, while the control and heated surface temperatures averaged -3.7±6.0 and 1.6±5.9°C, respectively, representing a mean 5.3°C difference between treatments. In comparison, the average difference between seedbed temperatures of the control (-0.4±2.5°C) and heated (1.6±3.3°C) plots was reduced to 2.0°C. While the heated treatment was programmed to limit warming to a maximum of 0°C between December 17th and March 15th, additional warming from the sun during clear days caused the surface temperatures of the heated plots to rise above 0°C several times (Fig. 2.2a). After March 15th, the removal of the 0°C temperature maximum constraint (mimicking an early spring onset) resulted in an abrupt increase in daily mean heated plot surface temperature, as the infrared heaters were reprogrammed to maintain a constant 6°C difference (Fig. 2.2a). The daily mean ambient air temperature, which fluctuated between -17.1 and 9.0°C, closely tracked the daily mean surface temperature of the control plots (Fig. 2.2a). Daily mean surface temperatures of control and heated plots ranged from -16.4 to 7.8°C and -10.3 to 12.9°C, respectively, but daily mean

seedbed temperatures ranged from -7.0 to 6.9°C and -2.3 to 10.3°C, respectively. Therefore, there were clear differences in both the surface and seedbed temperatures between the two treatments.

Over the course of the trial, snow depths of the heated and control plots ranged from 0.0 to 18.3 cm (mean = 3.0 cm) and 0.0 to 35.3cm (mean = 17.5 cm), respectively (Fig. 2.3). Due to significantly less snow accumulation and a higher frequency of snowmelt events in the heated plots, heated plot daily mean seedbed temperature experienced greater fluctuation than control plots, especially from early February to late March (Fig. 2.2b). During this period, control plot snow depth was approximately 15 cm or greater (Fig. 2.3) and the daily mean maximum seedbed temperature remained at or below 0°C (Fig. 2.4a). In contrast, over the same period, the daily mean maximum seedbed temperature of the heated treatment was frequently above 0°C (Fig. 2.4a). However, there were only eight days between December 17th and March 15th that recorded maximum daily mean seedbed temperatures greater than 5°C for the heated plots. Data collected from the seedbed moisture sensors indicated that all seedbeds in both treatments were above 50% soil moisture for the duration of the trial.

In the winter of 2019-2020, mean monthly temperatures for December, January, February, March and April were -4.1, -6.9, -7.5, -1.6 and 3.5°C, respectively (data acquired from the Fredericton International Airport weather station located in Lincoln, New Brunswick, Canada at 45.87° N and 66.54° W). Historical mean monthly temperatures (2012 to 2019 data from the Fredericton International Airport weather station) were -5.0, -8.6, -8.1, -2.6 and 4.2°C for December, January, February, March and

April, respectively. Although temperatures during the experiment were slightly warmer than past winters, they were not unusual.

2.4.2 Winter warming treatment effect and model comparison

Overall, warming had no significant effect on germination success of the tested tree species (Fig. 2.5). Interestingly, some balsam fir seedlots displayed strong responses to the warming treatment: one of the balsam fir seedlots exhibited an 88.5% reduction in mean germination success while another seedlot increased by 37.7% (Table 2.1, Fig. 2.6b). Similar inconsistencies in the response to warming were also observed in seedlots of other species, however, none were as large. Interestingly, yellow birch seedlots exhibited very little change (no more than 5%) in germination success under warming.

Under the control treatments, 97.1 and 76.5% of the total germinated sugar maple and red maple seeds, respectively, had germinated prior to removing the seed bags from the plots on April 15th. For the heated treatments, these values increased to 99.0 and 88.8% for sugar maple and red maple, respectively. Many of the sugar maple seedlings that germinated early had completely emerged from the seed coats with fully developed cotyledons, which indicated that requirements for seed dormancy release had been met well before to the end of the trial. No other species had germinated prior to removing the seed bags from the control plots; however, under warming, all species exhibited some germination prior to removing the seed bags from the plots, but no more than 10% of the total germinated seeds.

The model that provided the best fit for germination success differed between coniferous and deciduous species. For coniferous balsam fir, red spruce and white pine, the models that contained the single random effect of plot produced the lowest AICc values (Table 2.2). Conversely, for deciduous red maple, sugar maple and yellow birch, the models that contained the additional random effect of seedlot produced the lowest AICc values (Table 2.2). Furthermore, models for deciduous species that contained both random effects produced much larger conditional R^2 values than models with the single random effect of plot, while the conditional R^2 values of models for coniferous species exhibited minimal change with the additional random effect (Table 2.2). This suggests that seedlot accounted for much more of the variation in germination rates for red maple, sugar maple and yellow birch than it did for balsam fir, red spruce and white pine. This is clearly shown in Fig. 2.6 with deciduous species exhibiting a lot of variation in seedlot germination rates in both treatments, while coniferous species produced minimal variation in both the control and heated (except balsam fir) treatments.

2.5 Discussion

The results of our study do not support our first hypothesis that germination success of seeds that require long durations of cold stratification (i.e., balsam fir and sugar maple) will decrease with warming, but rather suggest the magnitude of winter warming projected for the Acadian Forest Region under RCP 8.5 may not affect germination success. Although we applied a 6.0°C warming treatment, the heated plots maintained average seedbed temperatures between 0 and 5°C for approximately 90 days, which studies have shown to be adequate for breaking balsam fir and sugar maple seed

dormancy (Bonner and Karrfalt 2008, Solarik et al. 2016). Additionally, the seedbed moisture content that was observed (approximately 50% or greater) in this trial was sufficient, as past literature has found that stratification substrate moisture content of 24% or greater to be adequate for breaking physiological dormancy (Luo et al. 2020). Therefore, because seedbed temperature and moisture of the heated plots were within suitable ranges for stratification, a significant reduction in the germination success of the tested species with deep dormancy was not likely to occur. Moreover, when comparing the winter of 2019-2020 to the historical climate data, it was consistently, albeit slightly, warmer than the historical trend, which, presumably, should have increased the likelihood of observing a significant effect due to warming. The tested tree species that produce seeds that are either non-dormant or lack lengthy cold stratification requirements were unaffected by the warming treatment, which supports our second hypothesis.

Admittedly, some of the control germination rates of the tested species (i.e., balsam fir, red maple and sugar maple) fell below the laboratory germination rates that were observed at the National Tree Seed Centre (Table 2.1). However, the stratification environment of a field trial is substantially different from that of a laboratory setting in terms of environmental constancy (Milberg and Andersson 1998), and reduced germination rates of dormant seeds have been observed in past studies that stratified seeds in outdoor environments (Milberg and Andersson 1998, Solarik et al. 2018). In our experiment, the largest differences between the germination rates of laboratory-stratified and field-stratified seeds were observed with species that produce seeds with either intermediate or deep physiological dormancy (i.e., balsam fir, red maple and sugar

maple). This is not surprising as laboratory stratification protocols are specifically designed to optimize dormancy release by providing consistent and ideal temperature, moisture and humidity levels (Bonner and Karrfalt 2008). Therefore, we would expect these two stratification methods to result in different germination rates of seeds with physiological dormancy.

Results of our study differ from several past field studies that examined the effects of future reductions in snow depth (due to winter warming) on seed germination success. Drescher and Thomas (2013) performed a snow removal trial and found germination success of balsam fir, red maple and sugar maple was significantly reduced when snow accumulation was restricted. In their experiment, the temperature that the seeds experienced in the snow-free plots closely tracked ambient air temperature, as no warming treatment was applied. Similarly, Milbau et al. (2009) tested the effects of reduced snow depth on the germination success of subarctic tundra species by exposing them to a "thin snow cover" stratification treatment. This treatment simulated real soil temperature data that was recorded in wind-swept subarctic plots with thin snow cover, and they found that germination success of some herbaceous and shrub species was reduced under the "thin snow cover" treatment. Although we did not remove snow from the plots, the heated plots had significantly less snow accumulation than the controls (Fig. 2.3), however, no treatment effect was observed. Unlike past snow manipulation trials, our experiment simulated future winter warming by heating plot surfaces with infrared heat. This prevented the seedbed temperature of the heated plots from closely tracking current ambient air temperature when snow was absent and resulted in warmer seed

stratification temperatures than those observed by other studies. Previous winter warming studies that attempt to determine the effect of reduced snow depths do not generally account for concurrent increases in ambient air temperature with climate change, which may increase the likelihood of observing a treatment effect due to suboptimal stratification temperatures. For instance, Drescher and Thomas (2013) and Milbau et al. (2009) recorded and applied stratification temperatures that were equal to or below -5°C—well outside the effective temperature range for relieving physiological dormancy in seeds (Seeley 1997, Bonner and Karrfalt 2008). Thus, the poor germination success of dormant seeds observed in these studies is not unexpected.

While sugar maple germination success appeared to be marginally affected by the heated treatment, it was not as affected by winter warming as past studies have suggested. Solarik et al. (2016) observed a significant reduction in sugar maple germination success when seeds from Sherbrooke, Québec, Canada were stratified in a +5°C winter warming treatment. Although, the locations of sugar maple seedlots we selected were climatically similar to those from Sherbrooke, our heated treatment did not significantly decrease sugar maple germination success. The discrepancy between studies may be due to differences in stratification temperatures—the +5°C winter warming treatment that Solarik et al. (2016) applied had a constant incubation temperature of 8.9°C, while the average seedbed temperature of our heated treatment was only 1.6°C. Considering the optimal temperature for sugar maple seed dormancy release ranges between -1.0 to 5°C (Solarik et al. 2016), a reduction in sugar maple germination success would be expected following stratification at 8.9°C, while a 1.6°C stratification temperature is not likely to

have a negative effect. Admittedly, due to the programmed warming limit of 0°C between December 17th and March 15th, surface temperatures of the heated plots were occasionally less than 6.0°C above the control plots. This limitation may have caused the average seedbed temperature of the heated treatment to be slightly cooler than what a continuous 6.0°C difference would have produced. Nonetheless, because the average ambient air temperature was -3.4°C over the course of the trial, the heated treatment would not have resulted in mean seedbed temperatures of more than 5°C under a 6°C warming scenario. Moreover, with the majority of sugar maple seeds of both treatments having germinated one to two weeks prior to the end of the trial, it is evident that dormancy of sugar maple seeds begins to diminish early in the winter months when the average air temperature is at or below 0°C.

For the deciduous species models, a high conditional R^2 was produced when the random effect of seedlot was added, suggesting that germination success among seedlots varied substantially. Although the balsam fir model with the additional random effect of seedlot did not produce a high conditional R^2, the large variation in germination success among balsam fir seedlots in the heated treatment (relative to the other conifer species) suggests that these seedlots may differ in their sensitivity to warming. As the balsam fir seedlots were collected from trees within a 75-km radius, it is unlikely that they came from non-breeding populations (Shea and Furnier 2002). However, environmental conditions during seed maturation (maternal climate) can influence seed dormancy and germination characteristics (Walck et al. 2011, Penfield and MacGregor 2017). Low temperatures during seed development result in higher levels of seed dormancy, which require longer

periods of moist chilling to overcome (Rampart 2019). Some of the balsam fir seedlots that exhibited substantial declines under the heated treatment may have required longer periods of moist chilling due to this effect. In addition, a high degree of intraspecific variation in seedlot dormancy traits may have increased the range of acceptable climatic conditions for breaking seed dormancy (Bolnick et al. 2011). The impact of these factors, together, may have contributed to the differences in seedlot germination success that we observed in deciduous species and the variation in sensitivity to winter warming exhibited by balsam fir seedlots. However, having observed no significant reduction in germination success due to warming at the species level, the different characteristics that seeds of these species may possess will not likely preclude them from persisting under the level of climate warming projected for the Acadian Forest Region over the 21st century.

2.6 Conclusions

While snow depth is predicted to decrease with winter warming along with the insulative properties it provides, reductions in soil temperature and the consequent impact on seed germination success may not be as severe as past field studies suggest. Additionally, physiological dormancy of species that require long periods of cold stratification, like sugar maple, may still be overcome under the shorter winter seasons that are projected for the end of the 21st century in the Acadian Forest Region. The field of seed dormancy and germination research has made a lot of progress in recent years; however, the genetic mechanisms that signal seeds to remain dormant or initiate germination are not yet fully understood (Nonogaki 2017). Our study showed that under the high-emission, business-

as-usual climate change scenario, natural regeneration of balsam fir, red spruce, white pine, red maple, sugar maple and yellow birch in the Acadian Forest Region may not be impeded by their individual seed characteristics. However, with the random nature in which seedlot locations were selected, the potential magnitude of variation in seed sensitivity to warming resulting from different environmental conditions during seed development or population genetics was not able to be measured. Thus, further research regarding specific environmental and genetic factors that may determine seed germination success under warmer winters is warranted.

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2.8 References

Barton, B. 2020. Package 'MuMIn' [online]. Available from https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf [accessed 25 May 2020].

Baskin, C.C., and Baskin, J.M. 1998. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, CA, USA.

Baskin, J.M., and Baskin, C.C. 2004. A classification system of seed dormancy. Seed Sci. Res. 14: 1-16.

Bates, D., Maecler, M., Bolker, B., Walker, S., Christensen, R., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., and Fox, J. 2017. Package "lme4" [online]. Available from https://cran.r-project.org/web/packages/lme4/lme4.pdf [accessed 4 February 2020].

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C., and Vasseur, D.A. 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol. Evol. 26: 183-192.

Bonner, F.T., and Karrfalt, R.P. 2008. The woody plant seed manual. Agriculture Handbook 727. USDA Forest Service, Washington, DC, USA.

Bush, E., and Lemmen, D.S. (Editors) 2019. Canada's Changing Climate Report. Government of Canada. Ottawa, ON, Canada.

Campbell, J.L., Mitchell, M.J., Groffman, P.M., Christenson, L.M., and Hardy, J.P. 2005. Winter in northeastern North America: a critical period for ecological processes. Front. Ecol. Environ. 3: 314-322.

Decker, K.L.M., Wang, D., Waite, C., and Scherbatskoy, T. 2003. Snow removal and ambient air temperature effects on forest soil temperatures in Northern Vermont. Soil Sci. Soc. Am. J. 67: 1234-1242.

Drescher, M., and Thomas, S.C. 2013. Snow cover manipulations alter survival of early life stages of cold-temperate tree species. Oikos 122: 541-554.

Environment Canada. 2019a. Canadian Climate Data and Scenarios [online]. Available from http://climate-scenarios.canada.ca/index.php?page=main [accessed 28 May 2020].

Environment Canada. 2019b. Canadian climate normals 1981–2010 [online]. Available from http://www.climate.weatheroffice.ec.gc.ca/climate_normals/ [accessed 27 March 2020].

Fay, P.A., and Schultz, M.J. 2009. Germination, survival, and growth of grass and forb seedlings: Effects of soil moisture variability. Acta Oecol. 35: 679-684.

Fenner, M., and Thompson, K. 2005. The ecology of seeds. Cambridge University Press, Cambridge, UK.

Foster, G., Royer, D., and Lunt, D. 2017. Future climate forcing potentially without precedent in the last 420 million years. Nat. Commun. 8(14845).

Godman, R.M., Yawney, H.W., and Tubbs, C.H. 1990. Sugar maple. Silvics of North America. Vol. 2, Hardwoods. Burns, R.M., and Honkala, B.H. USDA Forest Service, Washington, DC, USA. pp. 78-91.

