EFFECTS OF SOIL WARMING ON DECIDUOUS TREE PHENOLOGY IN THE GEORGIA PIEDMONT

by

REBECCA PARK

(Under the Direction of Jacqueline Mohan)

ABSTRACT

Phenology is the study of the annual and seasonal timing of plant and animal life cycle events, such as leaf-out and flowering of deciduous plants in the spring. Climate warming has shifted species distributions and advanced spring phenology worldwide, but questions remain about how responses to warming differ among populations across a species' natural range. In this two-year study, we examined intraspecific variation in survival, growth, and leaf-out phenology of red maple (*Acer rubrum*) seedlings in response to experimental forest soil warming. Overall, the provenances showed diverging responses to increases in temperature, indicating a genetic-by-environment interaction effect. In particular, the seedlings sourced from different regions displayed significantly different magnitudes of change in leaf unfolding date in response to the warming treatment. These findings support the premise that locally adapted populations respond differently to changes in temperature.

INDEX WORDS: Phenology, Climate change, Budburst, Leaf-out, Experimental soil warming, Phenological plasticity, Red maple (*Acer rubrum*)

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CHAPTER 1

INTRODUCTION

Phenology is the study of the annual and seasonal timing of plant and animal life cycle events, especially how it relates to weather and climate (Schwartz 2013). For deciduous trees, these phenological events include leaf-out and flowering in the spring, followed by senescence and abscission in the autumn. These events are controlled primarily by temperature, although photoperiod, precipitation, and biotic factors may contribute as well (Polgar & Primack 2011). My thesis examines the effects of experimental soil warming on deciduous tree phenology in a forest ecosystem in the Georgia Piedmont, USA.

Monitoring plant phenology is a popular approach for studying the effects of recent climate change because changes in plant phenology are some of the most sensitive and observable biological responses to climate change (Schwartz 2013). Unlike changes in population size or distribution, phenological changes can be relatively easy to detect, which is one reason for their popularity in climate change research (Polgar & Primack 2011, Schwartz 2013).

Scientists and naturalists have been monitoring leafing and flowering time in plants for hundreds of years. In Japan, records of the flowering dates of cherry trees date back to 705 BCE (Aono & Kazui 2008). In Europe, the earliest records of flowering date back to 371 BCE (Sparks et al. 2009). In North America, there is an extensive dataset of leaf-out dates compiled by Henry David Thoreau for the plant communities of Concord, Massachusetts in the 1850's (Miller-

Rushing & Primack 2008), and the dates of leaf emergence of individual trees and shrubs of several common species have been monitored at Harvard Forest each spring since 1990 (Richardson et al. 2009).

The close relationship between plant phenology and temperature leads to large annual variations in the timing of spring onset; however, while interannual variation is expected, anthropogenic climate change has led to significant directional changes in the onset of spring globally that cannot be attributed to normal variation alone (Ibáñez et al. 2010, Polgar & Primack 2011). This phenological shift is largely attributed to global climate warming, which has proceeded at ~0.1° C per decade since 1960, and faster in recent decades (Intergovernmental Panel on Climate Change (IPCC) 2021). In fact, the association between climatic warming and a seasonal advance in phenology has been documented across a wide range of taxa (Parmesan & Yohe 2003, Root et al. 2003).

Observational studies and experimental evidence agree that rising global temperatures have altered plant phenology such as flowering, germination, and leaf-out (Wolkovich et al. 2012, Piao et al. 2019). Data from long-term observational datasets show that during the last 50 years, leaf unfolding has, on average, advanced 1-3 days per decade, and leaf senescence has been delayed by 1-2 days per decade (Chung et al. 2013, Parmesan 2007). For example, in North America, at the Hubbard Brook Experimental Forest in New Hampshire, the onset of leaf-out of American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), and yellow birch (*Betula alleghaniensis*) has advanced on average 5-10 days over the past five decades (Richardson et al. 2006).

It is worth noting that shifts in phenology are not the only response to anthropogenic climate change experienced by plants. Other types of trait changes include: (a) an increase in species density poleward or upslope as species ranges shift to occupy areas within their metabolic temperature tolerances, (b) changes in morphology or behavior, or (c) a shift in gene frequencies (Root et al. 2003). It seems likely that climate change will impose strong selection on complex polygenic traits, potentially favoring phenological, physiological, and morphological trait values that enable stress tolerance and avoidance (Anderson et al. 2012). Moreover, given that higher latitudes have warmed more than the lower latitudes in recent decades, we may expect phenological responses to be larger near the poles and less pronounced closer to the equator (Root et al. 2003). However, other abiotic conditions, such as photoperiod and edaphic characteristics, will remain at historical levels, leading to future environments that have no current analog (Anderson et al. 2012).

Effects of phenology in deciduous forest ecosystems

Changes in vegetative phenology have implications for deciduous forests at the population, community, ecosystem, and global scales. Understanding how climatic warming influences deciduous tree phenology is important because forests provide numerous ecosystem services such as harboring biodiversity, regulating hydrologic cycles, sequestering carbon, and protecting soils from erosion (Brockerhoff et al. 2017). Forests cover approximately 30% of the world’s land surface and contribute approximately 50% of the net primary production (NPP) in terrestrial ecosystems (Chung et al. 2013). Thus, understanding the role of phenology in forest ecology is crucial to enhancing our understanding and management of this valuable resource.

At the individual level, the phenology of deciduous tree species in temperate climates is determined by local biotic and abiotic factors. The timing of spring leaf-out and fall senescence may affect individual plants by allowing for greater rates of photosynthesis, influencing production of biomass, or by putting the plant at risk of frost damage (Polgar & Primack 2011). In fact, several plant and ecosystem models suggest an increased risk of frost damage as a possible effect of climatic warming (Chamberlain et al. 2019). For example, in eastern and central North America in 2007, abnormally warm weather in March triggered early leaf-out. Subsequent freezing temperatures in early April caused the young leaves and flowers of woody plants to suffer serious frost damage. The immediate result was a dieback of young growth, and that year the canopy failed to reach the stage of development or NPP seen in other years (Gu et al. 2008).

At the population and community levels, changes in phenology also have important implications for species abundance and distribution (Chuine 2010, Körner et al. 2016, Iler et al. 2021). For example, if species with minimal photoperiod and chilling requirements continue to leaf out earlier in the spring, they may increase their abundance and distribution (Polgar & Primack 2011). Different phenological responses to temperature change among species could ultimately affect their competitive abilities and distributions (Chuine 2010, Vitasse et al. 2013). It is also important to note that species which successfully extend their ranges into previously unoccupied habitats will experience novel selective pressures to which they are not currently adapted (Anderson & Song 2020).

Another concern is the possibility of ecological mismatches between different groups of interdependent organisms because of rapid climatic changes (Both et al. 2009). For example, the time at which deciduous trees begin to leaf-out determines the availability of food and shelter for many species, particularly insects (Parmesan 2006, Polgar & Primack 2011). When interactions between plants and herbivorous insects are disrupted, animals such as birds, mammals, and spiders that depend on those insects for food may similarly decline in abundance, creating cascading effects through multiple trophic levels (Both et al. 2009, Polgar & Primack 2011). Additionally, if canopy trees leaf-out earlier in spring, the reduced light and water availability on the forest floor could have negative consequences for herbaceous understory plants, which generally need high intensities of light early in the growing season to complete their life cycle (Heberling et al. 2019). These types of changes in species interactions could have profound impacts on forest communities.

At the ecosystem level, climatic warming has lengthened the growing season by two days per decade since the 1950s in the Northern Hemisphere extratropics (IPCC 2021). The length of the growing season can be an important determinant of annual NPP (Chapin et al. 2012). A commonly held (albeit simplistic) view of the relationship between growing season length and carbon (C) sequestration is that if leaf-out occurs earlier in the spring & senescence later in the autumn, there will be an increase in the net amount of C sequestered in the forest (Richardson et al. 2010). Over two decades ago, Keeling et al. (1996) observed that the amplitude of the seasonal CO_2 cycle in the northern hemisphere has been increasing since the 1960's, and that the springtime decline in CO_2 has advanced by about 7 days, suggesting a lengthening of the growing season. However, the exact effect of phenological shifts on ecosystem processes is not always straightforward, and it could affect productivity in several ways (Richardson et al. 2010). For example, under warmer temperatures, leaves may show earlier expansion and delayed senescence (longer growing season), but facing severe high temperature stress, many plants decrease stomatal conductance and hence photosynthetic activity, especially at midday (Chung et al. 2013). These phenological changes not only impact the global C budget but also feedback to Earth's climate through changes in evapotranspiration and surface albedo (Richardson et al. 2013).

In sum, the timing of leaf flushing and the corresponding growing season length can have important impacts on all ecosystem processes, including the uptake of CO_2, tree growth, microclimate, and water movement (Polgar & Primack 2011). To fully understand the effect of climate change on forest ecosystems, we must consider not only the direct effect of temperature on leaf phenology but also how changes in leaf phenology subsequently affect plant growth and allocation to reproduction, light availability to other plants via shading, interactions with insects and pathogens, and CO_2 exchange between the atmosphere and ecosystems (Chung et al. 2013).

Ultimately, understanding ecological and evolutionary responses to climate change will enable more informed conservation and management decisions (Aitken et al. 2007). Additionally, as more data on phenological responses to climate change emerge, and a better understanding of physiological mechanisms controlling leaf-out develop, more accurate models of ecosystem dynamics will be possible (Polgar & Primack 2011). Understanding multiple factors such as the mechanisms and controls regulating leaf-out, how these mechanisms differ among species, and how the timing of leaf-out in plant species and populations will be affected by climatic changes, would be helpful in the management and conservation of natural areas and in forecasting future changes in the C budgets of ecosystems (Polgar & Primack 2011).

Experimental Ecosystem Warming

Experimental warming studies provide us with a useful tool to examine the cascade of ecological processes in forest ecosystems that change with increased temperatures (Chung et al. 2013). Experimental warming enables us to directly test the effects of increasing temperature on ecosystem functions with fewer confounding factors (*i.e.*, other variables that covary spatially and temporally with temperature) (Chung et al. 2013). Warming experiments have been used to extrapolate to future climate conditions for more than 25 years (e.g., Harte & Shaw 1995, Chapin & Shaver 1996, Mellilo et al. 2017). Results from warming experiments can be studied on their own, be used to validate model predictions, and serve as crucial information in constructing models (Rustad et al. 2001, Polgar & Primack 2011).

Several different methods of experimental warming can be used, such as (a) branch heating cables, (b) soil heating cables, (c) open-top canopy chamber, or (d) infrared (IR) lamp (Chung et al. 2013). In a meta-analysis by Wolkovich et al. (2012), experimental designs using above-canopy heating tended to produce the greatest phenological advances for flowering; otherwise, researchers found no evidence of differences in the sensitivities between passive and active designs (Wolkovich et al. 2012). A separate meta-analysis by Chung et al. (2013) found that across all warming methods investigated, effects on phenology depended more on the tree species or forest ecosystems in which the warming experiment was performed, and the degree of warming, than the method used. Conversely, a recent experimental study by Vitasse et al. (2021) convincingly demonstrated that temperature and light conditions are sensed locally at the bud level through an interesting technique of painting the buds black or white to influence bud albedo.

Experimental field manipulations have demonstrated that climate shifts alter ecological processes (Anderson & Song 2020). For example, the aforementioned meta-analysis by Chung et al. (2013) found that in response to higher temperatures in boreal and temperate forests, (1) leaves emerged earlier and senesced later, (2) the abundance of herbivorous insects increased, and their performance was enhanced, and (3) soil nitrogen mineralization and leaf litter decomposition were accelerated. Moreover, experimental warming causes shifts in plant communities that resemble those observed in longitudinal studies, demonstrating the ecological relevance of experimentally manipulating climatic factors (Elmendorf et al. 2015). However, a relevant meta-analysis suggests that warming experiments underpredict long-term shifts in phenology: Wolkovich et al. (2012) found that for the phenology of 1634 plant species, warming experiments underpredict advances in the timing of flowering and leafing by 8.5-fold and 4.0-fold, respectively, compared with long-term observations. That is to say, current estimates of phenology advancement are 1.9-3.3 days per °C for experiments and 2.5-5.0 days per °C for observations (Wolkovich et al. 2012). The difference between observational studies and experimental warming may be partially explained by a bias in the scientific literature against publishing "non-significant results," particularly for relatively less expensive observational studies (Machmuller et al. 2018). More specifically, experimental studies may be more likely to be published even without significant results, which would contribute to the discrepancy seen in Wolkovich et al. (2012).

