



**Michigan
Technological
University**

Michigan Technological University
Digital Commons @ Michigan Tech

Dissertations, Master's Theses and Master's Reports

2022

Common garden study reveals frost-tolerant, generalist northern seed sources are best suited to expand range of *Quercus rubra*

Emily Lindback

Michigan Technological University, eclindba@mtu.edu

Copyright 2022 Emily Lindback

Recommended Citation

Lindback, Emily, "Common garden study reveals frost-tolerant, generalist northern seed sources are best suited to expand range of *Quercus rubra*", Open Access Master's Thesis, Michigan Technological University, 2022.

<https://doi.org/10.37099/mtu.dc.etdr/1389>

Follow this and additional works at: <https://digitalcommons.mtu.edu/etdr>



Part of the [Forest Biology Commons](#), [Forest Management Commons](#), [Other Forestry and Forest Sciences Commons](#), [Other Physiology Commons](#), [Other Plant Sciences Commons](#), and the [Plant Biology Commons](#)

COMMON GARDEN STUDY REVEALS FROST-TOLERANT, GENERALIST
NORTHERN SEED SOURCES ARE BEST SUITED TO EXPAND RANGE OF
QUERCUS RUBRA

By

Emily Lindback

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Forest Ecology and Management

MICHIGAN TECHNOLOGICAL UNIVERSITY

2022

© 2022 Emily C. Lindback

This thesis has been approved in partial fulfillment of the requirements for the Degree of
MASTER OF SCIENCE in Forest Ecology and Management.

College of Forest Resources and Environmental Science

Thesis Advisor: *Molly Cavaleri*

Committee Member: *Carsten Kuelheim*

Committee Member: *Andrew Burton*

College Dean: *Andrew Storer*

Table of Contents

List of Tables	iv
List of Figures	v
Author Contribution Statement.....	vi
Acknowledgements	vii
Abstract	viii
Chapter 1. Introduction	1
Introduction.....	1
Chapter 2. Common garden study reveals frost-tolerant northern seed sources are best suited to expand range of <i>Quercus rubra</i>	4
Introduction.....	4
Methods	9
Results.....	14
Discussion.....	16
Conclusion	26
Tables.....	28
Figures	33
Chapter 3. Northern populations of <i>Quercus rubra</i> are best suited for expanding range due to wide photosynthetic thermal niche and greater stress tolerance	41
Introduction.....	41
Methods	46
Results.....	53
Discussion.....	55
Conclusion	63
Tables.....	64
Figures	70
Citations	79

List of Tables

Table 2.1 Common garden average temperatures and precipitation.....	28
Table 2.2 Seed sources and environmental variables of <i>Quercus rubra</i> seedlings.....	29
Table 2.3 Two-way ANCOVA results for biomass index and growth index.....	30
Table 2.4 Two-way ANCOVA results for senescence, budburst, and frost damage.....	31
Table 2.5 Regression results for leaf lifespan.....	32
Table 3.1 Seed sources and environmental variables of <i>Quercus rubra</i> seedlings.....	64
Table 3.2 Common garden average temperatures and precipitation.....	65
Table 3.3 Two-way ANCOVA and regression results A_{\max} and F_v/F_m	66
Table 3.4 Two-way ANCOVA and regression results for N_{area} , N_{mass}	67
Table 3.5 Two-way ANCOVA results for temperature curve parameters.....	68
Table 3.6 Two-way ANCOVA and regression results for A/g_s , ^{13}C and LWC.....	69

List of Figures

Figure 2.1 Map of <i>Quercus rubra</i> seed sources.....	33
Figure 2.2 Common garden design.....	34
Figure. 2.3 Interaction plots of growth index.....	35
Figure 2.4 Interaction plots of biomass index.....	36
Figure 2.5 Regression plots of heat sum required for budburst.....	37
Figure 2.6 Regression plots of Julian date of senescence.....	38
Figure 2.7 Regression plots of frost damage.....	39
Figure 2.8 Regression plots of leaf lifespan.....	40
Figure 3.1 Map of <i>Quercus rubra</i> seed sources.....	70
Figure 3.2 Common garden design.....	71
Figure 3.3 Interaction plots of A_{\max}	72
Figure 3.4 Regression plots of N_{mass} and N_{area}	73
Figure 3.5 Interaction plots of N_{mass} and N_{area}	74
Figure 3.6 Interaction and regression plots of F_v/F_m	75
Figure 3.7 Interaction plots of temperature curve parameters.....	76
Figure 3.8 Interaction plots of A/g_s (A_{\max} /stomatal conductance)	77
Figure 3.9 Interaction and regression plots of $\delta^{13}\text{CVPDB}$ (‰) and LWC	78

Author Contribution Statement

This thesis will be submitted to peer-reviewed journals for publication in the near future.

All Chapters were written by myself, Emily Lindback, with contribution from Molly Cavaleri. All experiments were designed in collaboration with Molly Cavaleri and Carsten Kuelheim. I performed the data collection, analysis, and writing. Chapter 2 is planned for publication as is. Chapter 3 will include data from colleagues at the University of Minnesota when submitted for publication.

Acknowledgements

Thank you to our funding agencies, the USDA and the Ecosystem Science Center. I would also like to thank Dr. Molly Cavaleri and Dr. Carsten Kuelheim for their support and guidance during my Master's. To Rob Tunison for helping with field campaigns and R as well as always being a source of plant physiology knowledge. To all those who assisted in lab work, particularly grinding and tinning hundreds of leaf samples for nutrient analysis. To James Rauschendorfer, Stephanie Frantti, Victoria Peck, Madalyn Tudor-Duncan, and Joel Van Diepenbos for making field work fun. To my partner, Mack Berkowitz, who almost has an honorary degree in plant physiology after two years of me talking about this thesis. Thank you for your love and support.