Goode, K., and Rey, K. 2019. ggResidpanel: Panels and Interactive Versions of Diagnostic Plots using 'ggplot2' [online]. Available from https://cran.r-project.org/web/packages/ggResidpanel/index.html [accessed 25 May 2020].

Greenwood, M.S., O'Brien, C.L., Schatz, J.D., Diggins, C.A., Day, M.E., Jacobson, G.L., White, A.S., and Wagner, R.G. 2008. Is early life cycle success a determinant of the abundance of red spruce and balsam fir? Can. J. For. Res. 38: 2295-2305.

Houle, D., Bouffard, A., Duchesne, L., Logan, T., and Harvey, R. 2012. Projections of future soil temperature and water content for three southern Quebec forested sites. J. Clim. 25: 7690-7701.

Kreyling, J. 2010. Winter climate change: a critical factor for temperate vegetation performance. Ecology 91: 1939-1948.

Liu, Y., and El-Kassaby, Y.A. 2015. Timing of seed germination correlated with temperature-based environmental conditions during seed development in conifers. Seed Sci. Res. 25: 29-45.

Loehle, C. 2000. Forest ecotone response to climate change: sensitivity to temperature response functional forms. Can. J. For. Res. 30: 1632-1645.

Luo, X.P., Yuan, Z., Bai, M.J., and Hu, X.W. 2020. Seed dormancy release of *Halenia elliptica* in response to stratification temperature, duration and soil moisture content. BMC Plant Biol. 20: 1-8.

Mazerolle, M.J. 2020. AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c) [online]. Available from https://cran.r-project.org/web/packages/AICcmodavg/index.html [accessed 22 July 2020].

Mellander, P.-E., Löfvenius, M.O., and Laudon, H. 2007. Climate change impact on snow and soil temperature in boreal scots pine stands. Clim. Change 85: 179-193.

Milbau, A., Graae, B., Shevtsova, A., and Nijs, I. 2009. Effects of a warmer climate on seed germination in the Subarctic. Ann. of Bot. 104: 287-296.

Milberg, P., and Andersson, L. 1998. Does cold stratification level out differences in seed germinability between populations? Plant Ecol. 134: 225-234.

Mosseler, A., Lynds, J.A., and Major, J.E. 2003. Old-growth forests of the Acadian Forest Region. Environ. Rev. 11: S47-S77.

Nakagawa, S., and Schielzeth, H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4: 133-142.

Nonogaki, H. 2017. Seed biology updates – highlights and new discoveries in seed dormancy and germination research. Front. Plant Sci. 8(524).

Pachauri, R.K., Meyer, L.A., and Core Writing Team (Editors) 2014. Climate Change 2014: Synthesis Report. IPCC, Geneva, Switzerland.

Penfield, S., and MacGregor, D.R. 2017. Effects of environmental variation during seed production on seed dormancy and germination. J. Exp. Bot. 68: 819-825.

R Core Team. 2020. R: A Language and Environment for Statistical Computing [online]. Available from https://www.R-project.org/ [accessed 22 May 2020].

Rampart, M. 2019. Seed germination variation among crop years from a *Pinus sylvestris* clonal seed orchard. Asian J. Agric. Res. 3: 1-14.

Rowe, J.S. 1972. Forest regions of Canada. Natural Resources Canada, Canadian Forest Service, Ottawa, Canada.

Seeley, S.D. 1997. Quantification of endodormancy in seeds of woody plants. HortScience 32: 615-617.

Sharratt, B.S., Baker, D.G., Wall, D.B., Skaggs, R.H., and Ruschy, D.L. 1992. Snow depth required for near steady-state soil temperatures. Agric. For. Meteorol. 57: 243-251.

Shea, K.L., and Furnier, G.R. 2002. Genetic variation and population structure in central and isolated populations of balsam fir, *Abies balsamea* (Pinaceae). Am. J. Bot. 89: 783-791.

Sokal, R., and Rohlf, F. 1995. Biometry: the principles and practice of statistics in biological research. W. H. Freeman, New York, NY, USA.

Solarik, K.A., Gravel, D., Ameztegui, A., Bergeron, Y., and Messier, C. 2016. Assessing tree germination resilience to global warming: a manipulative experiment using sugar maple (*Acer saccharum*). Seed Sci. Res. 26: 153-164.

Solarik, K.A., Messier, C., Ouimet, R., Bergeron, Y., and Gravel, D. 2018. Local adaptation of trees at the range margins impacts range shifts in the face of climate change. Glob. Ecol. and Biogeogr. 27: 1507-1519.

Sullivan, J. 1993. *Picea rubens* [online]. In: Fire Effects Information System. USDA, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer), Missoula, MT, USA. Available from http://www.fs.fed.us/database/feis/plants/tree/picrub/all.html (accessed 3 August 2018).

Taylor, A.R., Endicott, S., and Hennigar, C. 2020. Disentangling mechanisms of early succession following harvest: Implications for climate change adaptation in Canada's boreal-temperate forests. For. Ecol. Manage. 461(117926).

Tielborger, K., Petru, M., and Lampei, C. 2012. Bet-hedging germination in annual plants: a sound empirical test of the theoretical foundations. Oikos 121: 1860-1868.

United Nations Environment Programme (2019). Emissions Gap Report 2019. United Nations Environment Programme, Nairobi, Kenya.

Venalainen, A., Tuomenvirta, H., Heikinheimo, M., Kellomaki, S., Peltola, H., Strandman, H., and Vaisanen, H. 2001. Impact of climate change on soil frost under snow cover in a forested landscape. Clim. Res. 17: 63-72.

Voosen, P. 2019. New climate models forecast a warming surge. Science 364: 222-223.

van Vuuren, D., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G., Kram, T., Krey, V., Lamarque, J.-F., Masui, T., Meinshausen, M.,

Nakicenovic, N., Smith, S., and Rose, S. 2011. The representative concentration pathways: an overview. Clim. Change. 109: 5-31.

Walck, J.L., Hidayati, S.N., Dixon, K.W., Thompson, K., and Poschlod, P. 2011. Climate change and plant regeneration from seed. Glob. Change Biol. 17: 2145-2161.

Table 2.1. National Tree Seed Centre laboratory germination rates and the observed germination rates following control and heated treatments. Laboratory results were collected following the protocol for stratification (if necessary) and germination outlined in Bonner and Karrfalt (2008).

Species	Seedlot	Laboratory germination rate (%)	Germination rates following treatments (%)	
			Control	Heated
Abies balsamea	20021325	51.5	34.5	47.5
	20021327	66.5	30.0	14.5
	20021331	67.5	30.5	3.5
	20021332	61.0	46.5	36.0
Picea rubens	9610241	88.5	88.5	80.5
	9810257	72.0	74.5	91.5
	9810269	76.0	85.0	79.0
	20001207	98.5	98.5	90.0
Pinus strobus	20001173	90.0	95.5	96.5
	20001176	95.5	95.0	82.0
	20001177	100.0	98.0	99.0
	20001178	100.0	95.5	91.5
Acer rubrum	20021025	91.0	43.5	38.0
	20021026	80.0	28.5	26.0
	20021064	74.0	70.5	75.5
	20021067	96.0	72.0	74.0
Acer saccharum	20021132	62.0	54.0	50.5
	20021136	56.0	49.0	38.5
	20021144	57.0	41.5	33.0
	20081147	65.0	76.0	74.0
Betula alleghaniensis	20001104	60.5	71.0	69.0
	20021103	80.5	77.5	74.5
	20021124	91.0	80.5	79.5
	20021125	82.5	85.0	88.0

Table 2.2. Comparisons between species' models with the random effect of plot (π_{plot}) and models with the additional random effect of seedlot ($\pi_{seedlot}$). Small-sample-corrected Akaike's Information Criterion (AICc) were used to determine whether model fit significantly improved with the addition of the random effect of seedlot. The difference in criterion value with the best fit model is shown with ΔAICc (**0*** represents best fit model). Marginal and conditional R^2 values were calculated to quantify the variation explained by the fixed effect of treatment (T) and the additional variation accounted for by the random effects, respectively.

	$T + \pi_{plot}$			$T + \pi_{plot} + \pi_{seedlot}$		
Species	ΔAICc	R_M^2	R_C^2	ΔAICc	R_M^2	R_C^2
Balsam fir	**0***	0.04	0.06	2.33	0.04	0.16
Red spruce	**0***	0.00	0.12	3.30	0.00	0.14
White pine	**0***	0.02	0.13	1.17	0.01	0.31
Red maple	9.65	0.00	0.00	**0***	0.00	0.60
Sugar maple	16.81	0.02	0.19	**0***	0.02	0.78
Yellow birch	3.96	0.00	0.00	**0***	0.00	0.38



Fig. 2.1. Seed plot layout and design: (a) - arrangement of the 24 seed bags within one seedbed and locations of the soil temperature and moisture sensors; (b) - the seedbed after the seed bags were covered with peat moss; (c) - the entire site where the eight plots were set up in two rows of four (the closest plot was not used) and the location of one of the ambient air temperature sensors; and (d) - location of the seedbed relative to the infrared heater and infrared temperature sensor.

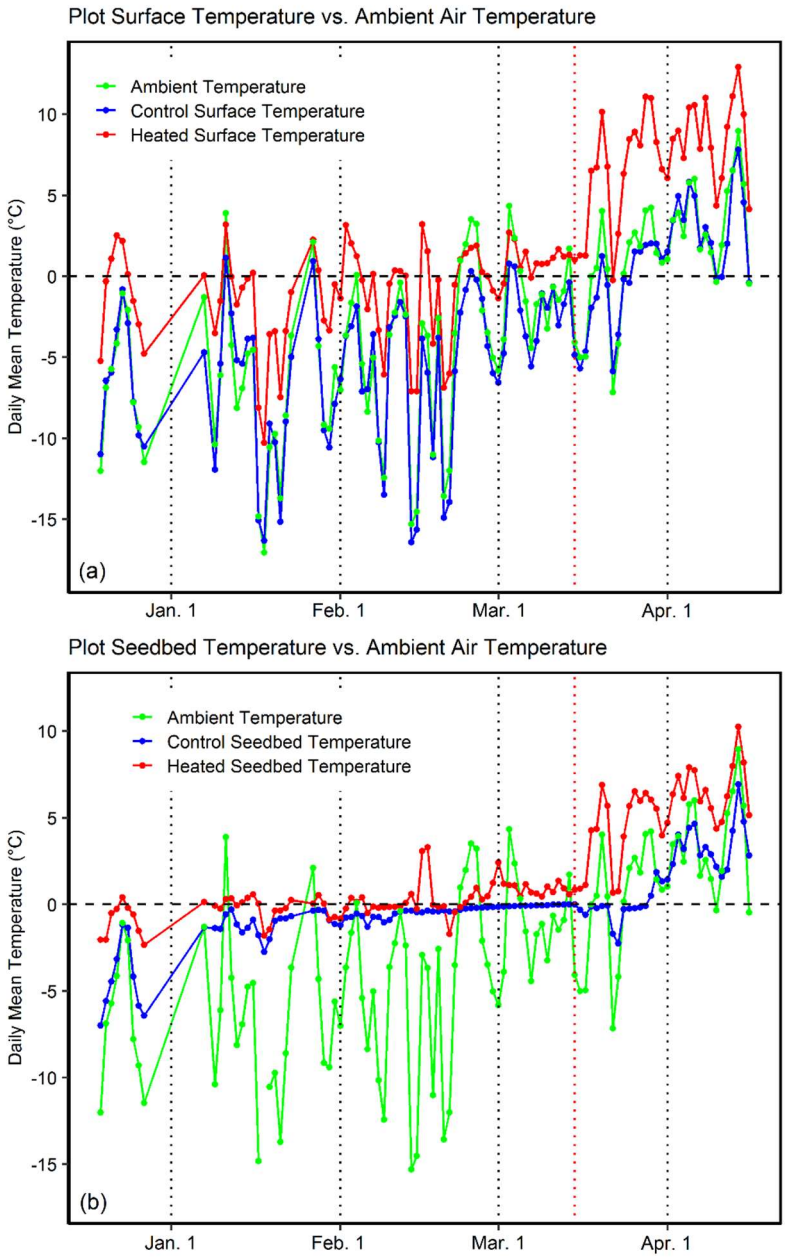


Fig. 2.2. Winter temperature variation between ambient air temperature and (a) surface temperature of control and heated plots, and (b) seedbed temperature of control and heated plots. Technical difficulties with the network resulted in missing data on the following dates: December 28th to January 6th, January 8th, and January 24th to 26th. Red dotted line indicates the end of the programmed 0°C temperature limit.

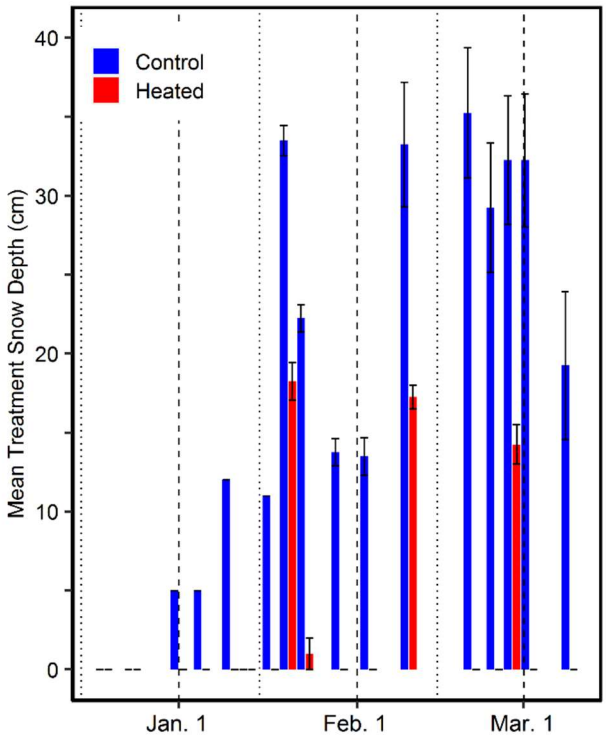


Fig. 2.3. Mean (± 1 SE) snow depth of control and heated plots. Dashed and dotted lines indicate the 1st and 15th of each month, respectively. Measurements ceased after March 9 due to COVID-19 lockdown.