In sum, experimental warming is a useful tool for testing whether trends observed in natural populations can be attributed to temperature. However, it is important to note that although warming methods have continually improved, all experimental manipulations unavoidably alter additional environmental factors. For example, the most common passive warming structures, open-top chambers, reduce light, wind, and often soil moisture (Wolkovich et al. 2012). One ongoing question is the role of microclimate (e.g., light and temperature) and resource availability (e.g., nutrient and water) on plants' phenological cycles (Vitasse et al. 2021). Ultimately, an enhanced understanding of phenological responses to climate change will come from a combination of experimental and observational evidence at the individual, community, and ecosystem levels.

CHAPTER 2

GEOGRAPHIC VARIATION AND PLASTICITY IN RED MAPLE (ACER RUBRUM) SEEDLING GROWTH, SURVIVORSHIP, AND PHENOLOGY[1]

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[1] Park, Rebecca, Megan DeMarche, Ricardo Holdo, Paul Frankson, and Jacqueline Mohan. To be submitted to *Global Change Biology*.

Abstract

Species with broad geographical ranges often demonstrate specific adaptations to local conditions, which may result in diverging responses among populations to climate change. Climate warming has shifted species distributions and advanced spring phenology worldwide, but questions remain about how responses to warming differ among populations across a species' natural range. In this two-year study, we examined intraspecific variation in survival, growth, and leaf-out phenology of red maple (*Acer rubrum*) seedlings in response to experimental forest soil warming. *Acer rubrum* is the most widely distributed and abundant tree species in eastern North America, displaying high levels of adaptability to a wide range of environments. In April 2020, two-year-old red maple seedlings sourced from three physiographic regions across the Southeastern United States (Coastal Plain, Piedmont, and Mountains) were transplanted into experimental forest soil warming plots located in the Georgia Piedmont. The seedlings were measured for size and survivorship in Fall 2020 and Fall 2021, and the leaf-out timing was recorded in Spring 2021. Overall, the provenances showed diverging responses to increases in temperature, indicating a genetic-by-environment interaction effect. In particular, the seedlings sourced from different regions displayed significantly different magnitudes of change in leaf unfolding date in response to the warming treatment. The difference in phenological plasticity may reflect geographic genetic differences in seedling response to environmental cues such as winter chilling and spring warming. These findings support the premise that locally adapted populations respond differently to changes in temperature, thus climate warming experiments need to consider individuals arising from multiple regions in order to forecast the response of a species.

Introduction

Climate warming up to 5° C is predicted to affect temperate deciduous forests in the next century (Intergovernmental Panel on Climate Change (IPCC) 2021). Numerous studies have used experimental warming to understand how temperature changes affect tree species and forest ecosystem processes (Chung et al. 2013). Broadly, tree species with wide geographical ranges often exhibit specific adaptations to local climates; locally adapted populations may show diverging responses to changing conditions (Patsiou et al. 2020). Climate change has shifted species distributions and advanced spring phenology worldwide (Parmesan & Yohe 2003, Root et al. 2003), but questions remain about whether and how responses to warming differ among individuals across the natural range of a species (Ensing & Eckert 2019, Gauzere et al. 2020, Zettlemoyer & Peterson 2021).

In this two-year study, we examined intraspecific variation in survival, growth, and spring leaf-out phenology of red maple (*Acer rubrum* L.) seedlings in response to experimental forest soil warming. Soil temperatures are important at the tree seedling stage because the low growth form of seedlings more closely track ground-level microclimate conditions (Körner 2003, Wheeler et al. 2016). Understanding how seedling performance changes in response to warmer soils is important for predicting future forest composition and diversity (Mohan et al. 2007, Clark et al. 2010). In addition to growth increment, leaf-out phenology plays an important role in deciduous tree fitness (Chuine 2010, Polgar & Primack 2011). Bud and leaf development in deciduous trees is an adaptive trait that ensures the maximization of growing season length while minimizing the risk of frost damage (Polgar & Primack 2011). Specifically, spring leaf-out phenology is known to be a heritable trait with high variability within species and populations, although the exact combination of genes and environmental factors involved in triggering leaf-out is largely unknown for most species (Howe et al. 2003, Vitasse et al. 2021). To our knowledge, this is the first study which examines the growth and phenology responses of intraspecific red maple populations to an experimental soil warming treatment. The combination of different populations with an experimental warming treatment allowed us to not only examine the geographic variation in thermal response, but also directly examine the environmental variation due to temperature.

Genotypic variation is one source of phenotypic variation that is often reflected as local adaptation. That is, specific plant populations are often adapted to highly local conditions (Savolainen et al. 2007, Anderson et al. 2012). Thus, individuals of the same species from different populations often have different requirements for growth increment and leaf-out based on geographic location (Polgar & Primack 2011). Over the past century, researchers have studied local adaptation in trees with the establishment of common garden experiments in which individuals from many provenances (populations from well-defined geographic areas) are grown at multiple sites (Savolainen et al. 2007). While historically these provenance trials were mostly established to provide information for forestry management, there is now renewed interest to assess the ability of tree populations to cope with climate change (Sáenz-Romero et al. 2017, Patsiou et al. 2020). Numerous common garden experiments have shown genetic differentiation among forest tree populations from different elevational or latitudinal provenances (Morgenstern 1996, Alberto et al. 2013, Sáenz-Romero et al. 2017, Patsiou et al. 2020).

Although plant populations are often highly locally adapted, many species also display phenotypic plasticity such that individual plants can alter their phenotypes in response to the environment they encounter (Anderson & Song 2020). This phenotypic variation due to the environment enables genotypes to prosper in heterogeneous environments by adjusting trait values to suit specific local conditions (Anderson et al. 2012). This plasticity will likely play an important role in the persistence of species in rapidly changing environments (*i.e.*, species can alter their phenotypes faster through plasticity than through adaptation) (Anderson et al. 2012). Since future warmer temperatures are inevitable, it is crucial to understand how common tree species will respond (Ellison et al. 2005, Chung et al. 2013). Some previous experimental soil warming studies have shown a positive effect of warming on woody seedling growth (Rogiers et al. 2014, Wheeler et al. 2016), while others have observed a detrimental effect (Danyagri & Dang 2014). As for leaf-out phenology, observational studies and experimental evidence agree that increasing temperatures generally advance spring phenological events such as leaf emergence in deciduous trees (Wolkovich et al. 2012).

One ongoing question is whether populations growing across a species' natural range have similar capacities to respond to environmental change such as warming temperatures (Zettlemoyer & Peterson 2021). Although numerous studies have reported genetic differentiation among tree populations from contrasting regions, only a few have addressed whether these populations also diverge in their phenotypic plasticity (Anderson & Song 2020). In other words, the variance due to the interaction of genotypes with the environment reflects genetic diversity in plasticity and is, thus, assumed to represent the heritable component of phenotypic plasticity (Schlichting 1986). It follows that some provenances (*i.e.*, genotypes) may show more and others less phenotypic plasticity in response to the same environmental gradient. For example, in a study of seven European tree species, Vitasse et al. (2013) found that tree populations sourced from lower elevations exhibited systematically greater plasticity of leaf-out date in response to temperature changes than populations from high elevations. The authors suggest that this difference may be attributed to the risk of frost damage varying across the elevational gradients;

put simply, high elevation individuals may demonstrate more conservative plasticity because the risk of frost damage increases with higher elevations (Vitasse et al. 2013).

This study examines the phenotypic variance due to seed source region, variance due to the temperature treatment, and variance due to the region-by-temperature interaction effect for *A. rubrum* seedlings sourced from different physiographic regions spanning 650 kilometers across Southeastern North America, with over 500 meters of difference in elevation. Specifically, we investigated the intraspecific differences in seedling growth, survival, and leaf-out phenology in response to experimental forest soil warming. We hypothesized that local adaptation has led to genetic differentiation in thermal response among red maple populations sourced from different physiographic regions (*i.e.,* regional variance). Furthermore, we predicted that experimental warming would induce earlier leaf-out in the seedlings (*i.e.*, environmental variance). Finally, we hypothesized that seedlings sourced from different physiographic regions would exhibit different degrees of phenological plasticity, reflecting a trade-off between maximizing growing season and avoiding frost damages (*i.e.*, region-by-environment interaction).

Materials and methods

Study species

Acer rubrum is a common deciduous tree species in eastern North American hardwood forests, occurring across a wide range of soil types, moisture, pH, and elevation (Walters & Yawney 1990). Its broad range spans 24° of latitude, 40° of longitude, and from sea level to 1370 m elevation (Sargent 1922, Walters & Yawney 1990). Moreover, red maple is becoming increasingly dominant in many forests in response to long-term fire suppression, land use changes, and reduced competition through deer browsing, insect herbivory, and pathogens (Abrams 1998). It has been described as a “super generalist” due to its high adaptability in many physiological and growth characteristics including cold hardiness, time of leaf-out and budset, growth rate, stem form, fall coloration, and drought tolerance (Abrams 1998). In fact, numerous studies have demonstrated great genetic and phenotypic variation among and within populations, as well as clinal variation in ecological traits such as phenology and growth related to temperature, length of growing season, site moisture, and atmospheric CO_2 (e.g., Perry & Wu 1960, Bauerle et al. 2003, Norby et al. 2003, Mohan et al. 2004, Townsend et al. 2011). Furthermore, a recent study demonstrated that *A. rubrum* shows the greatest adaptive capacity to climate change among the 26 northeastern North American tree species included in the study (Royer-Tardif et al. 2021). The extensive range and diversity within this species make it an interesting and relevant study species for examining intraspecific responses to warming. Moreover, understanding how this common tree species responds to warming is critical to predicting future consequences for North American deciduous forests (Abrams 1998, Mohan et al. 2004, Ellison et al. 2005, Royer-Tardif et al. 2021).

Seed collection

In April 2018, seeds were collected from *A. rubrum* trees from different physiographic regions across the southeastern United States. The three physiographic regions are the Coastal Plains of North Carolina (CP), the Piedmont of North Carolina (PD), and the Mountains of northern Georgia (MT) (Figure 1, Table 1). Populations within each region were separated from each other by at least 1 km, a distance greater than the relevant pollen source area (Calcote 1995) and the transport of wind-dispersed seeds known as samaras (Nave et al. 2021). Within each population, seeds were collected from one or more seed-producing maternal trees occurring within a 20 m^2 area, such that those individuals could have exchanged pollen. Seeds borne on a single maternal tree may result from pollen dispersed from different paternal trees; thus, seeds collected from a single tree are at least half-sibs, representing a specific maternal family (Mohan et al. 2004, Primack 2004). In principle, it would have been better to have a larger sample of maternal trees from each population, but the sample size was constrained by the number of trees bearing attainable seeds within an area small enough that genetic exchange was likely, as well as the ripeness of seeds at the time of collection. Many of the seeds that were collected were either under-ripe or over-ripe, causing a relatively low germination rate (Appendix S1). When possible, seeds were shaken from tree branches; otherwise, seeds were collected near the base of the tree. In these cases, only maternal trees that were separated by more than 15 m from other seed trees were used to avoid familial contamination. Seeds were stored at 4°C between collection and planting.

Depending on the success of germination, some regions were represented by fewer individuals (Appendix S1). The individuals that germinated and survived well enough to be included in this study represent seeds collected from 4 trees in the Coastal Plains (CP) representing 3 populations; from 5 trees in the Piedmont (PD) representing 3 populations; and from 4 trees in the Mountains (MT) representing 2 populations (Table 1).

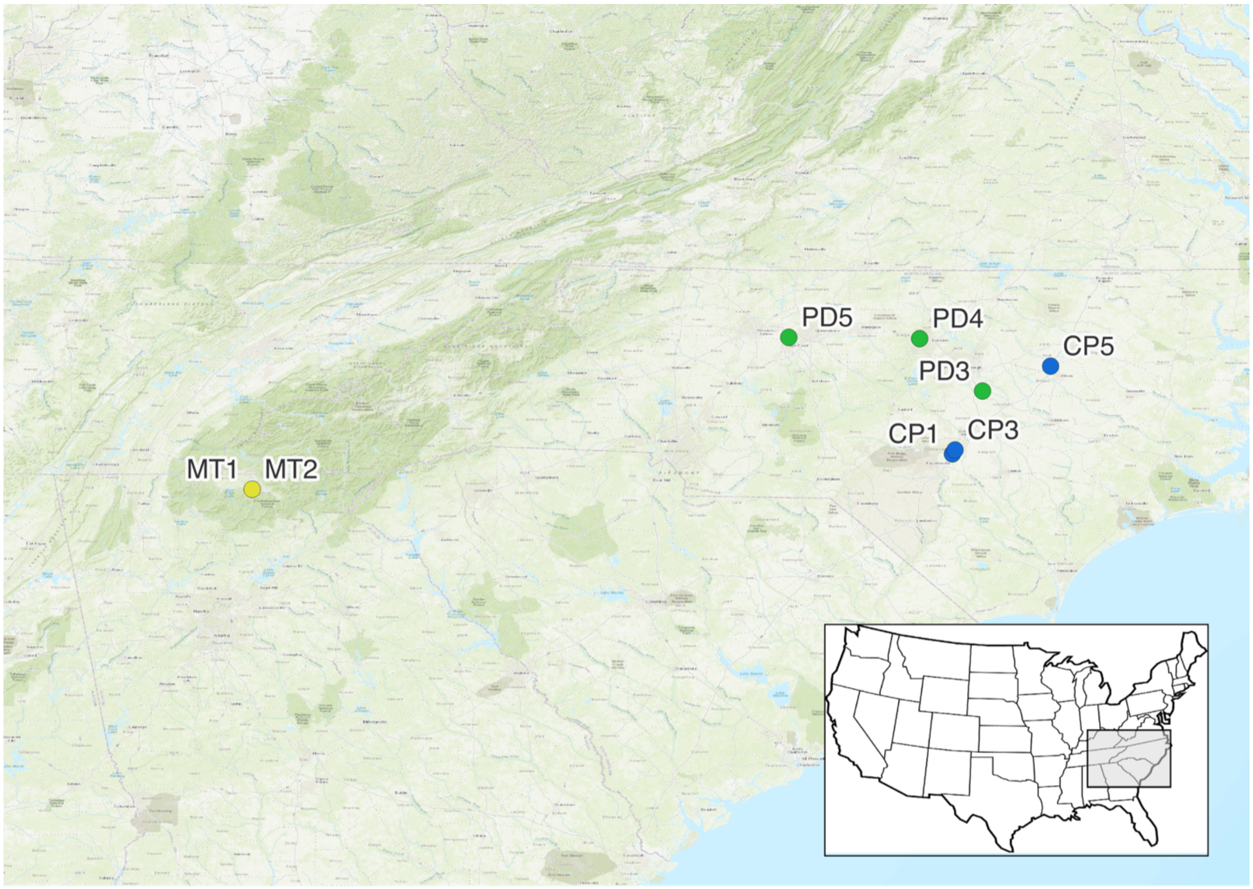

Fig. 1: Location of 8 *Acer rubrum* populations from 3 physiographic regions: Coastal Plain (CP), Piedmont (PD), and Mountains (MT).

Table 1: Site descriptions of the three regions and eight populations used as sources of *Acer rubrum* seeds. Temperature data represents 30-year normals describing average monthly conditions for each point location, retrieved from PRISM Climate Group, Oregon State University, https://prism.oregonstate.edu, data accessed April 2022.

Region	Population	Elevation above sea level (m)	Mean January temperature 1991-2020 (°C)	Mean July temperature 1991-2020 (°C)	Longitude and latitude
Coastal Plain, NC ('CP')	CP1	40	5.9	27.2	35°8'24" N 78°44'9" W
	CP3	42	5.9	27.2	35°10'9" N 78°43'26" W
	CP5	47	5.2	26.7	35°48'22" N 77°59'18" W
Piedmont, NC ('PD')	PD3	100	5.1	26.4	35°37'20" N 78°30'43" W
	PD4	162	4.4	25.8	36°1'10" N 78°59'21" W
	PD5	251	4.1	25.8	36°1'50" N 79°59'21" W
Mountains, GA ('MT')	MT1	581	3.2	23.1	34°52'12" N 84°5'42" W
	MT2	581	3.2	23.1	34°52'17" N 84°5'29" W

Germination and transplantation

The red maple seeds germinated in the summer of 2018 in unfertilized greenhouse soil mix in plant trays in a greenhouse. Red maple seeds do not require pregermination treatment and can germinate immediately after ripening (Walters & Yawney 1990). For the 2018 and 2019 growing seasons, the seedlings grew in a controlled greenhouse environment (Appendix S2). The seedlings were grown in Metro-Mix Professional Growing Mix, a soilless substrate containing vermiculite, sphagnum, and perlite. The seedlings were watered approximately daily and did not receive any additional fertilization. Although a large portion of the seeds collected did not germinate, we found no significant maternal effect based on the relationship of seed size to seedling height based on those that did successfully germinate (Appendix S1).

In April 2020, we transplanted the seedlings to the Whitehall Forest Soil Warming Facility (WFWF), located in the Piedmont region of Georgia (33°53’17.87”N, 83°21’40.92”W). Like most of the southeastern Piedmont, the site was previously used for agriculture and then abandoned in the early 1900s. The forest is dominated by upland hardwood species such as *Quercus alba* (white oak), *Quercus rubra* (red oak), *Liquidambar styraciflua* (sweetgum), and *Liriodendron tulipifera* (tulip poplar). The soils are classified as Typic Kanhapludults with 64% sand, 18% silt, 18% clay, low organic matter content (2.3% C, 0.09% N), bulk density of 0.87 g m^{-3}, and a pH of ~4.5 (0-10 cm) (Machmuller et al. 2016).

WFWF contains 9 open-top field chambers (5.2 × 3.5 m, or 18.2 m^2) in a shaded hardwood forest setting. There are 3 chambers warmed at +3°C, 3 chambers warmed at +5°C, and 3 ambient control chambers. The chambers are warmed with buried resistance heating cables; control chambers contain buried cables, but the cables are not turned on. The chambers are grouped into blocks (n = 3; one treatment per block) within specific landscape positions (upslope, midslope, and downslope). In each chamber, a TDR moisture sensor (Campbell Scientific (CS), Model CS616 water content reflectometer) and 3 temperature probes (CS, Type 109 thermistors), placed 10 cm deep, continuously measure soil moisture and temperature at 10-minute intervals with hourly averages recorded. Similarly, air temperature, relative humidity (Precon HS-2000V capacitive polymer sensors), and incoming photosynthetically active radiation (PAR) measurements (Apogee Model SQ110) are quantified and recorded.

Out of the 832 seeds that germinated in the greenhouse, 407 of those individuals survived to transplant in April 2020 (Appendix S1). We divided these individuals as equally as possible between the 9 chambers to create a complete block design of seedlings from each individual maternal tree represented in each temperature treatment. The sample size of each region depended on the number of seeds collected as well as the number that germinated; this discrepancy led to a highly unbalanced complete block design (Table 2).

Table 2: Complete block design. The experiment began with n = 407 individuals that survived to transplant in the warming chambers.

Seed source regions	Ambient	+3° C	+5° C
Coastal Plain	n = 46	n = 48	n = 44
Piedmont	n = 60	n = 53	n = 59
Mountains	n = 34	n = 30	n = 33

Response variable 1: Growth and survivorship

At the end of both the 2020 and 2021 growing seasons, we measured seedling sizes (heights) and recorded survivorship. In October 2020, we recorded the 2018 and 2019 heights using the terminal bud scars; we measured the 2020 height as the height from the root flare to the tallest apical bud. Additionally, we recorded the stem diameter at 5-cm height using digital calipers (6-inch Caliper, Model H-7352, General Tools & Instruments, Secaucus, NJ). In October 2021 we measured height and stem diameter at 5 cm using the same methods.

We recorded survival as a binomial variable. The cumulative survival rate describes the overall survival for all individuals over both growing seasons after transplant (n = 407). We calculated the cumulative relative growth rate (r) after transplanting to the warming chambers was calculated as: $r = \ln(H_{2021}) - \ln(H_{2019})$. Thus, this value represents the total cumulative growth rate for all individuals that survived both growing seasons (2020, 2021) in the warming chambers (n = 255).

Response variable 2: Phenology

In Spring 2021, we collected leaf-out phenology data for the red maple seedlings by observing the leaf bud burst. Through visual observation of the terminal buds on each seedling, we assigned a score representing the six stages of development as described by Norby et al. (2003) as the bud swells, breaks open, and the leaf unfurls (Figure 2). We assigned the score to each seedling based on the most advanced bud present at the time of observation. Additionally, as a separate metric, we recorded the percentage of buds that had opened on each seedling as "% bud break." We conducted the visual assessments every other day as the trees broke their winter dormancy during the months of March and April. The total number of phenology observations represents the number of individuals that survived the first year of transplantation (n = 274). Each seedling was monitored 30 unique days over the 2.5-month period (late February through April).

Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
Stage 6

Fig. 2: Visual assessment of red maple bud break using six stages as described by Norby et al. (2003): (1) no activity, (2) buds swelling, (3) buds just opening, (4) leaves unfolding, (5) leaves curled, and (6) leaves flat. Photo credit: Rebecca Park.

Data analysis

We performed the data analysis using the package *lme4* (v. 1.1.28, Bates et al. 2015) using R statistical software v. 4.1.0 (R Core Team 2021). We used linear and generalized linear mixed models to determine the effects on survival, growth, and spring budburst phenology data. For each of these response variables, we fit a model testing the effects of the region of origin (CP, PD, MT) and the warming treatment (Ambient, +3° C, +5° C), as well as the two-way interaction term. For the survival and growth models, the population and chamber identification were included as random effects. For leaf-out phenology, the maternal tree and chamber identification were included as random effects. Due to the limited number of maternal trees per population and populations per region, we fit models with either maternal tree or population (but not both) and selected the model with the lower Aike Information Criteria (AIC), in order to avoid overfitting the model. The design did not allow the quantification of both the seed family and population level effects because the number of replicates per region was too small and too variable to allow for proper estimation. Although the blocking factor of the chambers could have been considered as a random effect, we decided to treat it as a fixed effect due to the low number of factor levels (n = 3), which does not allow reliable estimation of the associated variance term when considered as a random effect (Bolker et al. 2009). Additionally, we tested seed source elevation as a fixed effect variable in place of seed source region; although these models beget similar conclusions, they had worse fit and thus less explanatory power (Appendix S4).

We fit the cumulative survival model using a binomial distribution; for cumulative growth and spring phenology, we used a Gaussian distribution. We examined the Gaussian data for assumptions of homogeneity of variance and normal distribution and found them to conform to model requirements. We determined significance of the main effects using type 3 F-tests. We tested the main effects of the seed source region and the experimental temperature treatment, as well as the region-by-temperature interaction effect.

Based on the linear models, we calculated estimated marginal means (least-squares means) for each of the interaction terms using the R package *emmeans* (v 1.7.2, Lenth 2021). Interaction plots thus contained 9 total combinations of the fixed effects from the model (3 × 3 complete block design). Pairwise differences among combinations were tested using a post-hoc Tukey test. The threshold of significance was set at P=0.05 for all models.

Results

Response variable 1: Growth and survivorship

For the red maple seedling cumulative growth rate over the two years, there was a significant interaction effect between seed source region and temperature treatment (P=0.017) (Table 3). In other words, the growth rates of seedlings from different regions responded differently to the increase in soil temperature. Specifically, seedlings sourced from the CP and MT regions on average displayed a positive relationship between growth rate and increasing temperatures, while the growth rate of the PD region seedlings decreased on average (Figure 3). The CP-Ambient growth rate was significantly lower than PD-Ambient and MT+5 (P<0.05), while MT+5 was significantly higher than CP-Ambient and PD+5 (P<0.05) (Table 5). A large portion of the variance in growth was explained by the residuals, meaning there was also substantial variation in growth rate at the individual seedling level.

Looking only at the main effects, the cumulative growth rate was marginally significantly different between regions (P=0.054). Averaged across all temperature treatments, the MT region had the highest growth rate (0.396 ± 0.07), the PD region had the second highest (0.307 ± 0.06), and the CP region had the lowest (0.267 ± 0.06). Notably, there was no difference in growth rates between any of the regions or temperature treatments in the first year after transplanting; only in the second growing season did the individuals sourced from the MT region exhibit more pronounced growth than the other regions (Table S3.1). There was no significant difference in cumulative growth rate for the main effect of temperature alone (P=0.128). There was no difference in stem diameter for any of the seedlings in either year (Table S3.3). Finally, there was no significant relationship between seedling growth and the seed source elevation (Appendix S4).