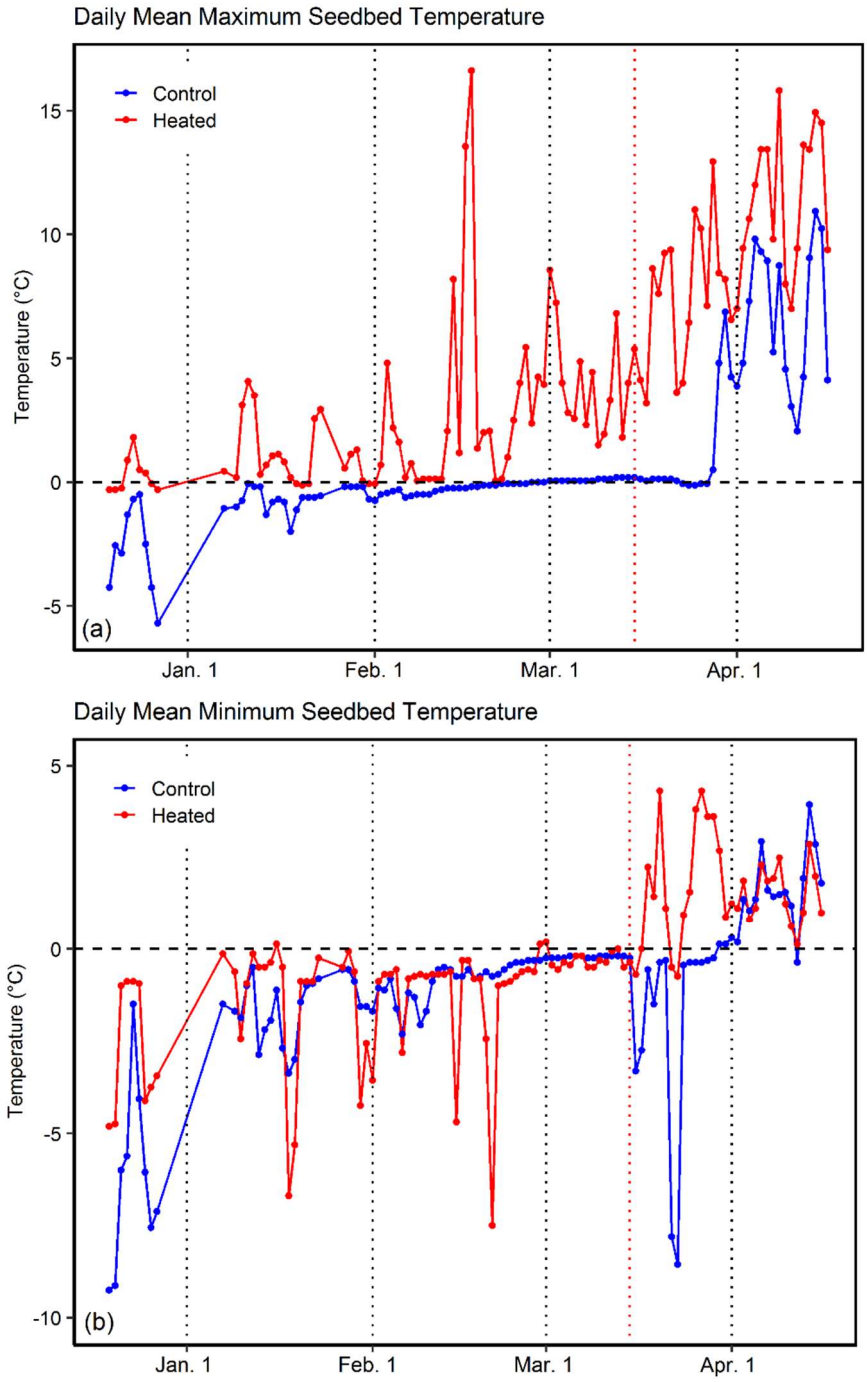


Fig. 2.4. Daily mean treatment maximum (a) and minimum (b) seedbed temperatures of control and heated plots. Technical difficulties with the network resulted in missing data on the following dates: December 28th to January 6th, January 8th, and January 24th to 26th. Red dotted line indicates the end of the programmed 0°C temperature limit.

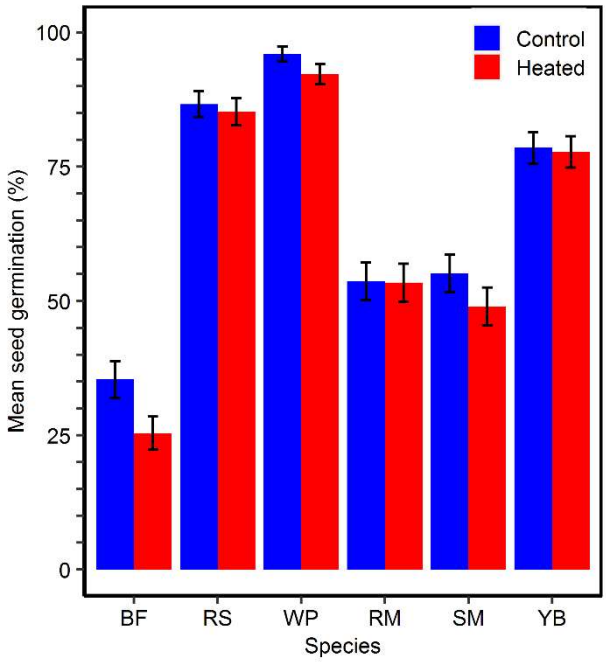


Fig. 2.5. Mean (± 1 SE) germination results for six species (BF = balsam fir, RS = red spruce, WP = white pine, RM = red maple, SM = sugar maple, YB = yellow birch) in control and heated plots. Each bar represents four replicate plots containing four seed bags (50 seeds per bag from one of four seedlots) totaling 800 seeds.

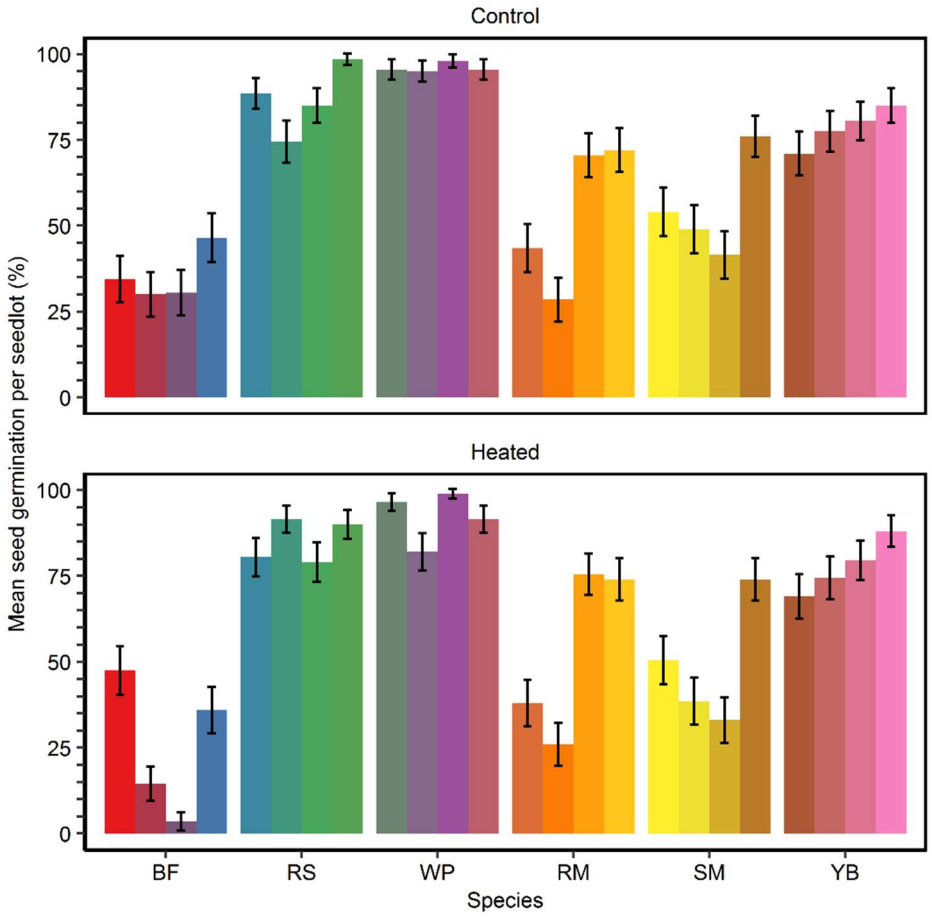


Fig. 2.6. Mean (± 1 SE) germination results for four seedlots (different colors) of six species (BF = balsam fir, RS = red spruce, WP = white pine, RM = red maple, SM = sugar maple, YB = yellow birch) in control and heated plots. Each bar represents four replicate plots, with each plot containing 50 seeds of one seedlot, totaling 200 seeds.

CHAPTER 3: EXPERIMENTAL WARMING OF ACADIAN FOREST TREE SEEDLINGS SUGGESTS DIVERGENT SPECIES RESPONSES TO 21st CENTURY CLIMATE CHANGE

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3.1 Abstract

The relative abundances of key commercial tree species in ecotones, like Canada's Acadian Forest Region, are projected to shift with climate change. However, models that predict these changes often lack empirically derived environmental thresholds of seedling growth and survival. In this study, we used chambers in a greenhouse to assess the growth and survival of 1-year-old balsam fir (*Abies balsamea* (L.) Mill.), red spruce (*Picea rubens* Sarg.) and red maple (*Acer rubrum* L.) seedlings in response to the interactive effects of warming, chronic drought, and elevated CO_2, as projected for the Acadian Forest.

In response to warming, only red spruce increased in height, however, this effect was influenced by CO_2 and soil moisture. Unexpectedly, warming did not improve red maple height growth; nonetheless it remained seven- to fifty-fold greater than that of conifers. Under drought, balsam fir biomass decreased by 24%, while red spruce biomass was unaffected, and red maple biomass decreased by 26% under +6°C warming. This decrease in red maple biomass under drought was accompanied by a 20.6% increase in root:shoot ratio, demonstrating trait plasticity in response to moisture stress. Overall, the drought effect was ameliorated under elevated CO_2 with seedlings exhibiting 23 to 31% increases in height growth. Red maple growth consistently improved under elevated CO_2, regardless of warming, while the positive effect of CO_2 was temperature-limited for conifer diameter growth. Balsam fir and red spruce mortality rates were seven and three percent in response to drought, and no mortality was observed for red maple. Balsam fir was the least adapted to projected warming and drought, while red spruce displayed some

positive responses to warming and was less affected by drought. Although red maple growth did not increase with warming, it displayed plasticity in response to drought and exhibited better growth and survival than the conifers.

Keywords: climate change, tree growth, drought, summer warming, carbon, balsam fir, red spruce, red maple.

3.2 Introduction

The early life stages of plant species are the most sensitive to environmental stress and may greatly influence forest community dynamics under a changing climate (Johnstone et al. 2010, Kroel-Dulay et al. 2015). By the end of this century, relative to the 1986 to 2005 average, northeastern North America's Acadian Forest Region (Rowe 1972) could experience a mean annual temperature increase of 4.2 to 5.4°C under the business-as-usual Representative Concentration Pathway (RCP; Van Vuuren et al. 2011) 8.5 (Environment Canada 2019a). The Acadian Forest lies within North America's boreal-temperate ecotone, an ecological transition zone where many local tree species are at their northern or southern climatic range limits. In particular, some currently abundant species such as balsam fir (*Abies balsamea* (L.) Mill.) and red spruce (*Picea rubens* Sarg.) are projected to become less suited to the local climate by the end of the 21st century, while warm-adapted temperate species with more southerly range limits, such as red maple (*Acer rubrum* L.), are expected to thrive (Bourque and Hassan 2008, Ashraf et al. 2015, Taylor et al. 2017). However, most forest simulation models used to predict future species' abundances under climate change lack empirically derived physiological limits of seedling growth and survival, undermining the accuracy of their predictions (Bugmann et al. 2001, Urban et al. 2016).

Atmospheric concentrations of carbon dioxide (CO_2) are projected to more than double, to 985 ± 97 ppm, by the year 2100 under the RCP 8.5 scenario (IPCC 2013). Free air CO_2 enrichment (FACE) experiments showed that increased levels of CO_2 can enhance tree growth and stem biomass (Ainsworth and Long 2005, Norby et al. 2005). Red spruce

seedlings grown under 713 CO_2 ppm were 40% larger in both size and weight compared to seedlings under ambient conditions (Samuelson and Seiler 1994). Similarly, in moist soil conditions, red maple seedlings grown in 700 ppm CO_2 produced 70% greater total biomass than seedlings exposed to 350 ppm CO_2 (Miao et al. 1992).

While both boreal and temperate tree species in the boreal-temperate ecotone have exhibited increased growth in response to warming (Fisichelli et al. 2014, Carles et al. 2015), rising temperature will also increase plant water use through its effect on vapour pressure deficit (VPD) (Will et al 2013). High VPD has been shown to decrease red spruce net photosynthesis and stomatal conductance by 26 and 43%, respectively (Day 2000), and increase tree seedling mortality risk during drought (Will et al. 2013). These effects may be counteracted to some degree by elevated CO_2, which has been found to increase water use efficiency (Brodribb et al. 2020) by reducing stomatal conductance (Long et al. 2004). It remains unclear how the complex interaction between increasing atmospheric CO_2 and temperature will affect seedlings of tree species in the Acadian Forest.

Compared to mature trees, young seedlings have smaller non-structural carbohydrate reserves (Hartmann et al. 2018), minimal surficial rooting depth and limited soil-to-leaf hydraulic conductance (McDowell et al. 2008). These characteristics make seedlings more susceptible to stress than mature trees when exposed to drought (Hartmann et al. 2018). Seedling mortality during drought can result from hydraulic failure when the xylem cavitates (develops air embolisms), carbon starvation, or an increase in harmful biotic agents resulting from drought conditions (McDowell et al. 2008). Plants will

attempt to avoid moisture stress through stomatal closure (McDowell et al. 2008) or, over the longer term, a reduction in shoot growth (Brunner et al. 2015). Reducing shoot growth increases the root:shoot ratio by reallocating resources to the roots and is an example of trait plasticity (Nicotra et al. 2010).

In this study, we used a controlled experiment with chambers in a greenhouse to determine the individual and interactive effects of temperature, CO_2 and soil moisture under current and projected climate for the Acadian Forest on seedling height, diameter, biomass and mortality of balsam fir, red spruce and red maple—three ecologically and commercially important tree species in the study area. We hypothesized that (1) warming will increase growth of species due to increased photosynthetic activity; (2) low soil moisture will reduce growth because of hydraulic stress-induced decreases in stomatal conductance; (3) elevated CO_2 will increase growth and counteract the drought effect through enhanced photosynthetic activity and improved water use efficiency; and (4) red maple will exhibit greater survival and relative growth than balsam fir and red spruce in response to increasing temperature due to its more southerly range limit (Tirmenstein 1991).

3.3 Methods

3.3.1 Study species

The tree species chosen to compare in our experiment coexist in the Acadian Forest, but their geographical ranges vary considerably throughout eastern North America (Fig. 3.1). Red spruce is predominantly found in Atlantic Canada and northeastern United States,

and grows in areas with cool, moist summers and cold winters (Sullivan 1993). Balsam fir and red maple, however, grow on a wider variety of soil types and have much larger distributions. Balsam fir grows in the Acadian Forest Region and Eastern Boreal Ecozone (Uchytil 1991), and red maple is found in the southern portion of the Eastern Boreal Ecozone, through the Acadian Forest Region and throughout the temperate forests of eastern North America, as far south as Florida (Tirmenstein 1991). Red maple has a superior capacity for indeterminant growth relative to the conifers (Abrams 1998), is moderately shade tolerant, and able to establish in a variety of successional stages, including pioneer and subclimax (Tirmenstein 1991). Balsam fir and red spruce are considered to be climax species and very shade tolerant (Uchytil 1991, Sullivan 1993).