For cumulative survival, there was a significant difference among seed source regions (P=0.034) (Table 3). In contrast to the growth rate, the CP seedlings had the highest survival rate (0.740 ± 0.08), the PD seedlings had the second highest (0.632 ± 0.08), and the MT seedlings had the lowest (0.509 ± 0.09) (Table 4). The interaction effect between region and temperature was marginally significant (P=0.086). On average, seedlings from the PD region displayed increasing survival with increasing temperatures, while the CP individuals did not show any change in mean survival with the change in temperature. Seedlings from the MT region had the most erratic response, with the +3° C treatment having the lowest survival rate among the three treatments (Figure 4). Like the growth response, there was no significant difference in survival rates among regions, temperatures, or the interaction effect in 2020; however, there was a significant interaction effect (P<0.001) for the 2021 survival rate (Table S3.2).

Response variable 2: Phenology observations

There was a significant interaction effect between region and temperature treatment for the spring leaf-out phenology (P=0.003) (Table 3, Figure 6). We observed that red maple seedlings from each of the regions responded to the increased warming with different magnitudes of change in leaf-out timing. The response variable represents the day of year to reach Stage 4, which is leaf-out (Figure 5; see also Appendix S5). With moderate warming (from Ambient to +3° C), each of the region's leaf out date advanced 1 to 4 days on average. However, with more extreme warming (+5° C), the regions each responded differently to the increase in temperature (Table 5, Figure 6). Specifically, the CP seedlings responded such that the mean leaf-out date was not further advanced, but rather delayed; thus, at extreme warming the leaf-out timing was similar to that of ambient temperatures. Similarly, the MT seedlings did not further advance their mean leaf-out date with more extreme warming, such that leaf-out timing was similar between +3°C and +5°C treatments. Only the PD seedlings further advanced their leaf-out date by an additional 3 days with the more extreme warming (Table 5, Figure 6).

Averaged across temperature treatments, the MT seedlings leafed out around three days later than the CP or PD seedlings, although these results were not statistically significant (P=0.101) (Table 3). Interestingly, there was a significant relationship between seed source elevation and leaf-out date (P=0.025) (Table S4.1); seedlings sourced from higher elevations leafed out later than those from the lower elevation sources (Figure S4.2). However, the interaction effect between elevation and temperature was not significant (P=0.823) (Table S4.1). Averaged across regions, the seedlings in the ambient chambers leafed out around three days later than those in the warmed (+3° C or +5° C) chambers, although this trend was also not significant (P=0.314) (Table 3). Like the growth response, a large portion of the variance in leaf-out date was explained by the residuals, as well as by the maternal tree to a lesser extent (Table 3). Therefore, even though there is a significant temperature-by-region interaction, there is a lot of unexplained variation among individuals in spring phenology. Finally, there was no significant relationship between the response variables of leaf-out date and growth increment.

Table 3: Model results for each of the three response variables (cumulative survival, cumulative growth, and day of year to leaf out). For Gaussian models, all parameters were calculated using REML. The blocking factor was treated as a fixed effect because there were not enough levels of the factor (n = 3) to base an estimate of the variance. Because maternal tree is nested within population, one or the other (but not both) was included in each model to avoid overfitting. The factor (population or maternal tree) yielding the lower AIC was selected for the model: population was included as a random effect for survival and growth, whereas maternal tree was included for the spring phenology model. $N_{[population]} = 8$, $N_{[maternal\ tree]} = 13$, $N_{[chamber]} = 9$. Significance of the fixed effects was determined using type 3 F-tests.

	Cumulative survival Binomial distribution	**Cumulative growth** Gaussian distribution	**Day of year to leaf out** Gaussian distribution
Fixed effects			
Temperature	NS	NS	NS
Region	P<0.05	NS	NS
Temperature * Region	NS	P<0.05	P<0.005
Blocking factor	NS	NS	NS
Random effects (Variance components)			
Population	0.0783	0.0048	-
Maternal tree	-	-	7.544
Chamber #	0.0360	0.0091	3.366
Residual	-	0.0701	25.837
Marginal R2	0.0983	0.0894	0.1376
Conditional R2	0.1285	0.2395	0.3937

Table 4: Model-based mean cumulative survival rates (± standard error) for each seed source region. Asterisks denote significant differences between CP and MT regions (* P<0.05). The region-by-temperature interaction effect was not significant for the survival response variable.

Seed source region	Mean cumulative survival rate
Coastal Plain	0.740 ± 0.08 *
Piedmont	0.632 ± 0.08
Mountains	0.509 ± 0.09 *

Table 5: Model-based estimated marginal means for cumulative growth rates and day of year to leaf-out. Estimated marginal means are given for each combination of seed source region (Coastal Plain, Piedmont, or Mountain) × temperature treatment (Ambient, +3° C, or +5° C). The leaf-out estimate is specifically the Julian day of year individuals of each region reached Stage 4 (leaf uncurling) Asterisks denote significant differences (* P<0.05, ** P<0.01). Refer to Figure 3 and Figure 6.

Temperature	Seed source region	Mean cumulative growth rate	Mean day of year to leaf-out
Ambient	Coastal Plain	0.150 ± 0.084 *	87.22 ± 1.98 *
+3° C	Coastal Plain	0.302 ± 0.083	84.87 ± 1.94 *
+5° C	Coastal Plain	0.349 ± 0.084	87.56 ± 1.97
Ambient	Piedmont	0.352 ± 0.086 *	88.81 ± 1.85
+3° C	Piedmont	0.325 ± 0.084	87.26 ± 1.85
+5° C	Piedmont	0.245 ± 0.080 *	83.98 ± 1.81 **
Ambient	Mountains	0.331 ± 0.100	92.52 ± 2.16 **
+3° C	Mountains	0.398 ± 0.108	88.46 ± 2.21
+5° C	Mountains	0.461 ± 0.095 *	88.38 ± 2.06

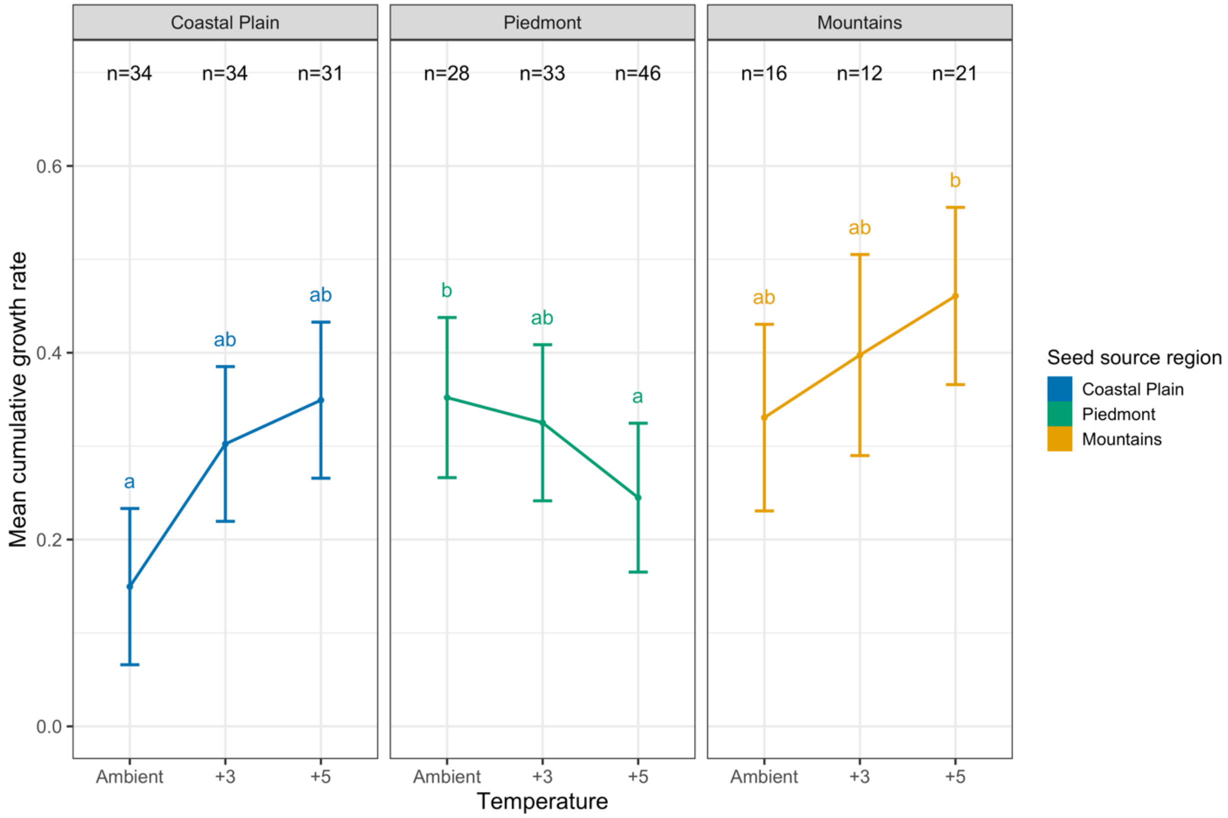

Fig. 3: Model-based estimated interaction means for cumulative growth rates over the two growing seasons. Error bars show ± standard error of the mean. The n indicates sample size for each factorial combination. Letters indicate significant differences in growth rate from post hoc Tukey test pairwise comparisons; region × temperature combinations with at least one matching letter in the label did not significantly differ from one another, while treatments with non-matched letter labels are significantly different at the $p = 0.05$ level.

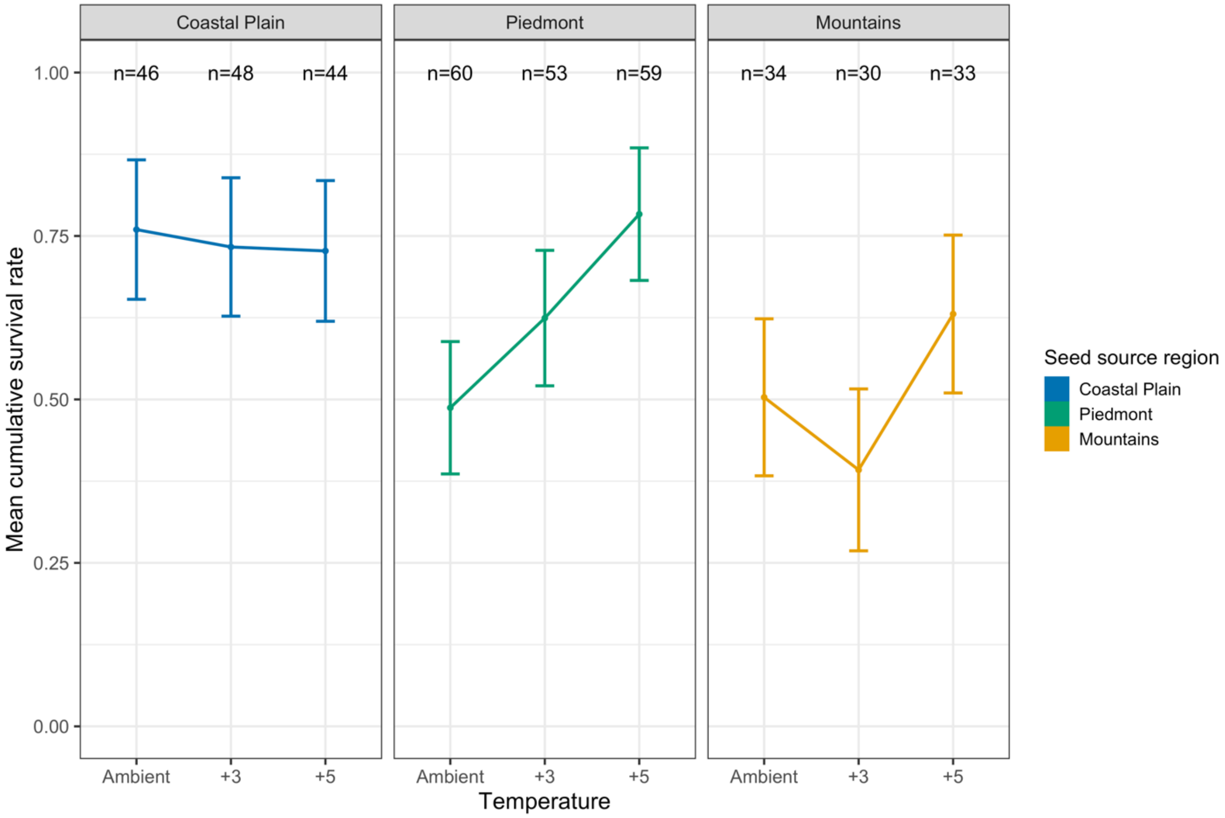

Fig. 4: Model-based estimated interaction means for cumulative survival rates over the two growing seasons. Error bars show ± standard error of the mean. The n indicates sample size for each factorial combination. There was no significant region-by-temperature interaction effect for the cumulative survival rate.