3.3.2 Experimental design

Six chambers in a greenhouse were constructed to compare seedling growth and survival of balsam fir, red spruce and red maple under current and projected climate conditions for the city of Fredericton, New Brunswick (45.96° N, 66.64° W), located approximately at the geographical center of the Acadian Forest. Current mean annual temperature is 5.6°C and mean January and July temperatures are -9.4 and 19.3°C, respectively. Mean annual precipitation is 1077.7 mm and the average annual frost-free period is 130 days (Environment Canada 2019b). The environmental conditions of the six chambers included three temperature treatments (baseline, baseline +3°C, and baseline +6°C) × two CO_2 levels (ambient and elevated), with each chamber split into two soil moisture treatments (moderate and drought). Each of the 12 unique treatment combinations contained 135 seedlings of both balsam fir and red spruce, and 15 red maple seedlings.

Balsam fir and red spruce seedlings were grown in 45-cell trays (110 cm^3/cell), and red maple were grown in six-inch square pots. The disparity between coniferous and deciduous seedling quantities was necessary because of the large difference in first year growth and the limited space within each chamber. The trial ran from June 4th to Oct 1st, 2019; however, red maple seedlings were removed from the chambers on Aug 21st due to height restrictions. The chambers were exposed to ambient light during the entire duration of the trial.

3.3.3 Chamber design

The chambers were built in the Atlantic Forestry Centre greenhouse, located in Fredericton, New Brunswick, constructed with pressure treated lumber and covered with clear greenhouse plastic. The chambers were 1.0 m wide by 3.0 m long and 1.2 m high. The ventilation system (Appendix, Fig. S1) for each chamber consisted of an inline fan that drew air from a galvanized steel container (mixing box) with two inlets (fresh air and recirculated chamber air) and one outlet. The elevated CO_2 chambers had an additional inlet for CO_2 installed, which injected pure CO_2 directly into the outlet pipe. Fresh air entered through the opening in the back of the mixing box and mixed with CO_2 from a storage tank (for elevated CO_2 treatments only) and air that was drawn from the chamber through a corrugated recirculation pipe. The fan pulled the mixed air from the mixing box through the outlet pipe into a 4-inch, perforated manifold that was located along the entire length of one side of the chamber. Each chamber had a space heater for warming and a window air conditioner for cooling. The space heater was installed inside the mixing box and the air conditioner was installed on the side of the chamber.

3.3.4 Experimental factors

The baseline temperature treatment was determined by averaging the last three years (2016, 2017 and 2018) of weekly temperature data collected from the Fredericton International Airport weather station located in Lincoln, New Brunswick, Canada at 45.87° N and 66.54° W. The +3°C and +6°C treatments represent the projected growing season temperature for Fredericton for the periods of 2041 to 2060 and 2091 to 2100, respectively, under the RCP 8.5 forcing scenario, using climate data from the Coupled Model Intercomparison Project phase 5 (Environment Canada 2019a). The temperature in each chamber was controlled by a temperature controller (model ITC-310T-B, Inkbird, Shenzhen, P. R. China), which cycled the heater and air conditioner on and off based on a programmed daily temperature schedule consisting of ten diurnal temperature steps. The minimum temperature occurred between 4 and 6 a.m. and increased every two hours until it reached the maximum temperature at 12 p.m. The maximum temperature was held for 6 hours (12 p.m. to 6 p.m.) and then decreased every two hours until 4 a.m. the next morning. The baseline temperature schedule was changed weekly to match the average weekly minimum and maximum temperatures from the Fredericton International Airport weather station. The +3°C and +6°C temperature schedules constantly maintained temperatures that were 3.0°C and 6.0°C above baseline temperature. Mean summer temperatures for the baseline, +3°C and +6°C treatments were 19.6, 22.6 and 25.6°C, respectively. Mean summer temperature of each species' southern geographic range boundary was 19.1 ± 0.9°C, 19.6 ± 1.8°C and 26.7 ± 1.2°C for balsam fir, red spruce and red maple, respectively. Species' southern boundary temperature data were derived by

overlaying tree species distribution maps (Little 1971) over North American interpolated climate grids obtained from Natural Resources Canada (McKenney et al. 2011) and calculating the average mean summer temperature for 100 randomly located points within the southern 1% of each species range. Unless further mitigation efforts are initiated to reduce greenhouse gas emissions beyond those currently in place, the high (RCP 8.5) emission scenario is becoming increasingly more likely (IPCC 2014, Foster et al. 2017, UNEP 2019, Voosen 2019).

The ambient CO_2 treatment, which represented the current atmospheric CO_2 concentration, had no additional CO_2 added. The elevated treatment held the CO_2 concentration at 900 ppm, mimicking the projected CO_2 concentrations for the end of the century for RCP 8.5 (IPCC 2013). Carbon dioxide levels in each of the elevated CO_2 chambers were regulated by a solenoid valve that was controlled by an Adafruit Feather Huzzah ESP 8266 microcontroller (Adafruit, New York, New York, USA). The microcontroller received data from a CO_2 sensor (model K30, SenseAir, Delsbo, Sweden), located in the recirculation pipe, and injected CO_2 into the chamber through the solenoid valve to maintain the target level.

The soil moisture treatments were applied to one half of every chamber. Field capacity soil moisture content in the seedling trays/pots was observed to be approximately 25% when measured with a digital soil moisture meter (model HSM50, OMEGA, Saint-Eustache, Quebec, Canada). Seedling trays in the moderate and drought soil moisture treatments were allowed to dry down to 15% and 5% soil moisture, respectively, before

being hand-watered to field capacity. The moderate soil moisture treatment was designed to provide adequate soil moisture and prevent negative effects due to overwatering.

3.3.5 Seed source and seedling preparation

Seed was acquired from the Canadian National Tree Seed Centre. Three parent trees (seedlots) of each of the three tree species were selected from locations within 75 km of Fredericton to minimize variation in the maternal climate. Balsam fir and red maple seeds were stratified according to standard procedures (Bonner and Karrfalt 2008), and red maple seeds were subsequently germinated in a germinator (model G1000, Conviron, Winnipeg, Manitoba, Canada). Red spruce and stratified balsam fir seeds were sown into 45-cell trays on January 31st and February 1st, respectively, and red maple germinants were planted into 15-cell trays on February 5th (all potting media contained a mixture of 50% vermiculite and 50% peat moss). Conifer seedlings from cells containing multiple seedlings were transplanted to empty cells within the first two weeks of germination, and all conifer cells were thinned to one seedling at the end of March. Red maple seedlings were grown until May 22nd and then re-potted in six-inch square pots. During the seedling preparation stage, prior to the start of the trial (January 31st to June 3rd), all seedlings were grown in a greenhouse with a 20°C/16°C (day/night) temperature schedule. Seedlings were watered as needed and daylight extenders ran from 8 a.m. to 8 p.m. (when ambient light was not adequate).

3.3.6 Seedling Growth and survival

Height and root-collar diameter of all seedlings were measured at the beginning and end of the trial to determine total height and diameter growth. After the trial, seedling mortality was recorded, and full seedling samples for mass measurement (n = 6 for red maple; n = 15 for red spruce; and n = 21 for balsam fir) were randomly selected from each of the 12 treatment combinations and rinsed of soil prior to being dried and weighed. Sample sizes varied between species as seedling sizes and processing times were substantially different.

3.3.7 Measurements of photosynthesis

Balsam fir photosynthesis (A_{net}) and stomatal conductance (g_s) were measured for all soil moisture and CO_2 treatments of the baseline and +6°C temperature levels. These data were only collected for balsam fir to assist with interpreting this species' growth and survival results as it is often projected to be one of the most severely impacted by climate change in the Acadian Forest (Boulanger et al. 2017, Taylor et al. 2017). Data were collected during the week with the hottest daily temperature schedule (baseline maximum temperature = 28.7°C; +6°C maximum temperature = 34.7°C), as we expected seedling stress levels to be greatest in the +6°C treatment during this period. Measurements were taken with an LI-6400XT portable photosynthesis system equipped with a 6400-22 opaque conifer chamber and a 6400-18A RGB light source (Li-Cor, Lincoln, Nebraska, USA). The entire aboveground portions of the seedlings were inserted into the 6400-22 opaque conifer chamber and exposed to 1000 μmol m^{-2} s^{-1} photosynthetic photon flux.

3.3.8 Measurements of xylem water potential

Seedling xylem water potential data were collected for all species and treatments with a model 1505D-EXP pressure chamber (PMS Instrument Company, Albany, Oregon, USA). These data were used to estimate differences in soil water potential between the soil moisture treatments for each species (Ritchie and Hinckley 1975; Major and Johnsen 1996). Three seedlings per species from each of the twelve treatments (totaling 36 seedlings per species) were randomly selected and placed in a dark room for two hours prior to being tested (mimicking predawn xylem water potential). Seedlings from the drought and moderate soil moisture treatments were sampled at 5% soil moisture and the day after watering, respectively. For each sample, a small, freshly cut branch section was placed into the pressure chamber and the pressure was slowly increased. When water was observed on the cut surface, the pressure was recorded.

3.3.9 Statistical analyses

Analysis of variance (ANOVA) was used to test the interactive effects of temperature, CO_2 and soil moisture treatments on seedling height and diameter growth, total biomass, and root:shoot ratio of each species. The four response variables were tested separately for each species using the following linear model:

$$Y = \beta_0 + \beta_1 T + \beta_2 C + \beta_3 S + \beta_4 T \times C + \beta_5 T \times S + \beta_6 C \times S + \beta_7 T \times C \times S + \varepsilon$$

where Y is one of the four response variables (i.e., seedling height growth, diameter growth, total biomass or root:shoot ratio); T represents temperature treatment; C is CO_2 treatment; S is soil moisture treatment, and ε is random error. Separate models were fit

for each species to ease interpretation and to assess the species-specific treatment effects. Significant differences between treatment combinations for all growth response variables were calculated using the "TukeyHSD" post-hoc test in R with the "multcompLetters4" function from the "multcompView" package (Graves et al. 2019). Additionally, the same ANOVA model was used to test the interactive effects of temperature, CO_2 and soil moisture on balsam fir A_{net} and g_s. Each ANOVA was fit with the "aov" function in R (R Core Team 2020). Non-significant three-way interactions were removed from the final model to increase degrees of freedom and simplify model interpretation. Heteroscedasticity and normality of model residuals were visually assessed by inspecting the model residuals versus fitted values and Normal Q-Q plots, and statistically analyzed with the Shapiro-Wilk test. If heteroscedasticity was found to be significant, the response variable was log-transformed.

Logistic regression was used to test the interactive effects of temperature, CO_2 and soil moisture on seedling mortality for balsam fir and red spruce (red maple mortality was not observed in any treatment), and a Chi-square test was used to determine the significance of each treatment factor. Mean xylem water potential values were compared between the two soil moisture treatments for each species using two-sample t-tests.

With some treatment combinations being nested within chambers (e.g., temperature and CO_2), there is the potential for pseudoreplication to produce spurious effects due to small variations in individual chamber environments (Hurlbert 1984). Hurlbert (1984), which addressed the issue of pseudoreplication in ecological studies, has been challenged with regard to the issue of pseudoreplication in growth chamber experiments (Oksanen 2001,

Munzbergova et al. 2017), and experiments using unreplicated growth chamber experimental designs have been successfully published (Bezemer et al. 1998, Cavieres and Arroyo 2000, Souther et al. 2012, Matias and Jump 2014, Munzbergova et al. 2017). Nonetheless, to help account for potential increase in Type I error and spurious treatment effects due to pseudoreplication, we chose to set α as 0.01 for all significance tests (as opposed to 0.05) (Banerjee et al. 2009).

3.4 Results

3.4.1 Seedling growth

Increasing temperature significantly influenced three out of four balsam fir growth parameters as either a main or interactive effect (Table 3.1). These effects included a significant interaction between temperature, CO_2 and soil moisture on height growth; a significant interaction between temperature and CO_2 on diameter growth; and a significant effect of increasing temperature that reduced root:shoot ratio (Table 3.1). The elevated CO_2 treatments had significant positive effects on all four balsam fir growth variables, with total biomass and root:shoot ratio showing 24.0% ($p=0.0062$) and 19.5% ($p<0.0001$) increases (Table 3.1, Fig. 3.2g, j). In contrast, the drought soil moisture treatment significantly reduced balsam fir diameter growth and total biomass by 9.2% ($p=0.00014$) and 23.5% ($p=0.00072$), respectively (Table 3.1, Fig. 3.2d, g). When compared with baseline temperature, balsam fir root:shoot ratio was 11.4% and 13.3% smaller in the +3°C and +6°C treatments ($p<0.01$, Fig. 3.2j). With warming, increases in balsam fir height growth were only detected under certain soil moisture and CO_2

conditions (Fig. 3.3a)—seedlings in the +6°C treatment exhibited a small, marginally significant ($p<0.05$) 14.0% (1.8 mm) increase in average height growth relative to the baseline treatment. When comparing the two CO_2 treatments, the increase in height growth in response to warming is much more evident under the elevated treatment (Fig. 3.3a). Furthermore, height growth of balsam fir under drought was 22.9% greater under elevated CO_2 relative to the ambient treatment ($p<0.01$, Fig. 3.3a). Balsam fir diameter growth under elevated CO_2 was 23.2% greater than the ambient CO_2 treatment under +3°C warming ($p<0.01$) but was similar under +6°C warming (Fig. 3.3c).

Warming had significant interactive effects on red spruce height and diameter growth, which included a significant interaction between temperature, CO_2 and soil moisture on height growth and a significant interaction between temperature and CO_2 on diameter growth (Table 3.1). Like balsam fir, all red spruce growth parameters increased under elevated CO_2, with total biomass and root:shoot ratio showing 19.9% ($p=0.0042$) and 10.4% ($p=0.0095$) increases (Table 3.1, Fig. 3.2h, k). Red spruce appeared to be much less sensitive to the drought soil moisture treatment than balsam fir with only a 2.3% decrease in diameter growth ($p<0.0001$) and no significant reduction in total biomass (Table 3.1, Fig. 3.2e, h). Warming increased red spruce average height growth but varied in intensity according to CO_2 and soil moisture conditions (Fig. 3.2b). Specifically, compared with baseline values, red spruce average height growth was 17.6% and 26.4% greater under +3 and +6°C warming, respectively ($p<0.01$, Fig. 3.2b). This was further demonstrated by the clear trend that red spruce height growth increased with warming under every CO_2 and soil moisture treatment combination (Fig. 3.3b). Additionally, in the

drought soil treatment, red spruce height growth was 23.1% greater under elevated CO_2 when compared with ambient ($p<0.01$, Fig. 3.3b). Similar to balsam fir, red spruce diameter growth in the elevated CO_2 treatment was 23.2% greater than in the ambient treatment under +3°C warming ($p<0.01$) but did not differ under +6°C (Fig. 3.3d). Interestingly, the positive combined effect of warming and elevated CO_2 that was observed at the +3°C level disappeared at the +6°C level, which suggests that this warming-induced increase in growth could be transitory for both conifers.

Red maple height growth and total biomass were influenced by significant interactions between temperature and soil moisture (Table 3.1). Red maple aboveground growth increased under elevated CO_2, however, unlike the conifers, root:shoot ratio was not affected (Table 3.1, Fig. 3.2l). Red maple showed 24.1% greater height growth ($p<0.0001$), 22.4% larger average diameter growth ($p<0.0001$), and 37.7% larger average total biomass ($p<0.0001$) under elevated CO_2 compared with ambient (Table 3.1, Fig. 3.2c, f, i)—roughly two to three times greater than the responses of the conifers. Moreover, the relative response to elevated CO_2 exhibited by red maple was consistently greater than the two conifers in both moderate and drought soil moisture treatments for all growth parameters except for root:shoot ratio. Additionally, no interactions between CO_2 and the other treatment factors were observed for any red maple response variable (Table 3.1). This suggests that the increase in red maple growth in response to elevated CO_2 was not significantly limited or affected by the other treatment factors, which differs from the temperature-limited positive effect of CO_2 that was observed with balsam fir and red spruce diameter growth. Under the drought treatment, red maple exhibited a

20.6% increase in root:shoot ratio ($p<0.0001$, Table 3.1, Fig. 3.2l); and, when comparing the baseline/ambient CO_2/drought soil moisture treatment with the +6°C/ambient CO_2/drought soil moisture treatment, root:shoot ratio increased by 49.2% ($p<0.01$, Fig. 3.2l). This response to increased moisture stress was not observed in the conifers. Occasionally, droughted red maple growth was significantly greater under elevated CO_2, while seedlings in the moderate soil treatment did not differ between CO_2 treatments (e.g., height growth, diameter growth and total biomass under baseline temperature; Fig. 3.2c, f, i). Although there was a negative effect of temperature on red maple total biomass overall (Fig. 3.2i), it varied in intensity between soil moisture treatments—under +6°C, average total biomass of droughted seedlings was 26.4% ($p<0.01$) smaller than that of seedlings in the moderate soil moisture treatment, while no significant differences were observed between soil moisture treatments at either baseline or +3°C (Fig. 3.3e). Under the moderate soil moisture treatment, warming did not significantly affect red maple total biomass (Fig. 3.3e). Similarly, red maple height growth was unaffected by temperature when soil moisture was adequate; however, under drought, we observed 17.3 and 27.4% reductions ($p<0.01$) in the baseline and +6°C treatments (Fig. 3.3f). Interestingly, the negative effect of drought on red maple height growth was less severe under elevated CO_2, which elicited a 30.7% increase relative to the ambient CO_2 treatment ($p<0.01$).

3.4.2 Impacts of soil moisture on xylem water potential

For all three species, significantly lower xylem water potentials (two-sample t-test, $p<0.01$) were observed under the drought treatment relative to the moderate treatment

(Fig. 3.4). Under drought, mean xylem water potentials were -1.01, -1.03 and -1.45 MPa for balsam fir, red spruce and red maple, respectively, and -0.42, -0.48 and -0.32 MPa in the moderate soil moisture treatment.

3.4.3 Balsam fir photosynthesis and stomatal conductance

Balsam fir average A_{net} under both CO_2 treatments decreased by 76.6% in response to moisture stress ($p<0.01$), and, under elevated CO_2, A_{net} of both soil moisture treatments increased by 120.6% ($p<0.01$, Fig. 3.5). No interaction was observed between soil moisture and CO_2 for balsam fir A_{net}, however, g_s response to moisture stress differed between CO_2 treatments. Under elevated CO_2, g_s of droughted seedlings improved, while seedlings in the moderate soil moisture treatment experienced a reduction in g_s. A_{net} and g_s values in the drought soil treatments of the ambient CO_2 treatments were nearly zero.

3.4.4 Seedling mortality

Across temperature and CO_2 treatments, average balsam fir and red spruce mortality increased significantly from 1.6 and 1.0% mortality under moderate soil moisture to 6.8 and 2.7% mortality under the drought treatment, respectively ($p<0.01$; Figure 6). Balsam fir mortality was significantly affected by an interaction between temperature and CO_2 ($p<0.01$): under ambient CO_2, mortality rates for balsam fir were 2.6, 1.5 and 13.3% for the baseline, +3°C and +6°C treatments, respectively, and 0.0, 3.0 and 4.8% under elevated CO_2. Balsam fir seedlings in the +6°C/ambient CO_2/drought soil treatment exhibited the greatest mortality at 22.2% (30 of 135 seedlings; Fig. 3.6). No red maple mortality occurred, so it was excluded from the analysis.

3.5 Discussion

3.5.1 Experimental effects of projected climate and CO_2 levels on growth

The observed increases in growth in response to warming (red spruce) and elevated CO_2 (all species) support our first and third hypotheses and agree with previous studies (Fisichelli et al. 2014, Carles et al. 2015, Resco de Dios et al. 2016). Red maple, predicted to become more abundant in the Acadian Forest under climate change (Ashraf et al. 2015, Taylor et al. 2017), did not show improved growth in response to warming, but maintained greater absolute growth relative to spruce and fir and was only negatively affected by warming when soil moisture was limiting. Interestingly, CO_2 did not interact with any other treatment factor for aboveground growth of red maple, which suggests that red maple growth will continue to benefit from rising atmospheric CO_2 regardless of the warming that is projected to occur in the Acadian Forest Region. In contrast, we found that there are limits to the increased growth effect of elevated CO_2 for balsam fir and red spruce. This was demonstrated by the temperature-limited increase in diameter growth that these species exhibited under elevated CO_2. The drought soil moisture treatment, which produced soil water potential estimates similar to those of drought stress field studies (Major and Johnsen 1996, Olesinski et al. 2011), generally reduced aboveground growth of all species; however, the negative effect of drought was counteracted under elevated CO_2, in accordance with previous studies (Swann et al. 2016, Sperry et al. 2019). This was evident in the height growth of balsam fir, red spruce and red maple seedlings in the drought treatments, which exhibited significant increases under elevated CO_2 relative to the ambient treatments.

Some of the observed increases in growth may be more pronounced as a result of the indeterminate growth pattern expressed during the first growing season (Carles et al. 2015). In the following years, the determinate growth habits typically expressed by red spruce and balsam fir juveniles (Pothier and Margolis 1991, Samuelson and Seiler 1993) may limit these species' competitiveness relative to red maple, which has a much greater capacity for indeterminate growth throughout its ontogeny (Abrams 1998). This characteristic will further benefit red maple if the growing season lengthens with climate change as projected (Peñuelas and Filella 2001). Although the red maple seedlings were removed from the trial 40 days before balsam fir and red spruce, average height growth during the trial was approximately fifty- and seven-fold greater than balsam fir and red spruce, respectively.

Under ambient CO_2 and drought soil conditions, red maple root:shoot ratio increased in response to warming. This shift in resource allocation to the roots was not observed in balsam fir and red spruce seedlings. An increase in root:shoot ratio limits stress when soil moisture availability is low and can be achieved by restricting shoot growth (Brunner et al. 2015). As the effects of typical drought events become more severe with climate warming (Will et al. 2013, Peñuelas et al. 2017), tree species that lack the ability to limit water stress by altering resource allocation may exhibit lower fitness. Although red maple height growth and total biomass generally decreased under drought, absolute growth remained much greater than fir and spruce. Moreover, when soil moisture was not limiting, red maple total biomass and height growth were not affected by warming. Therefore, if red maple is capable of superior growth, regardless of warming and soil

moisture, its competitiveness in the Acadian Forest, relative to balsam fir and red spruce, may improve with climate change.

Excess soil water resulting from CO_2-induced increases in water use efficiency (Assmann 1993, Long et al. 2004) could explain why red maple growth in the moderate soil moisture treatment did not significantly increase in response to elevated CO_2. This is similar to observations of Fisichelli et al. (2014), however, in their case, excessive soil moisture reduced the increased growth effect of warming. This result agrees with past studies that have observed an increase in water use efficiency resulting from rising atmospheric CO_2 (Peñuelas et al. 2011, Soh et al. 2019). Furthermore, the drought soil moisture treatment generally produced smaller seedlings in the ambient CO_2 chambers where improved water use efficiency, as a result of CO_2 enrichment, was absent.

3.5.2 Effects of climate change on balsam fir photosynthesis and stomatal conductance

Elevated CO_2 has been shown to decrease transpiration through a reduction in g_s (Long et al. 2004, Wertin et al. 2010, Franks et al. 2013); however, the decrease in g_s only negligibly offsets the increase in the rate of photosynthesis (Long et al. 2004). This is in line with the decrease in g_s and the increase in A_{net} that we observed in balsam fir seedlings under elevated CO_2. The low A_{net} and g_s values that were recorded in the ambient CO_2/drought soil treatments may be the result of stomatal closure due to the extremely low soil water potential (Franks et al. 2013). Interestingly, under elevated CO_2, seedlings exposed to similarly low soil water potentials produced larger A_{net} and g_s values, suggesting that the stomata were still open. When subjected to low soil water

potentials, plants exposed to elevated CO_2 have been observed to maintain better functionality and higher xylem water potentials than those under ambient conditions (Tyree and Alexander 1993). Thus, rising levels of atmospheric CO_2 over the 21st century may allow some tree species to continue photosynthesizing during periods of rainfall deficits that, under current atmospheric CO_2 concentrations, would significantly reduce A_{net}. Still, further research is needed to understand the effects of rising atmospheric CO_2 on species with differing stomatal regulation strategies (Duan et al. 2015) and to determine whether the observed increase in photosynthetic capacity will remain heightened or return to baseline levels as a result of photosynthetic adjustment responses (Rey and Jarvis 1998, Tissue et al. 1999).

3.5.3 Seedling survival in a changing environment

The lack of red maple mortality supports our fourth hypothesis that, under long-term RCP 8.5 climatic conditions, red maple seedlings will exhibit better survival than balsam fir and red spruce. With the highest (>20%/year) balsam fir mortality rates occurring in the +6°C/drought soil treatment under ambient CO_2 (Fig. 3.6), high temperature and reduced soil moisture most likely had the strongest impacts on survival. In comparison, the same treatment under elevated CO_2 produced substantially lower (5.2%/year) balsam fir mortality rates, providing further evidence of the positive effect of improved water use efficiency resulting from CO_2 enrichment. Seedling mortality is often the result of hydraulic failure when soil moisture is low because their limited root size exposes them to more negative soil water potentials (McDowell et al. 2008). The substantial balsam fir mortality that we observed in the +6°C/ambient CO_2/drought soil treatment will require

further testing to corroborate these results and may not be meaningful as +6°C warming is not expected to occur in the absence of increased atmospheric CO_2. However, recent modelling studies (Boulanger et al. 2017, Taylor et al. 2017) and field experiments (Reich et al. 2015) have predicted that this species, along with other boreal species in the boreal-temperate transition zone, will be severely impacted under a warming climate, and these findings generally agree with the species' geographic distributions. Balsam fir, having the most northern distribution, was most sensitive to the +6°C treatment, while red maple, the most southerly species, did not show any mortality. Red spruce, which is found in the southern portion of balsam fir's range, exhibited less mortality under the +6°C treatment than balsam fir. In the drought soil treatments, red maple seedlings produced the lowest xylem water potential (Fig. 3.4), however, no mortality was observed. This agrees with past studies that have observed low mortality rates of red maple during drought (Elliott and Swank 1994). Thus, given the potential increase in drought severity under a warming climate (Will et al. 2013, Peñuelas et al. 2017), red maple may be more likely to survive compared with balsam fir and red spruce.

3.6 Conclusions

Our experiment showed that key Acadian Forest tree species may exhibit different physiological responses to the RCP 8.5 radiative forcing scenario. Compared with balsam fir, red spruce exhibited better growth and survival in response to warming and was less sensitive to moisture stress. While diameter growth of balsam fir and red spruce increased under the short-term climate change projections (+3°C/elevated CO_2) and declined under long-term projections (+6°C/elevated CO_2), red maple growth was unaffected by

warming when soil moisture was adequate, and consistently maintained greater absolute growth in all treatments. Additionally, red maple did not exhibit mortality in any treatment and was the only species that altered resource allocation in response to increasing temperature and hydraulic stress. These results suggest that red spruce may be better adapted than balsam fir to the long-term projected warming for the Acadian Forest, while red maple seedlings will likely outperform both conifers. The results of this study are limited to first year seedling growth in response to the RCP 8.5 climate change scenario over the course of the 21st century. This experiment should be repeated with two- and three-year-old juveniles to better understand the variation between the determinate and indeterminate growth patterns expressed by these species and to determine the effects of long-term exposure to elevated CO_2 on photosynthetic activity.

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3.8 References

Abrams, M.D. 1998. The Red Maple Paradox. Bioscience 48:355-364.

Ainsworth, E.A., and Long, S.P. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. 165:351-371.

Ashraf, M.I., Bourque, C.P-A., MacLean, D.A., Erdle, T., and Meng, F-R. 2015. Estimation of potential impacts of climate change on growth and yield of temperate tree species. Mitig. Adapt. Strat. Glob. Change 20:159-178.

Assmann, S.M. 1993. Signal transduction in guard cells. Annu. Rev. Cell Biol. 9:345-375.

Banerjee, A., Chitnis, U.B., Jadhav, S.L., Bhawalkar, J.S., and Chaudhury, S. 2009. Hypothesis testing, type I and type II errors. Ind. Psychiatry J. 18:127-131.

Barber, V.A., Juday, G.P., and Finney, B.P. 2000. Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. Nature 405:668-673.

Bezemer, T.M., Thompson, L.J., and Jones, T.H. 1998. *Poa annua* shows inter-generational differences in response to elevated CO_2. Glob. Chang. Biol. 4:687-691.

Bonner, F.T., and Karrfalt, R.P. (Editors) 2008. The woody plant seed manual. U.S. Department of Agriculture, Forest Service, Washington, DC.

Boulanger, Y., Taylor, A.R., Price, D.T., Cyr, D., McGarrigle, E., Rammer, W., Sainte-Marie, G., Beaudoin, A., Guindon, L., and Mansuy, N. 2017. Climate change impacts on forest landscapes along the Canadian southern boreal forest transition zone. Landsc. Ecol. 32:1415-1431.

Bourque, C.P-A., and Hassan, Q.K. 2008. Projected impacts of climate change on species distribution in the Acadian Forest region of eastern Nova Scotia. For. Chron. 84:553-557.

Brodribb, T.J., Powers, J., Cochard, H., and Choat, B. 2020. Hanging by a thread? Forests and drought. Science 368:261-266.

Brunner, I., Herzog, C., Dawes, M.A., Arend, M., and Sperisen, C. 2015. How tree roots respond to drought. Front. Plant Sci. 6:547.

Bugmann, H.K.M., Wullschleger, S.D., Price, D.T., Ogle, K., Clark, D.F., and Solomon, A.M. 2001. Comparing the performance of forest gap models in North America. Clim. Change 51:349-388.

Bush, E., and Lemmen, D.S. (Editors) 2019. Canada's Changing Climate Report. Government of Canada. Ottawa, ON.

Carles, S., Groulx, D.B., Lamhamedi, M.S., Rainville, A., Beaulieu, J., Bernier, P., Bousquet, J., Deblois, J., and Margolis, H.A. 2015. Family variation in the morphology and physiology of white spruce (*Picea glauca*) seedlings in response to elevated CO_2 and temperature. J. Sustain. Forest. 34:169-198.

Cavieres, L.A., and Arroyo, M.T.K. 2000. Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae) - Altitudinal variation in the mediterranean Andes of central Chile. Plant Ecol. 149:1-8.