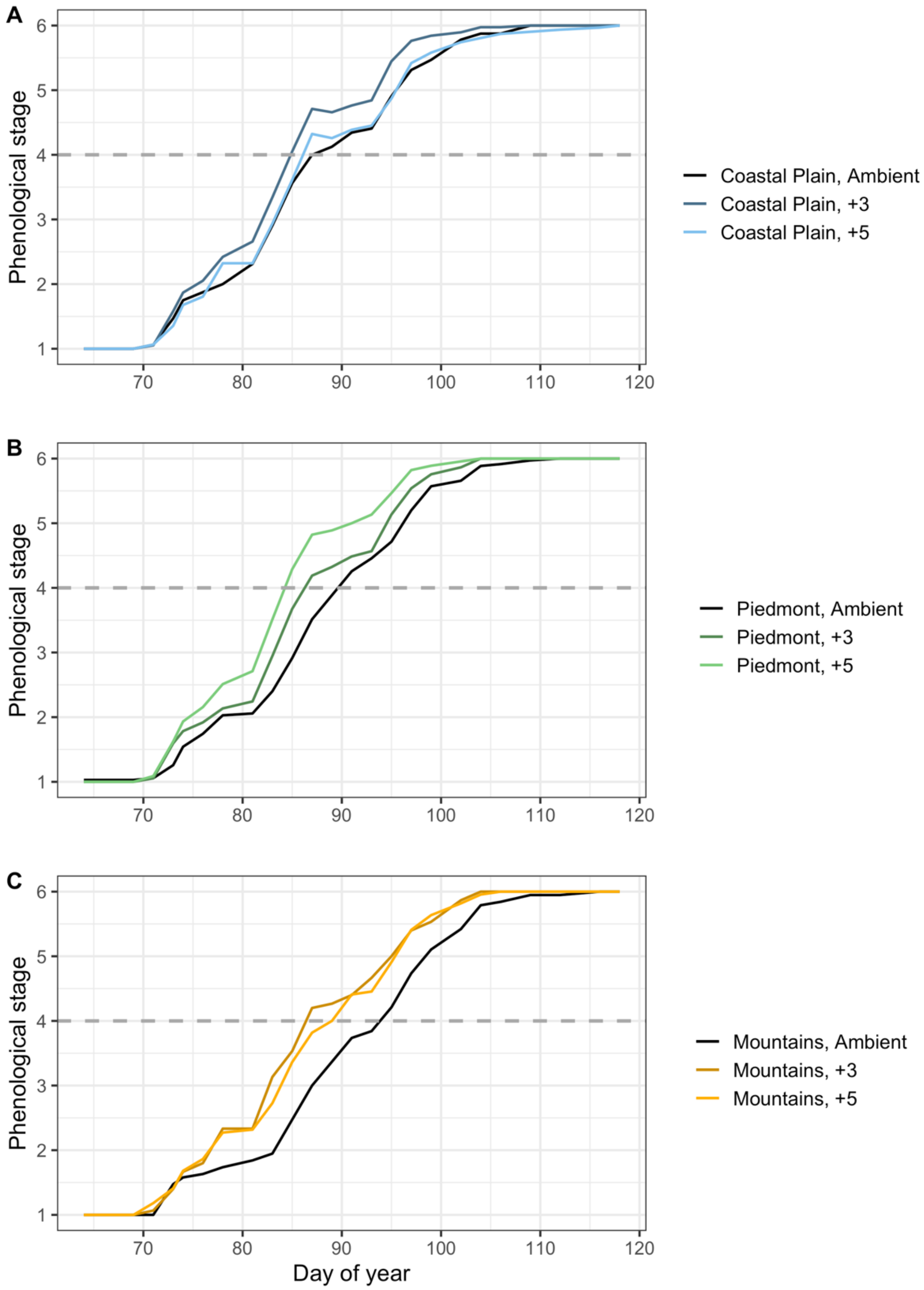

Fig. 5: Mean leaf-out development for each region under each temperature treatment. Separate panels are given for each seed source region: (A) Coastal Plain, (B) Piedmont, and (C) Mountains. The x-axis represents the Julian day of year. Phenological stages (1-6) are represented on the y-axis, thus higher values represent more developed leaf phenology for any given day of year (refer to Figure 2). Black lines represent the ambient treatment. Lighter colored lines represent warmer treatments. The dashed horizontal line at Stage 4 represents the day of year used as the response variable in the analysis (Figure 6).

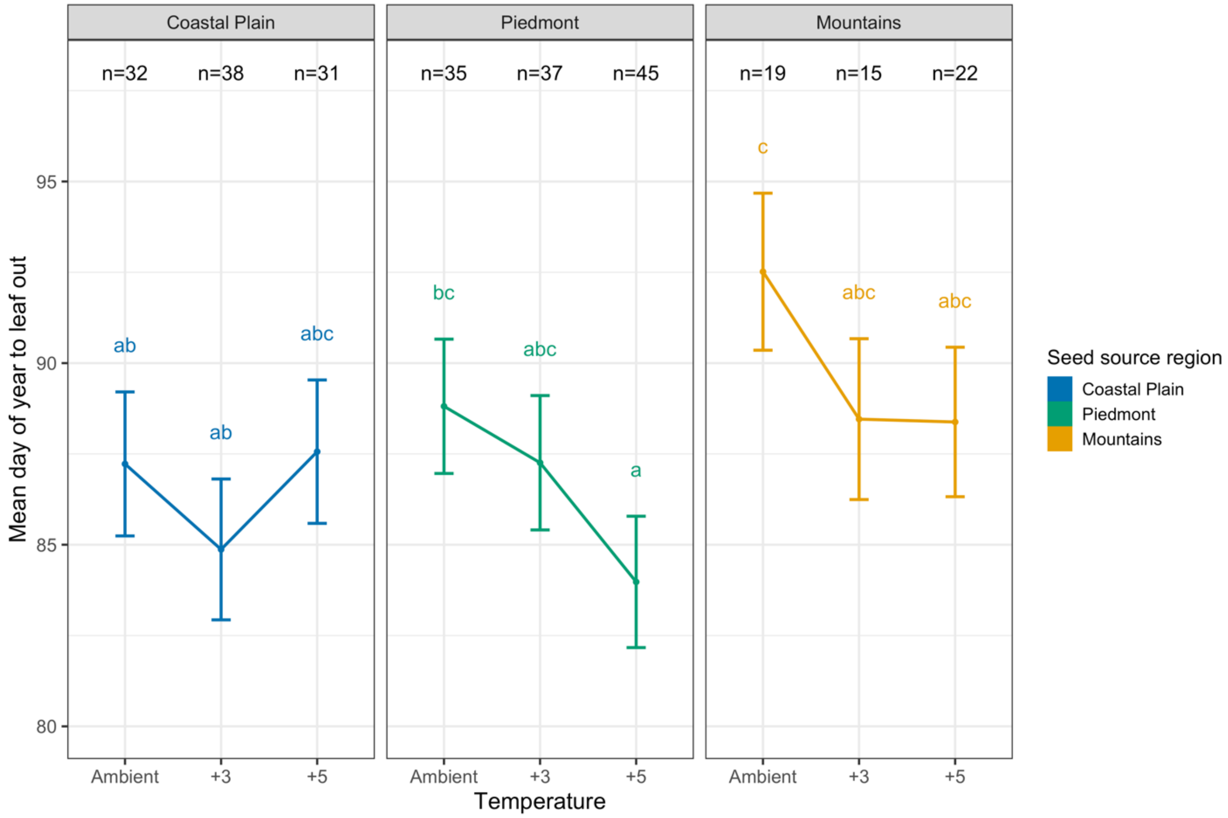

Fig. 6: Model-based estimated interaction means for day of year seedlings reached Stage 4 (leaf uncurling). Y-axis values represent the Julian day of year that each seedling reached Stage 4 (leaf unfolding), thus higher values represent later leaf emergence. Error bars show ± standard error of the mean. The n indicates sample size. Letters indicate significant differences in growth rate from post hoc Tukey test pairwise comparisons; region × temperature combinations with at least one matching letter in the label did not significantly differ from one another, while treatments with non-matched letter labels are significantly different at the p = 0.05 level.

Discussion

The combination of seed source locations and warming treatments allowed us to examine the environmental and geographic components of phenotypic plasticity in *Acer rubrum* seedlings in an upland temperate deciduous forest environment. Overall, our results showed that regions had different magnitudes, and sometimes direction, of response to the warming treatments for growth and phenology, indicating a region-by-temperature interaction effect. In contrast, survival differed strongly among regions but was not significantly affected by temperature.

The CP and MT seedlings showed increasing growth rates on average in response to soil warming. On the other hand, the PD seedlings showed no significant change in growth rate across the temperature treatments. In the literature, *A. rubrum* growth responses to soil warming are mixed: no effect or reduced growth under warming conditions (Farsworth et al. 1995, Norby & Luo 2004), positive initial growth followed by higher fine root turnover and mortality (Wan et al. 2004), as well as increased growth (Butler et al. 2012, Wheeler et al. 2016, Wheeler et al. 2017) have all been reported. Although carbon (C) gain is expected to increase for many temperate trees under warmer conditions (Way & Oren 2010, Mellilo et al. 2011), it is worth noting that climate change could negatively impact these species if temperatures exceed optimal ranges (Sendall et al. 2015). The results of this study suggest that growth responses to warming temperatures may be regionally dependent, and intraspecific variations in sensitivity to warming must be considered when projecting *A. rubrum* growth responses.

The regions showed significantly different survival rates, with the MT seedlings having the lowest survival overall, especially in the +3° C chambers (see Appendix S6). Ecologists have long noted that growth increment is an important predictor of survival in hardwood trees (Monserud 1976, Wycoff & Clark 2002). Interestingly, the regional responses are opposite for growth and survival: MT seedlings had the highest growth and the lowest survival, PD seedlings were intermediate, and CP seedlings had the lowest growth and highest survival. This could indicate differences in life-history strategies among the regions, such as allocation of resources over the seedlings' ontogeny (Murren 2002). Importantly, the different allocation of resources is not reflected in the survival and growth responses alone; for example, belowground growth, storage, and defense compounds are not accounted for in this study but could help explain the discrepancy (for example, see Santini et al. 2020). Worth noting, a study comparing the relationship between growth and mortality for native tree species in the southern Appalachian Mountains found that *A. rubrum* stood out in its ability to both grow rapidly and to tolerate slow growth (Wyckoff & Clark 2002). This finding may shed light on the apparent disconnection between growth and survival rates found in this study.

In addition to growth increment, phenology plays an important role in individual tree fitness (Polgar & Primack 2011) and species distribution (Chuine 2010, Iler et al. 2021). Specifically, early flushing lengthens the period for growth and subsequently increases competitive ability and the chance of producing mature seeds, while it simultaneously increases the risk of being exposed to late frosts that could damage flowers, leaves, and meristem tissues (Gu et al. 2008, Vitasse et al. 2013). Quantifying the effect of temperature on leaf-out timing is challenging because the environmental and genetic mechanisms underlying leaf-out phenology are still not fully understood (Wolkovich et al. 2012, Vitasse et al. 2021). Research suggests that once a critical photoperiod has been attained and winter chilling requirements are met, bud development progresses more rapidly with warmer temperatures (Ettinger et al. 2020). The term "chilling requirement" refers to a period of obligate exposure of plants to cold temperatures before they break dormancy (Polgar & Primack 2011). In other words, when chilling during the winter months is insufficient, bud expansion is retarded and abnormal (Ettinger et al. 2020). For example, a similar study of red maple phenology by Norby et al. (2003) found that temperature by itself is not sufficient to predict the response; rather, the sequence of temperature changes in relation to the plant's growth stage is important. Overall, timing of budburst is a biological tightrope as plants must balance the advantages of a longer growing season with the risks of late frost damage (Chamberlain et al. 2019).

The effects of belowground warming on leaf-out phenology are mixed in the literature. A meta-analysis by Chung et al. (2013) concluded that the effects on leaf-out phenology depended more on the species and the degree of warming than the method of warming used (e.g., soil versus air warming). On the other hand, some studies have shown no effect of soil warming of leaf-out phenology (Farnsworth et al. 1995, Bergh & Linder 1999, Bronson et al. 2009). Moreover, several studies have shown that air temperature is the most important factor in regulating budburst and leaf-out in temperate and boreal woody plants (Linkosalo et al. 2006, Polgar & Primack 2011). In fact, a recent study by Vitasse et al. (2021) convincingly demonstrated that temperature is sensed locally within the bud. However, soil warming could have a stronger effect on leaf-out in regions where frozen soils determine spring phenology (Chung et al. 2013). Interestingly, similar studies by Wheeler et al. found a significant advancement of leaf-out in one-year old *A. rubrum* seedlings grown in small soil-warming pots in a greenhouse setting (Wheeler et al. 2016), but no change in leaf-out date for two-year old *A. rubrum* seedlings grown in forest soil warming chambers (Wheeler et al. 2017).

In this study, the changes in phenological response from the moderate warming scenario to the more extreme warming indicated a significant region-by-temperature interaction effect. The MT and CP seedlings did not further advance their leaf-out date with more extreme warming. This could be due to an insufficient overwinter chilling, causing leaf-out to be delayed; additionally, it could represent a limit to the amount of phenotypic plasticity for seedlings from these regions (Clark et al. 2014). Specifically at higher elevations of the mountains, late frosts in spring are more frequent. Researchers have posited that bud burst timing is constrained at higher elevations to minimize the danger of frost damage (Vitasse et al. 2013). Hence, the optimal window for budburst is narrower at increasing elevations (Gauzere et al. 2020). This may explain why the MT seedlings exhibited a limit to their phenological plasticity despite warmer conditions. Conversely, some studies have shown that different elevational clines do not display different levels of plasticity when transplanted along an elevational gradient (Ensing & Eckert 2019). Moreover, when the selected target temperature is too high, it may reduce the phenological response, leading to an underprediction of phenological changes, as shown by Wolkovich et al. (2012) for multiple species. In conclusion, only the PD seedlings further advanced their leaf-out date with more extreme warming, which was the response we had predicted.

Until recently, there has been a disparity between species' forecasts, which generally consider a species as a single entity, and population studies, which have long shown intraspecific differences in climate response (DeMarche 2020). Numerous studies have similarly shown that the intraspecific variation in plasticity has important implications for species distributions under future conditions (Valladares et al. 2014, Patsiou et al. 2020, Zettlemoyer & Peterson 2021). The intraspecific differences in response to warming observed in this study underline the fact that climate warming experiments need to consider individuals arising from multiple regions in order to forecast the response of a species.

Conclusion

Adaptive capacity to climate change exists at multiple spatial and temporal scales: individual adaptation, population phenotypic diversity, and genetic exchange within and between populations (Royer-Tardif et al. 2021). Previous field studies have shown *A. rubrum* to exhibit individual plasticity: seedlings were responsive to temperature, with leaf phenology, photosynthetic rates, respiration and growth all acclimating rapidly to both atmospheric and soil warming (Norby et al. 2003, Sendall et al. 2015, Wheeler et al. 2017). However, at the population level, a recent meta-analysis by Royer-Tardif et al. (2021) suggested that *A. rubrum* has relatively low levels of genetic diversity and population differentiation when compared to 26 other northeastern North American tree species. In contrast, the results of our study do indicate some level of regional genetic differentiation in phenotypic plasticity in response to soil warming. The differences among regions in this study are especially noteworthy given the relatively restricted area of our study compared with the full extent of red maple's range (Walters & Yawney 1990). Moreover, *A. rubrum* shows great adaptive capacity to climatic change (e.g., individual plasticity and genetic exchange within populations) when compared to other common northeastern North American tree species (Royer-Tardif et al. 2021). Understanding geographic differences in *A. rubrum* is important because this foundational species is increasingly dominant in eastern North American forests. Despite red maple's epithet as a "super-generalist" (Abrams 1998), our study suggests that trees from different regions respond differently to changes in temperature, which will likely influence which populations succeed across regions. Our findings highlight the fact that intraspecific variations in temperature sensitivity must be considered when projecting *A. rubrum* and other common tree species responses to future climate conditions.

CHAPTER 3

CONCLUSIONS

Phenology plays a key role in ecosystem function and countless ecosystem services. For example, phenology is an important determinant of species abundance and distribution (Chuine 2010, Heberling et al. 2019, Iler et al. 2021). Additionally, by determining the length of the growing season, phenology plays a key role in annual net primary productivity (NPP) (Chapin et al. 2012). Phenology also plays a role in evapotranspiration, surface albedo, and feedback to Earth's climate (Richardson et al. 2013).

As atmospheric and soil temperatures increase due to anthropogenic climate change (IPCC 2021), changes in vegetative phenology could have cascading effects on deciduous forest ecosystems. For example, if interacting species do not respond by the same magnitude to changes in temperature, the possibility arises of ecological mismatches (Both et al. 2009). Furthermore, if populations across a species' range respond differently to climate change, this could dramatically alter species-level forecasts of performance or distribution under future conditions (Patsiou et al. 2020, DeMarche 2020). Therefore, particular ecotypes may come to dominate in future climates if certain populations are more or less vulnerable to climate change (DeMarche 2020).

In sum, my research contributes to a growing body of work examining the differences in intraspecific responses to climate change (Sáenz-Romero et al. 2017, Ensing & Eckert 2019, Patsiou et al. 2020, Zettlemoyer & De Marche 2021). My research demonstrated that various populations of red maple (*Acer rubrum*), a common tree species across Eastern North America, responded differently to changes in soil temperature. Specifically, there was a significant region-by-temperature interaction effect for seedling growth and leaf-out phenology. In contrast, survival differed only by region, not by temperature treatment. The findings of my research agree with previous work suggesting that plant populations across a species' range do not necessarily respond in the same magnitude to environmental changes. Phenotypic changes for widespread and ecologically diverse trees may be genetically based and not exclusively the result of a uniform plastic response (Patsiou et al. 2020).

Future directions

Because intraspecific populations may respond differently to changes in temperature, climate warming experiments need to consider individuals arising from multiple regions in order to forecast the response of a species. Although many forecasts have been published for species across the globe, relatively few of these studies take into account the intraspecific variation in response to climate change (DeMarche 2020). For example, studies have shown that intraspecific variation is related to climate, yet climate change may drive nonintuitive shifts in range, as different populations respond differently to these changes (Valladares et al. 2014, Patsiou et al. 2020). This type of information is crucial for informed land management decisions and estimating the impact of climate change on global biodiversity.

Secondly, although it is well understood that spring phenology is advancing worldwide due to rising temperatures (Parmesan & Yohe 2003, Wolkovich et al. 2012), the response of autumn phenology to climatic warming is relatively understudied (Gallinat et al. 2015). Fall phenology is more difficult to study because of the protracted nature of autumn events, coupled with less-predictable factors that may help explain variation (Gallinat et al. 2015). However, fall phenology plays an important role in the annual carbon (C) balance of temperate ecosystems. For example, Wu et al. (2013) found that changes in autumn leaf phenology better explain variation in annual net ecosystem productivity (NEP) than do changes in spring phenology. Although long term observational studies do suggest that fall phenology is delayed by climate warming (Ibanez et al. 2010), experimental evidence is lacking (but see Fu et al. 2018, Vitasse et al. 2021).

Another gap in knowledge relates to the species-specific fitness effects of changes in phenology. Not surprisingly, the influence of warmer temperatures on phenology often differs among species. However, there is a lack of studies of both phenology and fitness, meaning that the fitness consequences of shifts in phenology remain unclear for most ecosystems. Additionally, given the large stature and long generation time of trees, most investigations of warming impacts focus on smaller, often faster reproducing graminoids, forbs, and/or shrubs (Mohan et al. 2019). Therefore, the fitness consequences of phenological changes in larger, longer-lived plants such as trees remains relatively understudied (Chung et al. 2013).

Given the amplified warming observed in and projected for the Arctic (IPCC 2021), it is important to understand phenological responses to ecosystem warming in high-latitude and high-altitude systems. However, there is already a bias toward higher latitude, fertile soils in the literature (Rustad et al. 2001, Mohan et al. 2019). Therefore, future research is needed in lower latitudes and varying soil types, as tropical plants may already exist close to the upper limits of thermal tolerance, placing these tropical species at higher risk (Sunday et al. 2011, Sunday et al. 2019). However, there are relatively few soil warming experiments in the tropics or Southern Hemisphere (van Gestel et al. 2018, Mohan et al. 2019). A more complete understanding of the effects of ecosystem warming will rely on a combination of long-term observation studies and warming experiments (Chung et al. 2013). Through an integrated approach, as well as broadening the scope of research to incorporate understudied ecosystems and longer-lived species, we can enhance our understanding of climate change impacts on plant phenology and the associated fitness consequences for ecosystems globally.

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APPENDICES

Appendix S1: Seed collection and germination information

Red maple seeds were collected from 36 unique maternal trees across North Carolina and Georgia. Seeds require no pre-germination treatment and can germinate immediately after ripening. Seeds from 24 maternal trees germinated in the greenhouse (n=832) (Table S1.1). Many seeds did not germinate because they were either not yet ripe or became contaminated with mold after collection. After being grown out in the greenhouse for two years, seeds from 13 maternal trees survived and were transplanted to the soil warming chambers (n=407) (Table S1.1). No significant relationship was found between seed size and germination rate and/or mean height before transplant (Appendix S2).

Table S1.1: Seed collection and germination count data. Region indicates physiographic region: Coastal Plain (CP), Piedmont (PD), or Mountains (MT). All CP trees were in North Carolina, all MT trees were in GA, and the PD trees are indicated by 'GA' or 'NC' in the population name. Tree # is the unique identification code of each maternal tree. Seed size is an ordinal variable indicating small=1, med=2, med-big=3, big=4, very big=5. '# Germinated' are the total number of seeds which germinated in the greenhouse in Summer 2018 (n=832). '# Transplanted' are the total number per mother tree which survived to transplant into the soil warming chambers in Spring 2020 (n=407). No data indicates a count of zero.

Region	Population	Tree #	Seed size	# Germinated (2018)	# Transplanted (2020)
CP	CP 1	CP 1.1	2	23	21
CP	CP 1	CP 1.2	1	6	-
CP	CP 2	CP 2.1	3	-	-

CP	CP 3	CP 3.1	4	55	51
CP	CP 3	CP 3.2	3	2	-
CP	CP 3	CP 3.3	3	-	-
CP	CP 4	CP 4.1	4	2	-
CP	CP 4	CP 4.2	2	-	-
CP	CP 4	CP 4.3	5	-	-
CP	CP 4	CP 4.4	2	-	-
CP	CP 5	CP 5.1	4	-	-
CP	CP 5	CP 5.2	2	19	16
CP	CP 5	CP 5.3	3	101	50
PD	GA PD 1	PD 1.1	2	4	-
PD	GA PD 1	PD 1.2	1	-	-
PD	GA PD 1	PD 1.3	4	-	-
PD	GA PD 1	PD 1.4	2	-	-
PD	GA PD 1	PD 1.5	2	95	-
PD	GA PD 1	PD 1.6	1	110	-
PD	NC PD 2	PD 2.1	2	-	-
PD	NC PD 2	PD 2.2	3	-	-
PD	NC PD 3	PD 3.1	4	11	-
PD	NC PD 3	PD 3.2	4	31	30
PD	NC PD 3	PD 3.3	5	3	-
PD	NC PD 4	PD 4.1	3	88	63
PD	NC PD 4	PD 4.2	4	49	28
PD	NC PD 4	PD 4.3	2	3	-
PD	NC PD 5	PD 5.1	2	41	31
PD	NC PD 5	PD 5.2	2	4	-
PD	NC PD 5	PD 5.3	2	38	20
MT	MT 1	MT 1.3	2	46	28
MT	MT 1	MT 1.4	1	26	14
MT	MT 1	MT 1.5	2	8	-
MT	MT 2	MT 2.1	1	21	15
MT	MT 2	MT 2.2	1	46	40
MT	MT 2	MT 2.3	1	-	-

Appendix S2: Mean height before transplant

After the red maple seeds germinated in Spring 2018, the seedlings grew in a controlled greenhouse environment for two growing seasons (2018, 2019) before being transplanted into the Whitehall Forest Soil Warming Facility (WFWF) in Spring 2020. The mean height prior to transplant (Fig. S2.1) was determined using the bud scar. No significant relationship was found between seed size and seedling height.