Chen, L., Huang, J.G., Alam, S.A., Zhai, L.H., Dawson, A., Stadt, K.J., and Comeau, P.G. 2017. Drought causes reduced growth of trembling aspen in western Canada. Glob. Chang. Biol. 23:2887-2902.

Day, M.E. 2000. Influence of temperature and leaf-to-air vapor pressure deficit on net photosynthesis and stomatal conductance in red spruce (*Picea rubens*). Tree Physiol. 20:57-63.

Duan, H., O'Grady, A.P., Duursma, R.A., Choat, B., Huang, G., Smith, R.A., Jiang, Y., and Tissue, D.T. 2015. Drought responses of two gymnosperm species with contrasting stomatal regulation strategies under elevated [CO_2] and temperature. Tree Physiol. 35:756-770.

Elliott, K.J., and Swank, W.T. 1994. Impacts of drought on tree mortality and growth in a mixed hardwood forest. J. Veg. Sci. 5:229-236.

Environment Canada 2019a. Canadian Climate Data and Scenarios [online]. Available from http://climate-scenarios.canada.ca/index.php?page=main [accessed 28 May 2020].

Environment Canada 2019b. Canadian climate normals 1981–2010 [online]. Available from http://www.climate.weatheroffice.ec.gc.ca/climate_normals/ [accessed 27 March 2020].

Fisichelli, N., Wright, A., Rice, K., Mau, A., Buschena, C., and Reich, P.B. 2014. First-year seedlings and climate change: species-specific responses of 15 North American tree species. Oikos 123:1331-1340.

Foster, G., Royer, D., and Lunt, D. 2017. Future climate forcing potentially without precedent in the last 420 million years. Nat. Commun. 8:14845.

Franks, P.J., Adams, M.A., Amthor, J.S., Barbour, M.M., Berry, J.A., Ellsworth, D.S., Farquhar, G.D., Ghannoum, O., Lloyd, J., McDowell, N., Norby, R.J., Tissue, D.T., and von Caemmerer, S. 2013. Sensitivity of plants to changing atmospheric

CO_2 concentration: from the geological past to the next century. New Phytol. 197:1077-1094.

Graves, S., Piepho, H-P., Selzer, L., and Sundar, D-R. 2019. Package 'multcompView'. Available from https://cran.r-project.org/web/packages/multcompView/multcompView.pdf [accessed 6 March 2020].

Hartmann, H., Adams, H.D., Hammond, W.M., Hoch, G., Landhausser, S.M., Wiley, E., and Zaehle, S. 2018. Identifying differences in carbohydrate dynamics of seedlings and mature trees to improve carbon allocation in models for trees and forests. Environ. Exp. Bot. 152:7-18.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54:187-211.

IPCC 2013. Climate change 2013: the physical science basis. In: Stocker, T.F., Qin, D., Plattner, G-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgle, P.M. (Editors) *Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, UK and New York, NY, USA.

IPCC 2014. Climate change 2014: synthesis report. Core Writing Team, Pachauri, R.K., and Meyer, L.A. (Editors) *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Geneva, Switzerland.

Johnstone, J.F., McIntire, E.J.B., Pedersen, E.J., King, G., and Pisaric, M.J.F. 2010. A sensitive slope: estimating landscape patterns of forest resilience in a changing climate. Ecosphere 1:14.

Kroel-Dulay, G., Ransijn, J., Schmidt, I.K., Beier, C., De Angelis, P., de Dato, G., Dukes, J.S., Emmett, B., Estiarte, M., Garadnai, J., Kongstad, J., Kovacs-Lang, E., Larsen, K.S., Liberati, D., Ogaya, R., Riis-Nielsen, T., Smith, A.R., Sowerby, A., Tietema, A., and Penuelas, J. 2015. Increased sensitivity to climate change in disturbed ecosystems. Nat. Commun. 6:6682.

Little, E.L. 1971. Atlas of United States trees. Volume 1. Conifers and important hardwoods. Miscellaneous publication 1146. US Department of Agriculture, Forest Service, Washington, DC.

Loehle, C. 2000. Forest ecotone response to climate change: sensitivity to temperature response functional forms. Can. J. For. Res. 30:1632-1645.

Long, S.P., Ainsworth, E.A., Rogers, A., and Ort, D.R. 2004. Rising atmospheric carbon dioxide: Plants FACE the Future. Annu. Rev. Plant Biol. 55:591-628.

Major, J.E., and Johnsen, K.H. 1996. Family variation in photosynthesis of 22-year-old black spruce: a test of two models of physiological response to water stress. Can. J. For. Res. 26:1922-1933.

Matias, L., and Jump, A.S. 2014. Impacts of predicted climate change on recruitment at the geographical limits of Scots pine. J. Exp. Bot. 65:299-310.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., and Yepez, E.A. 2008. Mechanisms of

plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol. 178:719-739.

McKenney, D.W., Hutchinson, M.F., Papadopol, P., Lawrence, K., Pedlar, J., Campbell, K., Milewska, E., Hopkinson, R.F., Price, D., and Owen, T. 2011. Customized spatial climate models for North America. B. Am. Meteorol. Soc. 92:1611-1622.

Miao, S.L., Wayne, P.M., and Bazzaz, F.A. 1992. Elevated CO_2 differentially alters the responses of co-ocurring birch and maple seedlings to a moisture gradient. Oecologia 90:300-304.

Munzbergova, Z., Hadincova, V., Skalova, H., and Vandvik, V. 2017. Genetic differentiation and plasticity interact along temperature and precipitation gradients to determine plant performance under climate change. J. Ecol. 105:1358-1373.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F., and van Kleunen, M. 2010. Plant phenotypic plasticity in a changing climate. Trends. Plant. Sci. 15:684-692.

Norby, R.J., DeLucia, E.H., Gielen, B., Calfapietra, C., Giardina, C.P., King, J.S., Ledford, J., McCarthy, H.R., Moore, D.J.P., Ceulemans, R., De Angelis, P., Finzi, A.C., Karnosky, D.F., Kubiske, M.E., Lukac, M., Pregitzer, K.S., Scarascia-Mugnozza, G.E., Schlesinger, W.H., and Oren, R. 2005. Forest response to elevated CO_2 is conserved across a broad range of productivity. Proc. Natl. Acad. Sci. USA 102:18052-18056.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E., and McMurtrie, R.E. 2010. CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proc. Natl. Acad. Sci. USA 107:19368-19373.

Oksanen, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? Oikos 94:27-38.

Olesinski, J., Lavigne, M.B., and Krasowski, M.J. 2011. Effects of soil moisture manipulations on fine root dynamics in a mature balsam fir (*Abies balsamea* L. Mill.) forest. Tree Physiol. 31:339-348.

Peñuelas, J., Canadell, J.G., and Ogaya, R. 2011. Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. Glob. Ecol. Biogeogr. 20:597-608.

Peñuelas, J., Ciais, P., Canadell, J.G., Janssens, I.A., Fernández-Martínez, M., Jofre, C., Obersteiner, M., Piao, S., Vautard, R., and Sardans, J. 2017. Shifting from a fertilization-dominated to a warming-dominated period. Nat. Ecol. Evol. 1:1438-1445.

Peñuelas, J., and Filella, I. 2001. Responses to a warming world. Science 294:793-795.

Pothier, D., and Margolis, A. 1991. Analysis of growth and light interception of balsam fir and white birch saplings following precommercial thinning. Ann. For. Sci. 48:123-132.

R Core Team 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reich, P.B., Sendall, K.M., Rice, K., Rich, R.L., Stefanski, A., Hobbie, S.E., and Montgomery, R.A. 2015. Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. Nat. Clim. Change 5:148-152.

Resco de Dios, V., Mereed, T.E., Ferrio, J.P., Tissue, D.T., and Voltas, J. 2016. Intraspecific variation in juvenile tree growth under elevated CO_2 alone and with O_3: a meta-analysis. Tree Physiol. 36:682-693.

Rey, A., and Jarvis, P.G. 1998. Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. Tree Physiol. 18:441-450.

Ritchie, G.A., and Hinckley, T.M. 1975. The pressure chamber as an instrument for ecological research. Adv. Ecol. Res. 9:165-254.

Rogers, B.M., Jantz, P., and Goetz, S.J. 2017. Vulnerability of eastern US tree species to climate change. Glob. Chang. Biol. 23:3302-3320.

Rowe, J.S. 1972. Forest regions of Canada. Natural Resources Canada, Canadian Forest Service, Ottawa, Canada.

Samuelson, L.J., and Seiler, J.R. 1993. Interactive role of elevated CO_2, nutrient limitations, and water stress in the growth responses of red spruce seedlings. For. Sci. 39:348-358.

Samuelson, L.J., and Seiler, J.R. 1994. Red spruce seedling gas exchange in response to elevated CO_2, water stress, and soil fertility treatments. Can. J. For. Res. 24:954-959.

Soh, W.K., Yiotis, C., Murray, M., Parnell, A., Wright, I.J., Spicer, R.A., Lawson, T., Caballero, R., and McElwain, J.C. 2019. Rising CO_2 drives divergence in water use efficiency of evergreen and deciduous plants. Sci. Adv. 5:eaax7906.

Souther, S., Lechowicz, M.J., and McGraw, J.B. 2012. Experimental test for adaptive differentiation of ginseng populations reveals complex response to temperature. Ann. Bot. 110:829-837.

Sperry, J.S., Venturas, M.D., Todd, H.N., Trugman, A.T., Anderegg, W.R.L., Wang, Y., and Tai, X. 2019. The impact of rising CO_2 and acclimation on the response of US forests to global warming. Proc. Natl. Acad. Sci. USA 116:25734-25744.

Sullivan, J. 1993. *Picea rubens* [online]. In: Fire Effects Information System. USDA, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer), Missoula, MT, USA. Available from http://www.fs.fed.us/database/feis/plants/tree/picrub/all.html (accessed 3 August 2018).

Swann, A.L.S., Hoffman, F.M., Koven, C.D., and Randerson, J.T. 2016. Plant responses to increasing CO_2 reduce estimates of climate impacts on drought severity. Proc. Natl. Acad. Sci. USA 113:10019-10024.

Taylor, A.R., Boulanger, Y., Price, D.T., Cyr, D., McGarrigle, E., Rammer, W., and Kershaw, J.A. 2017. Rapid 21st century climate change projected to shift composition and growth of Canada's Acadian Forest Region. For. Ecol. Manag. 405:284-294.

Tirmenstein, D.A. 1991. *Acer rubrum* [online]. In: Fire Effects Information System. USDA, Forest Service, Rocky Mountain Research Station, Fire Sciences

Laboratory (Producer), Missoula, MT, USA. Available from https://www.fs.fed.us/database/feis/plants/tree/acerub/all.html [accessed 26 June 2020].

Tissue, D.T., Griffin, K.L., and Ball, J.T. 1999. Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO_2. Tree Physiol. 19:221-228.

Two Countries, One Forest 2014. Northern Appalachian/Acadian Ecoregion Conservation Atlas [online]. Available from https://2c1forest.databasin.org/datasets/425508ee1f55464aacc2a2d4be4d0d69 [accessed 16 October 2020].

Tyree, M.T., and Alexander, J.D. 1993. Plant water relations and the effects of elevated CO_2: a review and suggestions for future research. Vegetatio 104:47-62.

Uchytil, R.J. 1991. *Abies balsamea* [online]. In: Fire Effects Information System. USDA, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer), Missoula, MT, USA. Available from https://www.fs.fed.us/database/feis/plants/tree/abibal/all.html [accessed 26 June 2020].

UNEP 2019. Emissions Gap Report 2019. United Nations Environment Programme, Nairobi.

Urban, M.C., Bocedi, G., Hendry, A.P., Mihoub, J.B., Pe'er, G., Singer, A., Bridle, J.R., Crozier, L.G., De Meester, L., Godsoe, W., Gonzalez, A., Hellmann, J.J., Holt, R.D., Huth, A., Johst, K., Krug, C.B., Leadley, P.W., Palmer, S.C., Pantel, J.H.,

Schmitz, A., Zollner, P.A., and Travis, J.M. 2016. Improving the forecast for biodiversity under climate change. Science 353:aad8466.

Van Vuuren, D.P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G., Kram, T., Krey, V., Lamarque, J-F., Masui, T., Meinshausen, M., Nakicenovic, N., Smith, S., and Rose, S. 2011. The representative concentration pathways: an overview. Clim. Change 109:5-31.

Voosen, P. 2019. New climate models forecast a warming surge. Science 364:222-223.

Wertin, T.M., McGuire, M.A., and Teskey, R.O. 2010. The influence of elevated temperature, elevated atmospheric CO_2 concentration and water stress on net photosynthesis of loblolly pine (*Pinus taeda* L.) at northern, central and southern sites in its native range. Glob. Chang. Biol. 16:2089-2103.

Will, R.E., Wilson, S.M., Zou, C.B., and Hennessey, T.C. 2013. Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest-grassland ecotone. New Phytol. 200:366-374.

Table 3.1. Effects of temperature, CO_2, soil moisture treatments and their interactions on height growth, root-collar diameter growth, total biomass and root:shoot ratio of seedlings. Statistically significant terms ($p < 0.01$) are in bold. Three-way interaction terms that were not significant were removed from the model (NA).

Fixed Effect	Height growth		Diameter growth		Total Biomass		Root:shoot Ratio	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Balsam Fir								
Temperature	3.82	0.022	4.07	0.017	0.86	0.43	12.70	**<0.0001**
CO_2	8.66	**0.0033**	20.11	**<0.0001**	7.62	**0.0062**	48.85	**<0.0001**
Soil moisture	2.30	0.13	14.56	**0.00014**	11.73	**0.00072**	1.63	0.20
Temp x CO_2	2.93	0.054	5.20	**0.0056**	0.52	0.60	0.42	0.65
Temp x Soil moisture	0.34	0.71	2.00	0.13	2.01	0.14	3.11	0.047
CO_2 x Soil moisture	2.49	0.11	3.00	0.08	0.37	0.55	0.16	0.69
Temp x CO_2 x Soil	6.36	**0.0017**	NA	NA	NA	NA	NA	NA
Red Spruce								
Temperature	58.62	**<0.0001**	24.65	**<0.0001**	1.24	0.29	1.76	0.17
CO_2	53.03	**<0.0001**	20.07	**<0.0001**	8.40	**0.0042**	6.87	**0.0095**
Soil moisture	1.12	0.29	39.85	**<0.0001**	1.27	0.26	0.063	0.80
Temp x CO_2	3.09	0.046	29.82	**<0.0001**	0.81	0.45	2.26	0.11
Temp x Soil moisture	5.84	**0.0030**	1.70	0.18	2.74	0.067	0.38	0.68
CO_2 x Soil moisture	20.54	**<0.0001**	1.01	0.32	5.71	0.018	0.008	0.93
Temp x CO_2 x Soil	6.82	**0.0011**	NA	NA	NA	NA	NA	NA
Red Maple								
Temperature	1.97	0.14	3.14	0.046	5.82	**0.0048**	1.00	0.38
CO_2	49.92	**<0.0001**	74.75	**<0.0001**	52.11	**<0.0001**	0.13	0.72
Soil moisture	38.78	**<0.0001**	2.45	0.12	7.72	**0.0072**	17.63	**<0.0001**
Temp x CO_2	0.41	0.66	4.57	0.012	0.59	0.56	4.25	0.019
Temp x Soil moisture	4.83	**0.0091**	4.73	0.010	5.01	**0.0096**	3.84	0.027
CO_2 x Soil moisture	0.72	0.40	3.38	0.068	0.14	0.71	0.44	0.51
Temp x CO_2 x Soil	NA	NA	NA	NA	NA	NA	NA	NA

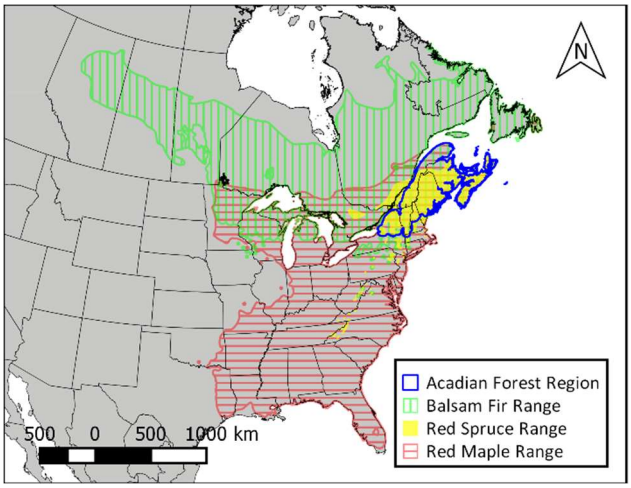


Fig. 3.1. Map showing the Acadian Forest Region (Two Countries, One Forest 2014) and the distribution of balsam fir, red spruce and red maple in Canada and the United States (Little 1971).

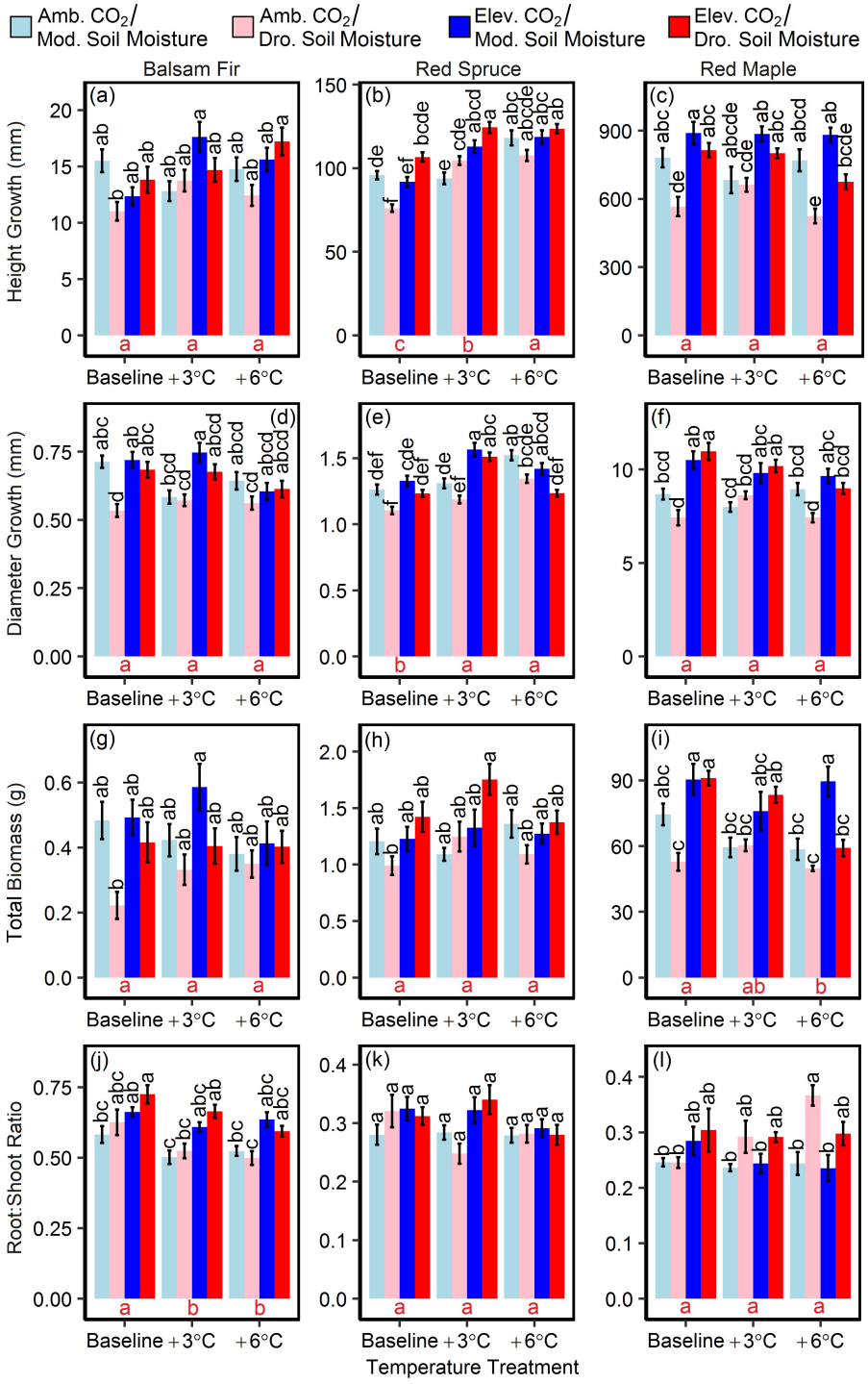


Fig. 3.2. Average height growth, root-collar diameter growth, total biomass, and root:shoot ratio of all species in all treatment combinations. Each panel compares a

response variable of one species to all combinations of chamber treatments. Colours represent CO_2 and soil moisture treatment combinations and error bars indicate standard error. Letter displays report the results of a Tukey test with a 99% confidence interval. Groups that share the same letters do not have a significant difference between means. Pairwise comparisons were conducted between temperature treatments (red letters) and between all treatment combinations (black letters).

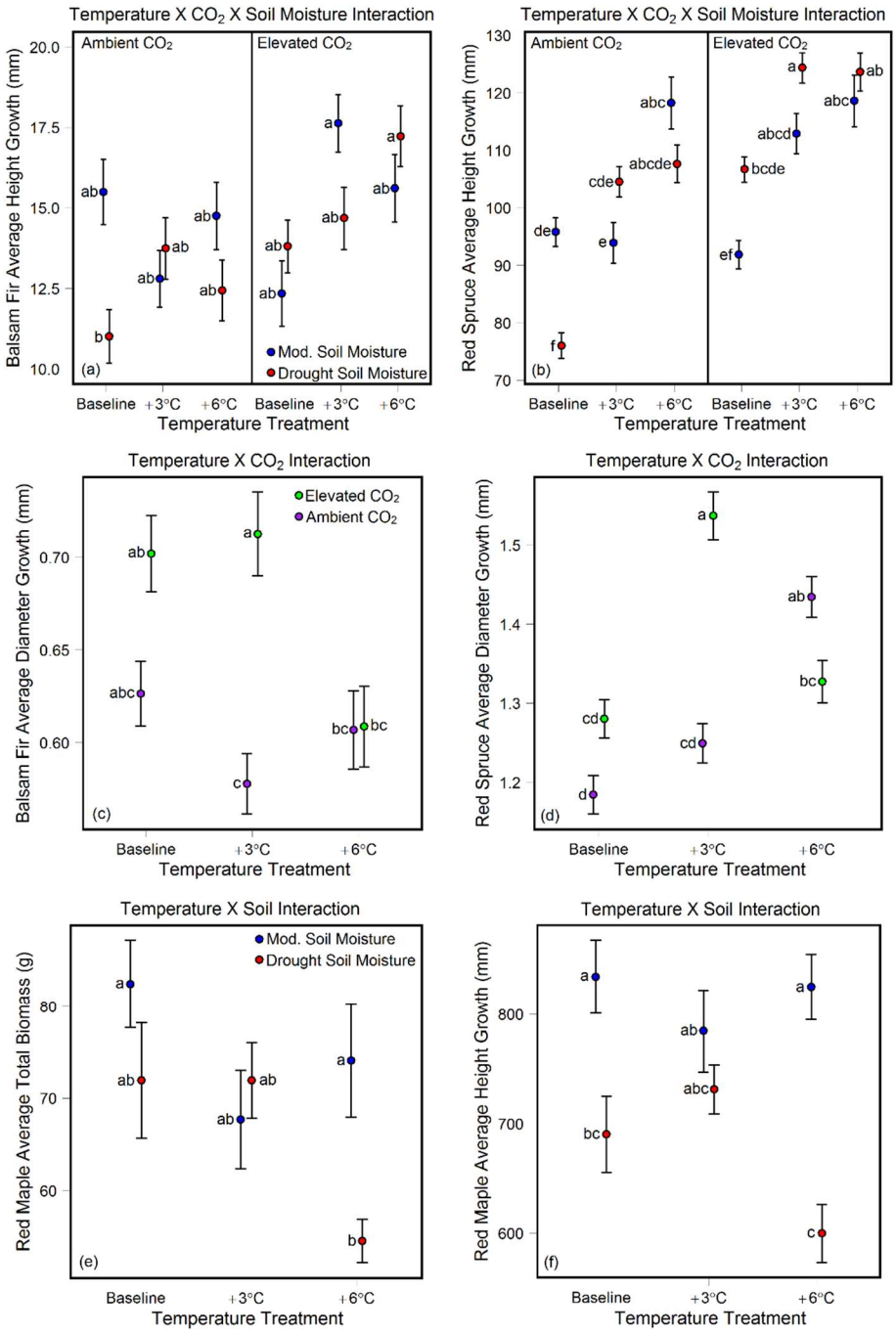


Fig. 3.3.Comparison of significant interactions between explanatory variables (temperature, CO_2 and soil moisture). Each panel demonstrates the effects of a significant two-way or three-way interaction on a response variable (average height growth, average diameter growth or average total biomass) for one species. Colours represent CO_2 and

soil moisture treatments and error bars indicate standard error. Letter displays report the results of a Tukey test with a 99% confidence interval. Points that share the same letters do not have a significant difference between means.

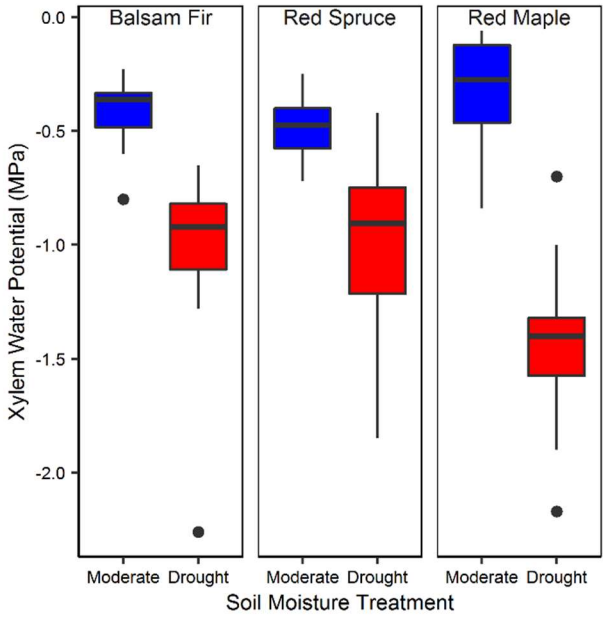


Fig. 3.4. Boxplots showing the xylem water potential for seedlings growing in moderate (blue) and drought (red) soil moisture treatments. The horizontal lines within the boxes are the medians, and the box represents the upper and lower quartiles. Upper and lower whiskers extend to the most extreme observed points above and below the box that fall within 1.5 times the interquartile range, and all points that fall outside of the whiskers are outliers.

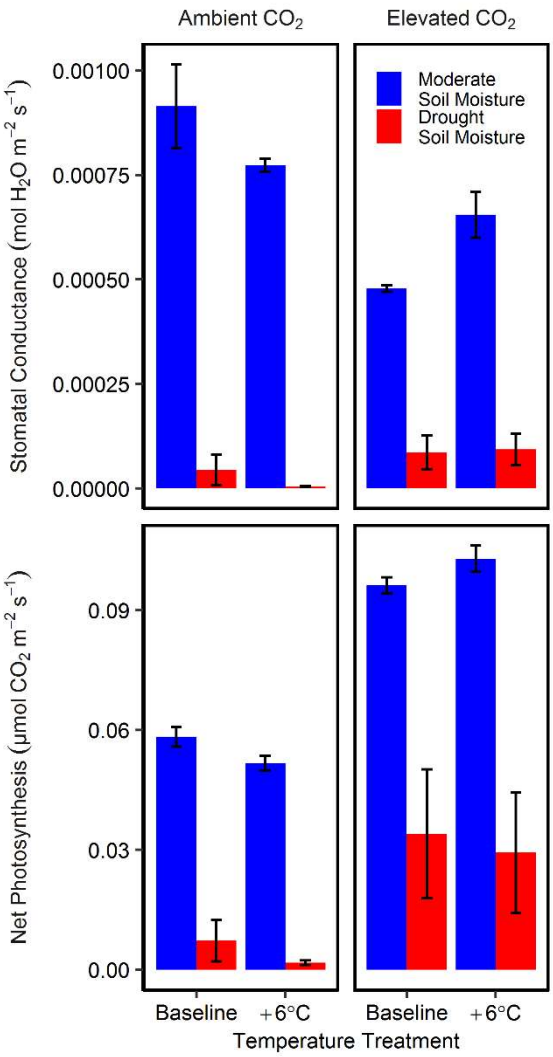


Fig. 3.5. Mean ± 1 standard error net photosynthesis and stomatal conductance of three balsam fir seedlings from ambient and elevated CO_2 treatments for the baseline and +6°C temperature and drought and moderate soil moisture treatments.

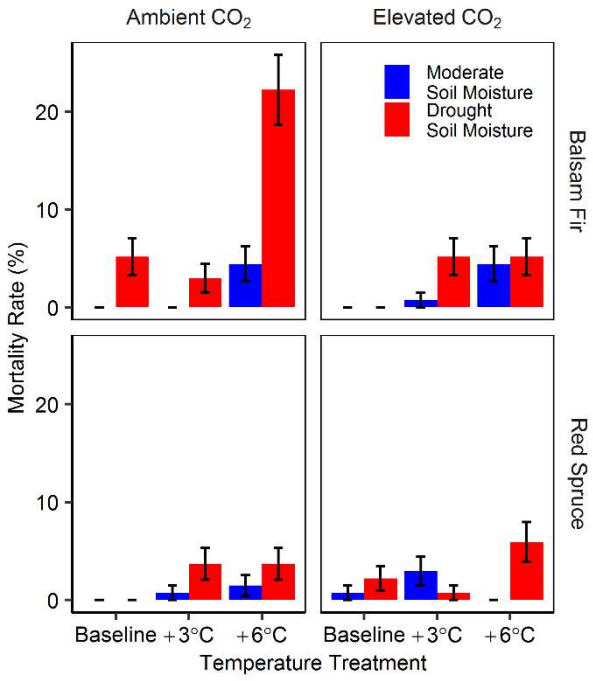


Fig. 3.6. Mean ± 1 standard error mortality of balsam fir and red spruce seedlings for temperature, CO2, and soil moisture treatment combinations. Red maple seedlings did not exhibit mortality.