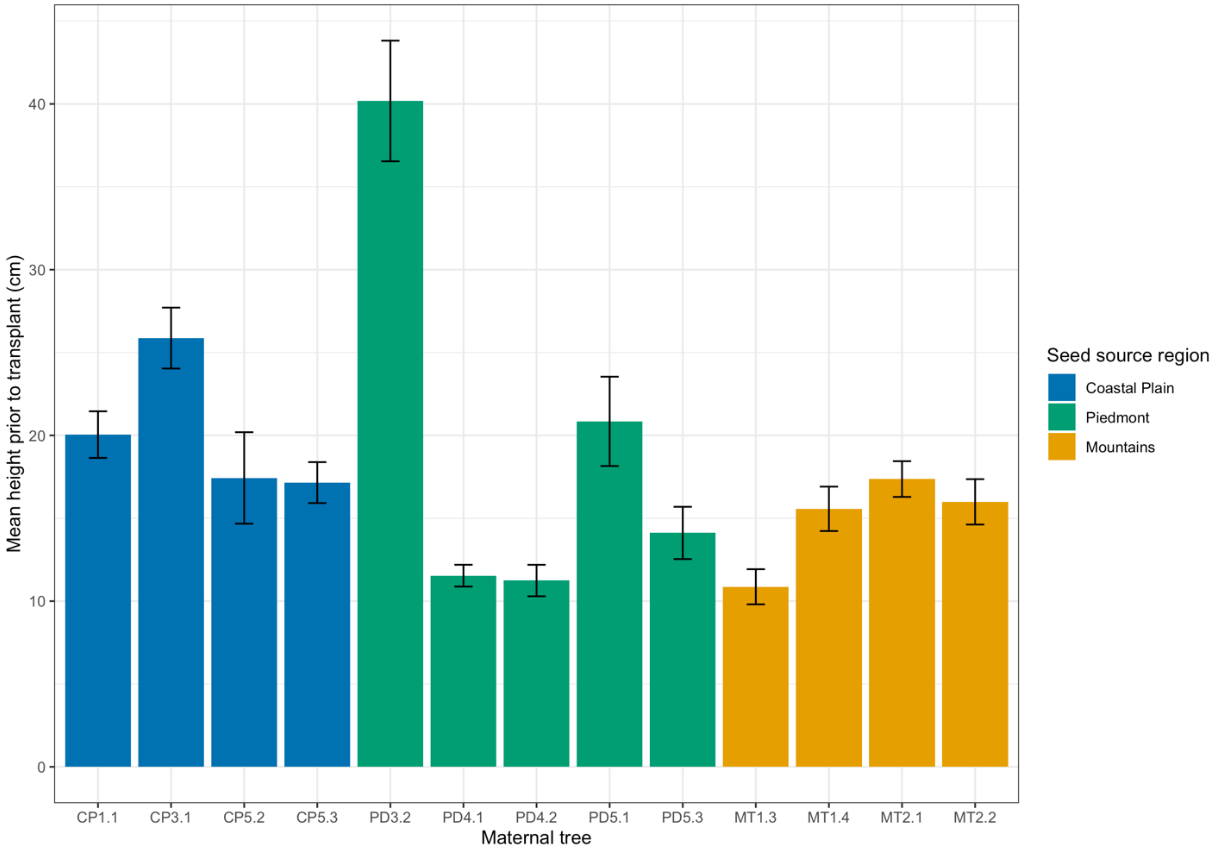

Figure S2.1: Mean height of seedlings from each maternal tree prior to transplant into the soil warming chambers. Error bars represent standard error of the mean. Each maternal tree is given a unique identifying code that contains the factorial region: Coastal Plain (CP), Piedmont (PD), or Mountain (MT); the population number (before the decimal); and the maternal tree number (after the decimal).

Appendix S3: Mean annual survival and growth rates

Red maple seedlings were transplanted into the soil warming chambers in April 2020 after being grown in a common greenhouse environment for two years. In the main text, we reported growth and survival rates as cumulative means over the two-year study period. Below are the marginal means for growth (Table S3.1), survival (Table S3.2), and stem diameter (Table S3.3) for each growing season (2020, 2021) reported separately. The survival rate represents the proportion of individuals that survived to the end of the growing season that year, out of those alive starting the season that year. The growth rate is given by the formula: $\ln(\text{height}_{\text{Year2}}) - \ln(\text{height}_{\text{Year1}})$. The stem growth rate was similarly calculated: $\ln(\text{stem diameter}_{2021}) - \ln(\text{stem diameter}_{2020})$.

Table S3.1: Mean annual growth rates (± standard error) for each growing season after transplant into the soil warming chambers. Estimated marginal means are given for each combination of seed source region (Coastal Plain (CP), Piedmont (PD), or Mountain (MT)) × temperature treatment (Ambient, +3° C, or +5° C). There was no significant difference in growth rates for region, temperature, or the interaction in either 2020 or 2021. The last column is the cumulative growth rate (± standard error) for all individuals which survived both growing seasons (N=255).

	Mean 2020 growth rate	**Mean 2021 growth rate**	**Mean cumulative growth rate**
CP, Ambient	0.25 ± 0.03	-0.05 ± 0.07	0.15 ± 0.08
CP, +3	0.27 ± 0.03	0.00 ± 0.08	0.30 ± 0.08
CP, +5	0.25 ± 0.03	0.09 ± 0.08	0.35 ± 0.08
PD, Ambient	0.29 ± 0.03	0.03 ± 0.08	0.35 ± 0.09
PD, +3	0.27 ± 0.03	0.04 ± 0.08	0.33 ± 0.08
PD, +5	0.23 ± 0.03	0.00 ± 0.07	0.24 ± 0.08
MT, Ambient	0.25 ± 0.04	0.08 ± 0.10	0.33 ± 0.10
MT, +3	0.28 ± 0.04	0.11 ± 0.10	0.40 ± 0.11
MT, +5	0.31 ± 0.04	0.18 ± 0.09	0.46 ± 0.09

Table S3.2: Mean annual survival rates (± standard error) for each growing season after transplant into the soil warming chambers. Estimated marginal means are given for each combination of seed source region (Coastal Plain (CP), Piedmont (PD), or Mountain (MT)) × temperature treatment (Ambient, +3° C, or +5° C) based on the best-fitting model, which was selected based on Aike's Information Criteria (AIC). There was no significant difference in survival rates for region, temperature, or the interaction in 2020. However, there was a significant interaction effect (P<0.001) for the 2021 survival rate.

	Mean 2020 survival rate	**Mean 2021 survival rate**	**Mean cumulative survival rate**
CP, Ambient	0.79 ± 0.07	0.94 ± 0.08	0.76 ± 0.11
CP, +3	0.78 ± 0.07	0.93 ± 0.08	0.73 ± 0.11
CP, +5	0.80 ± 0.07	0.89 ± 0.08	0.72 ± 0.11
PD, Ambient	0.71 ± 0.06	0.65 ± 0.07	0.49 ± 0.10
PD, +3	0.71 ± 0.07	0.86 ± 0.07	0.62 ± 0.10
PD, +5	0.79 ± 0.06	0.97 ± 0.07	0.78 ± 0.10
MT, Ambient	0.58 ± 0.08	0.84 ± 0.09	0.50 ± 0.12
MT, +3	0.70 ± 0.09	0.54 ± 0.09	0.39 ± 0.12
MT, +5	0.66 ± 0.08	0.95 ± 0.09	0.63 ± 0.12

Table S3.3: Mean stem diameter (± standard error) for each growing season after transplant into the soil warming chambers. The growth rate was calculated using the formula: ln(stem diameter$_{2021}$) – ln(stem diameter$_{2020}$). Estimated marginal means are given for each combination of seed source region (Coastal Plain (CP), Piedmont (PD), or Mountain (MT)) × temperature treatment (Ambient, +3° C, or +5° C). There was no significant difference in mean stem diameters nor mean growth rates for region, temperature, or the interaction in either year.

	Mean 2020 stem diameter (mm)	**Mean 2021 stem diameter (mm)**	**Mean stem growth rate**
CP, Ambient	3.65 ± 0.84	3.64 ± 0.89	0.07 ± 0.03
CP, +3	3.94 ± 0.83	4.10 ± 0.89	0.08 ± 0.03
CP, +5	3.90 ± 0.84	4.25 ± 0.89	0.06 ± 0.03
PD, Ambient	4.15 ± 0.83	4.38 ± 0.90	0.05 ± 0.03
PD, +3	4.65 ± 0.83	4.39 ± 0.89	0.05 ± 0.03
PD, +5	4.11 ± 0.82	4.37 ± 0.88	0.04 ± 0.02
MT, Ambient	3.08 ± 1.03	3.24 ± 1.10	0.01 ± 0.04
MT, +3	3.48 ± 1.02	3.46 ± 1.12	0.07 ± 0.04
MT, +5	3.21 ± 1.01	3.45 ± 1.08	0.11 ± 0.03

Appendix S4: Seed source elevation as an explanatory variable

Over the last century, there have been numerous experiments using common gardens to demonstrate genetic differentiation among tree populations from along elevational gradients (Morgenstern 1996, Vitasse et al. 2009, Bresson et al. 2011). Elevational changes result in variation in growing season length, as the duration of suitable temperatures for plant growth decrease at higher elevations (Körner 2007). At increasing elevations, cold conditions strongly constrain tree phenology: late frosts may be more frequent, thus bud burst timing must be regulated to minimize the danger of frost damage (Vitasse et al. 2013). It follows that tree populations at upper elevational limits may undergo selective abiotic pressure, leading to genetic differentiation (*i.e.*, local adaptation) in timing of phenological events. Plants growing at higher elevations often exhibit different resource allocation and shorter growth forms than those at low elevations (Körner 2003). Overall, elevational gradients provide a unique opportunity for exploring the impact of temperature on tree fitness and phenology because the relatively short distance between elevations with different temperatures enables the analysis of temperature effects on plants at otherwise similar conditions (Körner 2000).

We were interested in exploring the relationship between elevation of the maternal tree and the response of the seedling to the soil warming treatment. To investigate this question, instead of using the factorial combination of seed source region (Coastal Plain, Piedmont, or Mountains) with the soil warming treatment (Ambient, +3° C, or +5° C), the seed source region was replaced by the continuous explanatory variable of elevation (see Table 1). To test the role of elevation, we fit linear and generalized linear mixed models using the package *lme4* (v. 1.1.28, Bates et al. 2015) using R statistical software v. 4.1.0 (R Core Team 2021). We tested the same response variables reported in the main body of the text: cumulative survival, cumulative growth, and spring budburst phenology. We fit the models using the explanatory variables: elevation of the maternal tree (continuous), temperature treatment (factorial), and the interaction effects. Although the blocking factor of the chambers could have been considered as a random effect, we decided to treat it as a fixed effect due to the low number of factor levels (n = 3), which does not allow reliable estimation of the associated variance term when considered as a random effect (Bolker et al. 2009). Maternal tree, population, and chamber number were again included as random effects.

We decided to exclude these analyses from the main text because the seed source elevation proved to have less explanatory power than the physiographic region as a whole. For cumulative growth, the elevation models fit worse than the region models (Table S4.1); furthermore, elevation did not prove to be a significant predictor of growth (Fig. S4.1) for the seedlings. For cumulative survival, the limited number of seedlings did not allow for reliable linear regression using elevation while also including the random effects. On the other hand, for the spring leaf-out date, there was a significant difference in leaf-out phenology based on the seed source elevation (P=0.025) (Table S4.1). Interestingly, the interaction between elevation and temperature treatment was not significant for leaf-out phenology. However, there does appear to be a stronger correlation between seed source elevation and leaf-out date (Fig. S4.2) than for the other response variables.

Elevation is not the best predictor of seedling response in this experiment. Although the seed source regions do represent a regional elevation gradient, the populations represented in this experiment experience more ecosystem-level differences than elevation alone (assuming elevation as a proxy for temperature or growing season length (Körner 2007)). These environmental differences include abiotic factors such as soil type and moisture, as well as biotic factors such as community composition. Species spread over wide geographic regions develop functional traits that are genetically adapted to the local biotic and abiotic conditions (Anderson & Song 2020); these adaptations can sometimes be better explained by factors other than temperature or growing season (e.g., Patsiou et al. 2020). In sum, we posit that the regional differences observed among the red maple seedlings are not synonymous with elevation alone, but rather represent a broader environmental gradient across physiographic regions.

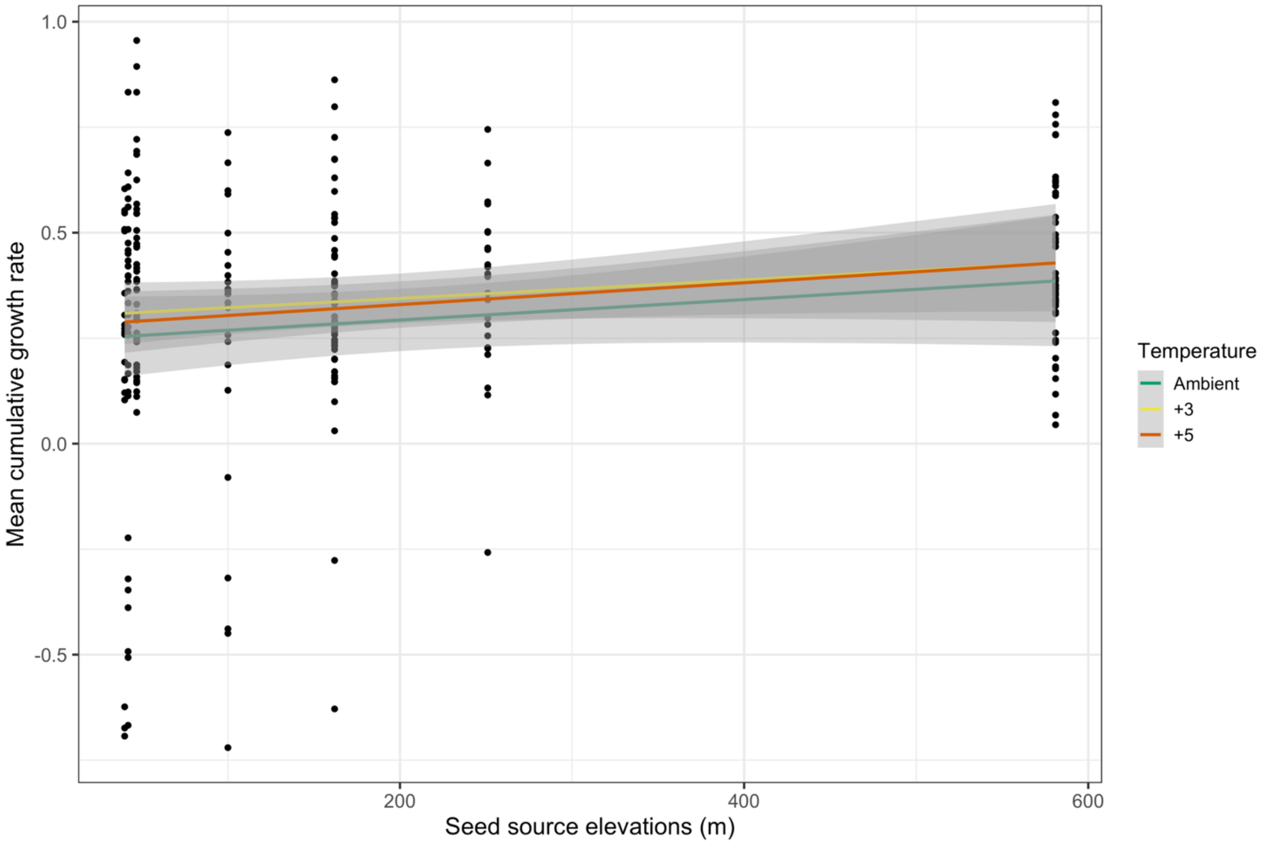

Figure S4.1: Regression line showing cumulative growth rate for red maple seedlings based on the elevation of their seed source locations. Colors represent the temperature treatment of the forest chambers. Conditional R-squared = 0.187.

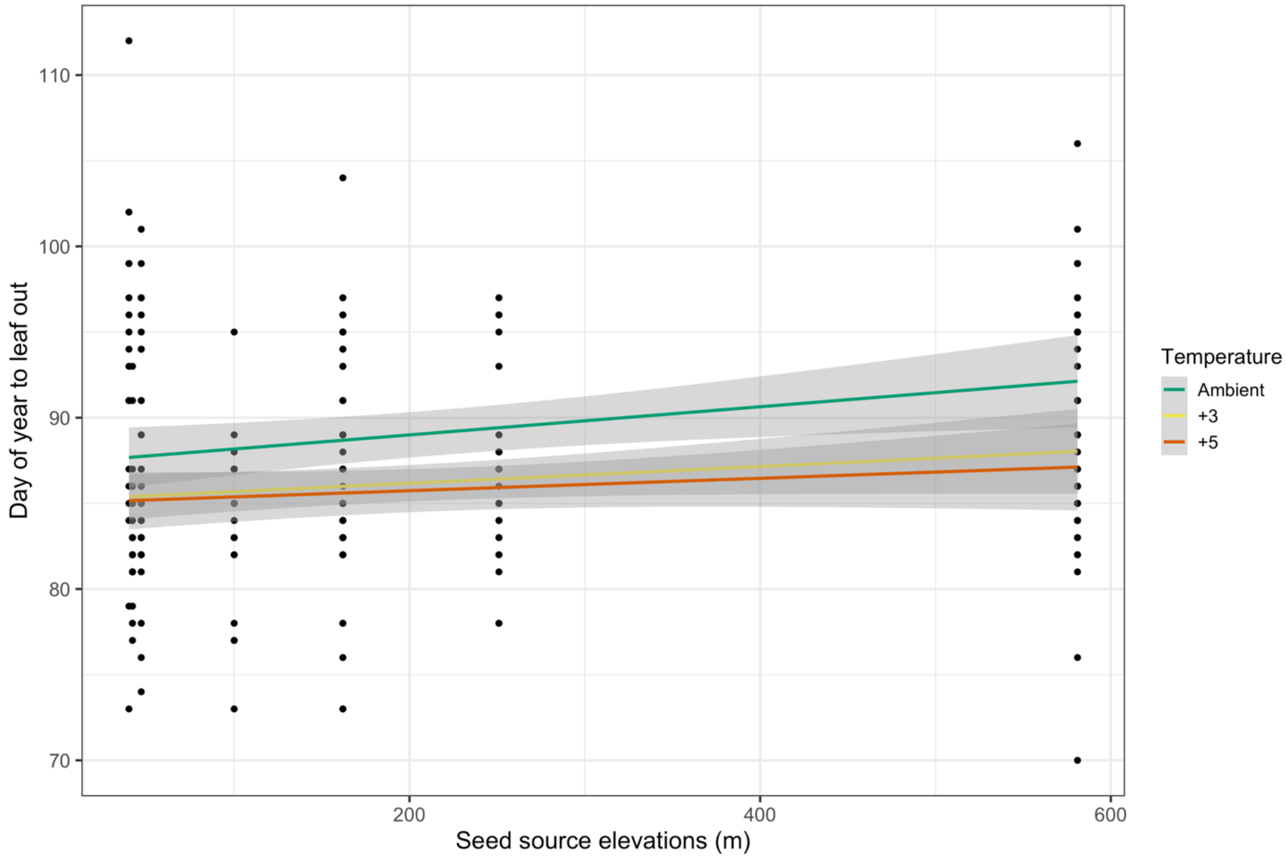

Figure S4.2: Regression line showing day of year to leaf-out (Stage 4) for red maple seedlings based on the elevation of their seed source locations. Colors represent the temperature treatment of the forest chambers. Conditional R-squared = 0.352.

Table S4.1: Model selection table for red maple seedling cumulative growth and spring leaf-out phenology. All parameters were calculated using REML (except AIC values). Cumulative survival is excluded from the table because the limited number of seedlings did not allow for a model to fit using elevation while also including the random effects. The blocking factor was treated as a fixed effect because there were not enough levels of the factor (n = 3) to base an estimate of the variance. Because maternal tree is nested within population, one or the other (but not both) was included in each model to avoid overfitting. The factor (population or maternal tree) yielding the lower AIC was selected for the model. $N_{[population]} = 8$, $N_{[maternal\ tree]} = 13$, $N_{[chamber]} = 9$. Significance of the fixed effects was determined using type 3 F-tests.

	Cumulative growth Gaussian distribution		**Day of year to leaf out** Gaussian distribution	
	Null model	**Full model**	**Null model**	**Full model**
Fixed effects				
Temperature	-	NS	-	NS
Elevation	-	NS	-	P<0.05
Temperature * Region	-	NS	-	NS
Blocking factor	-	NS	-	NS
Random effects (Variance components)				
Population	0.0053	0.0031	-	-
Maternal tree	-	-	8.400	6.834
Chamber #	0.0048	0.0086	3.664	3.274
Residual	0.0729	0.0731	27.046	26.991
Marginal R2	-	0.0569	-	0.1098
Conditional R2	-	0.1866	-	0.3518
AIC	79	85.1	1726.3	1727.9

References in Appendix S4:

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Appendix S5: Phenology response variables

We chose to report results from our phenology observation in Julian day of year the seedlings reached Stage 4 (leaf unfolding; Fig. 2). Other metrics such as day of year to Stage 3 (bud break), 50% bud break, 100% bud break, etc. yielded similar results; however, the most significant differences in leaf-out timing were observed when the seedlings reached Stage 4 (Fig. 5). We observed leaf emergence to be the most ephemeral stage; in other words, the seedling may be in Stages 1-2 for weeks, then move through stages 3-4 in a couple days (Fig. 5). In this way, Stage 4 was the most sensitive metric and most representative of seedling sensitivity to temperature change and clinal variation.

Appendix S6: Chamber moisture

Notably, the MT seedlings had a very depressed survival rate in the +3° C chambers when compared to the other two temperature treatments. This could be attributed in part to the +3° C chambers having lower moisture on average than the other chambers (Figure S6.1); in other words, perhaps the MT seedlings could not cope with *both* warmer temperatures *and* lower moistures. In theory, the soil warming plots evaluate plant adaptation to a single stress factor: temperature. However, the natural setting of the forest chambers can result in a combination of stresses, eliciting a response that cannot be extrapolated from each individual stress alone; rather, plants may show non-additive responses to interacting stresses, including unique expression profiles (Anderson & Song 2020).

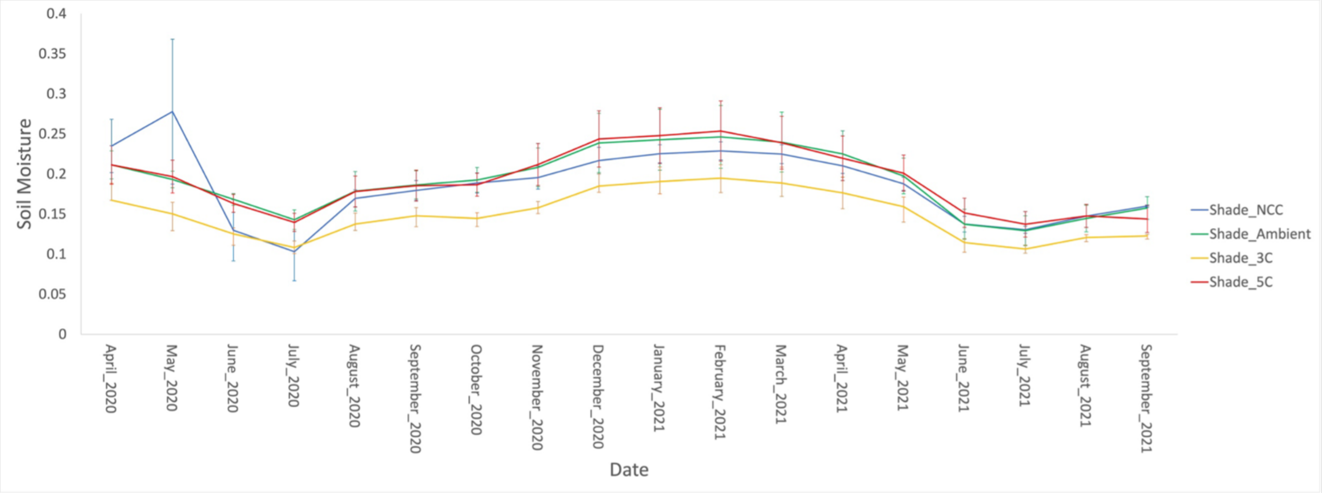

Figure S6.1: Monthly average soil moisture by temperature treatment for the study period. N = 3 chambers per temperature (Non-chamber control plots, Ambient plots, +3° C plots, and +5° C plots). There were no red maple seedlings transplanted into the non-chamber control plots. Notably, the +3° C chambers have lower soil moisture on average.

References in Appendix S6:

Anderson, J. T., & Song, B.-H. (2020). Plant adaptation to climate change—Where are we? *Journal of Systematics and Evolution, 58*(5), 533-545. doi:10.1111/jse.12649