CHAPTER 4: GENERAL DISCUSSION

4.1 Summary of results

This thesis examined the effects of projected climate change on germination and early developmental stages of tree species in the Acadian Forest Region under the RCP 8.5 radiative forcing scenario. Firstly, effects of winter warming on germination success of six common Acadian Forest tree species were examined. Overall, our results indicated that winter warming will not significantly affect germination success of balsam fir, red spruce, white pine, red maple, sugar maple and yellow birch at the species level. Interestingly, the response of some balsam fir seedlots varied under the warming treatment. Specifically, under the heated treatment, one of the balsam fir seedlots exhibited an 88.5% reduction in mean germination success while another seedlot increased by 37.7%. The random effect of seedlot explained much more of the variation in germination success of red maple, sugar maple and yellow birch than it did for balsam fir, red spruce and white pine.

The second experiment focused on the interactive effects of warming, elevated CO_2 and reduced soil moisture on growth and survival of balsam fir, red spruce and red maple, three economically important Acadian Forest tree species, under RCP 8.5. Red spruce exhibited a positive height growth response to warming; however, the extent of the response was influenced by CO_2 and soil moisture. While the effect of warming on balsam fir height growth varied with CO_2 and soil moisture, no clear temperature effect was observed. Unexpectedly, warming did not improve red maple height growth, however, average height growth was seven- to fifty-fold greater than the conifers. Under drought, balsam fir biomass decreased by 23.5%, while red maple biomass decreased by

26.4% under +6°C warming, and red spruce biomass did not show a response. Average height growth of droughted seedlings improved by 22.9, 23.1 and 30.7% under elevated CO_2 for balsam fir, red spruce and red maple, respectively. However, the positive effect of CO_2 was temperature-limited for conifer diameter growth, while red maple growth consistently improved under elevated CO_2, regardless of warming. Interestingly, under ambient CO_2, droughted red maple root:shoot ratio increased by 49.2% with warming, indicating a capacity to alter resource allocation in response to moisture stress. Balsam fir mortality was greater than red spruce in response to drought, and red maple did not exhibit mortality.

The results of our germination trial disagree with past field (Drescher and Thomas 2013) and lab studies (Solarik et al. 2016) that observed significant effects on germination success due to winter warming. Although I applied a 6.0°C warming treatment, the heated plots maintained average seedbed temperatures between -1.0 and 5.0°C for approximately 90 days, which studies have shown to be adequate for breaking balsam fir and sugar maple seed dormancy (Bonner and Karrfalt 2008, Solarik et al. 2016). In contrast, the experiments carried out by Drescher and Thomas (2013) and Solarik et al. (2016) observed seed temperatures that were outside of this optimal range; therefore, the poor germination successes observed in these studies is not unexpected. Because the average ambient air temperature that I observed was -3.4°C over the course of the trial, the heated treatment, which warmed seedbed surfaces to 6.0°C above ambient temperature, would not likely have resulted in unfavourable seedbed temperatures. The variable response to warming that I saw with balsam fir seedlots suggests that they may

differ in their sensitivity to warming. Although these seedlots were collected from trees that are in close proximity to one another, intraspecific variation in seedlot dormancy traits and dissimilarities in maternal climate may have affected seedlot germination characteristics (Bolnick et al. 2011, Walck et al. 2011, Penfield and MacGregor 2017). These effects may also explain why adding the random effect of seedlot explained much more of the variation in germination success for some species.

Under a warming climate, seedlings of balsam fir, red spruce and red maple may vary in terms of growth and survival. Red maple, predicted to become more abundant in the Acadian Forest under climate change (Ashraf et al. 2015, Taylor et al. 2017), did not show improved growth in response to warming, but maintained greater absolute growth relative to spruce and fir and was only negatively affected by warming when soil moisture was limiting. The drought soil moisture treatment generally reduced above-ground growth of all species; however, the negative impact on height growth was ameliorated under elevated CO_2 as a result of increased water use efficiency (Assmann 1993, Long et al. 2004). The temperature-limited diameter growth response of balsam fir and red spruce suggest that these species may improve with short-term projected warming but begin to decline as growing season temperatures continue to rise over the course of the 21st century. Moreover, with only balsam fir and red spruce exhibiting mortality, the results of our experiment align with past modelling studies (Boulanger et al. 2017, Taylor et al. 2017) and field experiments (Reich et al. 2015) that have predicted reductions in relative abundance of these species, along with other boreal species in the boreal-temperate transition zone, under a warming climate. Under elevated CO_2, red

maple growth consistently increased in all temperature and soil moisture treatments, suggesting that this species will continue to benefit from rising atmospheric CO_2 regardless of the warming that is projected to occur in the Acadian Forest Region. In addition, red maple did not exhibit mortality in any treatment and was the only species that altered resource allocation in response to increasing temperature and reduced water availability, which allows seedlings to avoid hydraulic stress (Brunner et al. 2015). Overall, these results suggest that red spruce may be better adapted than balsam fir to the long-term projected warming for the Acadian Forest, while red maple seedlings will likely outperform both conifers in terms of growth and survival.

4.2 Limitations and future research

Results from the seed experiment suggest the existence of high variability in germination success within local tree populations; however, due to the random nature in which seedlot locations were chosen around the City of Fredericton, it is difficult to determine what caused these variations. Likewise, growth and survival results observed in the seedling trial may have differed had seedlot locations been selected based on a specific criterion such as latitude, for example. With only running the seedling trial for one growing season, I was unable to answer questions regarding CO_2 acclimation and changes in the indeterminate growth patterns expressed in older seedlings. Additionally, any carry-over effects resulting from the environmental conditions during the first growing season, which are commonly referred to as legacy effects (Zweifel et al. 2020), were not able to be measured. To improve the quality of predictions regarding the effects of climate change on early developmental stages of tree species in the Acadian Forest Region,

testing individuals from a range of genetically diverse populations over multiple stages of ontogeny is required. To that end, seedlots should be selected throughout the Acadian Forest to assess the effects of both winter and summer warming on seeds and seedlings from geographically separated populations. Additionally, to determine the influence of maternal climate and the magnitude of intraspecific variation in seed dormancy characteristics, seeds that develop and mature under different environmental conditions should be tested. To examine the effects of summer warming on growth and survival of older seedlings, and to determine whether species differ in terms of CO_2 acclimation, expression of indeterminate growth patterns, and legacy effect, future seedling trials should run for multiple years with the same seedlings.

4.3 Management implications

Over the course of the 21st century, the Acadian Forest Region is expected to warm by roughly 6°C under RCP 8.5 (Environment Canada 2019). Our study demonstrated that seed germination is not likely to be hindered by this level of winter warming, however, rising temperatures during the growing season may affect seedling growth and survival of cold-adapted tree species. Specifically, the overall competitiveness of seedlings of balsam fir and red spruce, two economically important tree species in the Acadian Forest, may be reduced, while red maple, an important source of fuelwood and hardwood lumber (Nova Scotia Department of Natural Resources 2008), may be unaffected. While these cold-adapted tree species may continue to successfully regenerate from seed, sensitivity to climate warming during the growing season may reduce their abundance, which will significantly affect the forestry sector. To mitigate these impacts and prepare for future

conditions, adaptive management strategies, such as assisted migration or selective pre-commercial thinning treatments that favour warm-adapted temperate tree species (Gauthier et al. 2014), should be considered to maintain a continuous and sustainable wood supply.

4.4 References

Ashraf, M.I., Bourque, C.P-A., MacLean, D.A., Erdle, T., and Meng, F-R. 2015. Estimation of potential impacts of climate change on growth and yield of temperate tree species. Mitig. Adapt. Strat. Glob. Change 20:159-178.

Assmann, S.M. 1993. Signal transduction in guard cells. Annu. Rev. Cell Biol. 9:345-375.

Bonner, F.T., and Karrfalt, R.P. (Editors) 2008. The woody plant seed manual. U.S. Department of Agriculture, Forest Service, Washington, DC.

Boulanger, Y., Taylor, A.R., Price, D.T., Cyr, D., McGarrigle, E., Rammer, W., Sainte-Marie, G., Beaudoin, A., Guindon, L., and Mansuy, N. 2017. Climate change impacts on forest landscapes along the Canadian southern boreal forest transition zone. Landsc. Ecol. 32:1415-1431.

Brunner, I., Herzog, C., Dawes, M.A., Arend, M., and Sperisen, C. 2015. How tree roots respond to drought. Front. Plant Sci. 6:547.

Denney, D.A., Jameel, M.I., Bemmels, J.B., Rochford, M.E., and Anderson, J.T. 2020. Small spaces, big impacts: contributions of micro-environmental variation to population persistence under climate change. AoB Plants 12:plaa005.

Drescher, M., and Thomas, S.C. 2013. Snow cover manipulations alter survival of early life stages of cold-temperate tree species. Oikos 122:541-554.

Environment Canada 2019. Canadian Climate Data and Scenarios [online]. Available from http://climate-scenarios.canada.ca/index.php?page=main [accessed 28 May 2020].

Gauthier, S., Bernier, P., Burton, P.J., Edwards, J., Isaac, K., Isabel, N., Jayen, K., Le Goff, H., and Nelson, E.A. 2014. Climate change vulnerability and adaptation in the managed Canadian boreal forest. Environ. Rev. 22:256-285.

Long, S.P., Ainsworth, E.A., Rogers, A., and Ort, D.R. 2004. Rising atmospheric carbon dioxide: Plants FACE the Future. Annu. Rev. Plant Biol. 55:591-628.

Nova Scotia Department of Natural Resources 2008. State of the Forest Report 1995–2005, Nova Scotia Forests in Transition. Report FOR 3. Nova Scotia Department of Natural Resources, Truro, Nova Scotia.

Penfield, S., and MacGregor, D.R. 2017. Effects of environmental variation during seed production on seed dormancy and germination. J. Exp. Bot. 68:819-825.

Reich, P.B., Sendall, K.M., Rice, K., Rich, R.L., Stefanski, A., Hobbie, S.E., and Montgomery, R.A. 2015. Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. Nat. Clim. Change 5:148-152.

Solarik, K.A., Gravel, D., Ameztegui, A., Bergeron, Y., and Messier, C. 2016. Assessing tree germination resilience to global warming: a manipulative experiment using sugar maple (*Acer saccharum*). Seed Sci. Res. 26:153-164.

Taylor, A.R., Boulanger, Y., Price, D.T., Cyr, D., McGarrigle, E., Rammer, W., and Kershaw, J.A. 2017. Rapid 21st century climate change projected to shift

composition and growth of Canada's Acadian Forest Region. For. Ecol. Manage. 405:284-294.

Walck, J.L., Hidayati, S.N., Dixon, K.W., Thompson, K., and Poschlod, P. 2011. Climate change and plant regeneration from seed. Glob. Change Biol. 17:2145-2161.

Zweifel, R., Etzold, S., Sterck, F., Gessler, A., Anfodillo, T., Mencuccini, M., von Arx, G., Lazzarin, M., Haeni, M., Feichtinger, L., Meusburger, K., Knuesel, S., Walthert, L., Salmon, Y., Bose, A.K., Schoenbeck, L., Hug, C., De Girardi, N., Giuggiola, A., Schaub, M., and Rigling, A. 2020. Determinants of legacy effects in pine trees – implications from an irrigation-stop experiment. New Phytol. 227: 1081-1096.

Appendix

Supplementary data

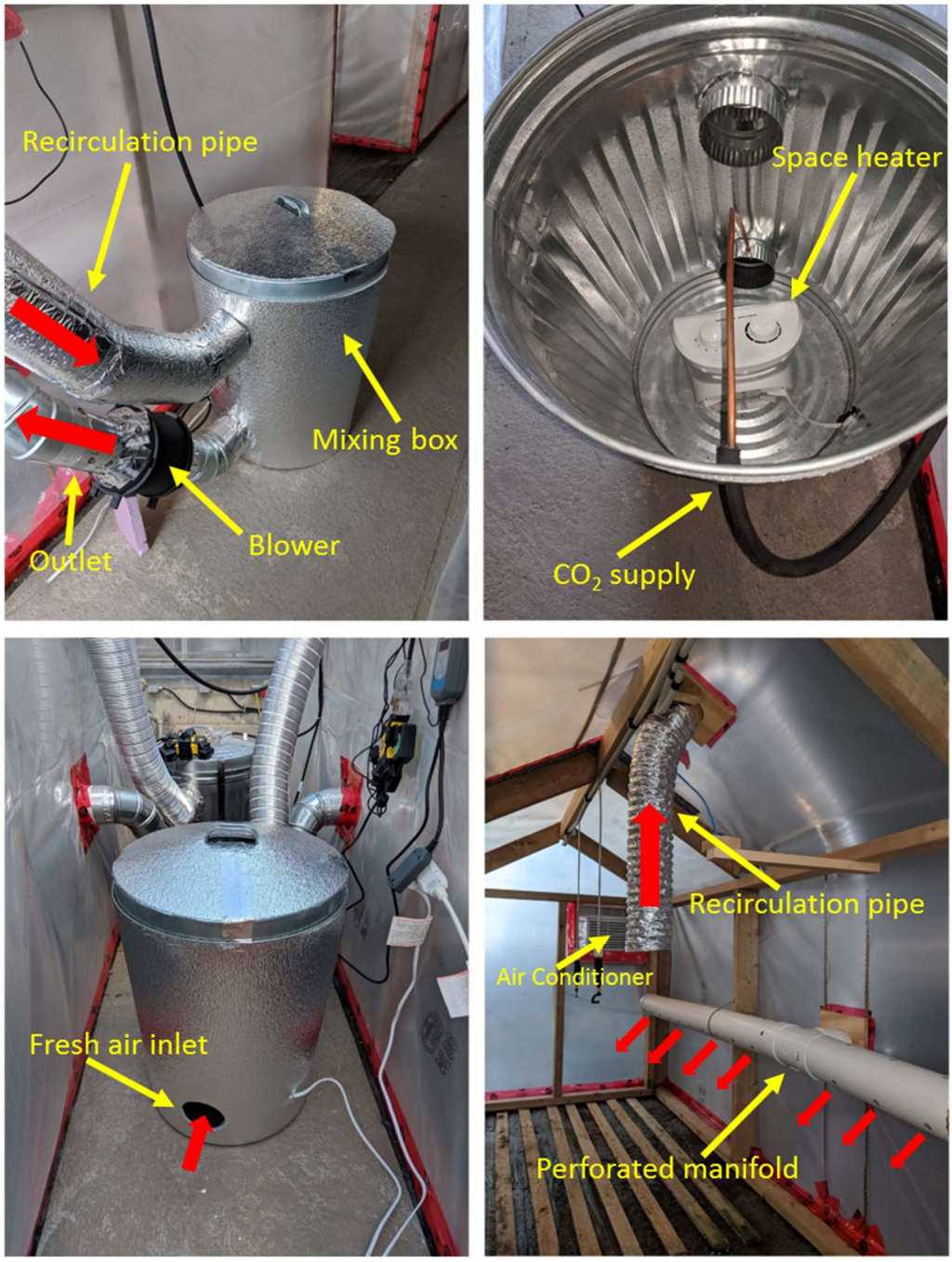


Fig. S1. Chamber ventilation design. Red arrows indicate the direction of airflow.

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