Abstract

Climate change is shifting the potential suitable range of northern red oak (*Quercus rubra* L.) faster than it can migrate, creating an adaptation lag. *Quercus rubra* is prominent in eastern North American forests and important for its carbon sequestration, ecological roles, and economic significance. Our study addresses which populations of *Q. rubra* are best suited for climatic conditions at the northern and central range limits. Our findings can inform forestry management policies such as assisted migration, where species are transferred within their native range to mitigate the effects of climate change. We planted over 800 *Q. rubra* seedlings from twelve populations across the current *Q. rubra* geographic range in two common gardens, one at the northern range limit (Alberta, Michigan) and one in the central range (Kalamazoo, Michigan). We observed *Q. rubra* aboveground biomass growth, phenology (senescence, budburst), and frost damage for three years. We also measured photosynthetic capacity, stress response, photosynthetic temperature response and water use efficiency to understand the physiological limits of the populations and how they responded to the environmental conditions of the common garden.

Southern seed source populations, those from warmer and wetter locations, showed greater growth increments in all years. However, southern seed sources generally had later phenological events which, coupled with their acquisitive growth strategy, resulted in them sustaining more frost damage than northern seed source populations. Northern seed sources, from colder and drier locations, had earlier budburst and were able to utilize early growing season snow melt and higher solar radiation. Northern seed

sources also senesced earlier, avoiding autumn frosts. While southern seed sources accumulated more aboveground biomass, it was a trade off with greater evidence of frost damage. Northern populations also seemed to have a more conservative, generalist strategy for physiology. Northern populations had higher leaf mass per area, foliar leaf nitrogen, and photosynthetic capacity. They also had a wider photosynthetic temperature range compared to southern seed sources. More extreme weather events are predicted with climate change, which favors the more conservative generalist strategy and greater frost tolerance of northern seed sources for assisted migration at the northern range limit.

Chapter 1. Introduction

Introduction

Increased temperatures, extreme weather events and decreased precipitation are changing the composition of forests in eastern North America (Anderson and Tomlinson 1998, IPCC 2021). Populations that were once suited to a past environment may now be maladapted as the climate changes. The suitable climate for *Quercus rubra* L., a dominant eastern species, is shifting north and east faster than the species can migrate, causing an adaptation lag at the northern range limit (Anderson and Tomlinson 1998, Frelich and Reich 2010, Zhu et al. 2012, Sittaro et al. 2017).

To address the inherent ability of different *Q. rubra* populations to cope with this problem, we created two common gardens with *Q. rubra* seedlings. Over 800 seedlings from 12 genetically diverse populations across the geographic range of *Q. rubra* were planted as seedlings at Kellogg Experimental Forest in Lower Peninsula Michigan, near the center of *Q. rubra* range, and at Ford Forest in Upper Peninsula Michigan, near the northern range limit. These sites' mean annual temperatures differ by 5 °C, creating two distinct growth environments.

This body of work focuses on comparing growth, phenology (Chapter 2) and physiology (Chapter 3) of the *Q. rubra* seedlings across seed sources in the two common gardens. The strategies seed sources use help to determine which populations are best suited to common garden conditions. For example, there is a known tradeoff of cold tolerance and biomass accumulation (Aitken et al. 2008), and cold tolerance may be more

important for plant survival at a northern site. Phenology also determines which seed sources are best suited for a site. A longer growing season, established through earlier budburst and later senescence, puts plants at greater risk of frost damage (Howe et al. 2003, Aitken et al. 2008). While a longer growing season allows more time for photosynthesis, it may also expose trees to harsh weather events in the shoulders of the growing season.

Physiological capacity and thresholds are also important for determining which populations are best suited for a site. If plants are exposed to temperatures outside of their thermal range and optimum temperatures at a site, for example, there will be decreased carbon assimilation or the potential of harm to photosystems (Gunderson et al. 2010, Mau et al. 2018). Water use efficiency and drought resistance should also be considered, as extreme weather events are likely to become more frequent and intense (IPCC 2021). Drought resistance strategies often seen in dry seed source plants, such as low stomatal conductance during gas exchange and greater leaf mass per area, allow plants to conserve water (Kerr et al. 2015).

The objectives of this study were to: (1) determine differences in growth, phenology, and physiology of *Q. rubra* seed sources from across a latitudinal climate gradient; and (2) determine which seed sources are best suited for the changing climate at the northern range limit of *Q. rubra*.

This research will help us better predict how *Q. rubra* seedlings from seed sources across a latitudinal gradient will respond to climate change, which in turn will have significant impacts on the ecology and economy of eastern forests of North America, as

Q. rubra is a valuable source of timber and wildlife habitat. In addition, knowing which seed sources do well in which transplanted locations can be used for management decision-making. For example, forestry assisted migration is the practice of moving populations of a species within its native range to locations likely to be better suited under predicted climate change (Williams and Dumroese 2013, Park and Talbot 2018, Etterson et al. 2020). This study will add to a growing body of work determining if assisted migration is an appropriate strategy for *Q. rubra*, and if so, which seed sources are the best candidates for northern locations.

Chapter 2. Common garden study reveals frost-tolerant northern seed sources are best suited to expand range of *Quercus rubra*

Introduction

Northern red oak (*Quercus rubra* L.) is a prominent mid-successional tree in forests of eastern North America, valued for its ecological, economic, and cultural significance. It is a dominant canopy species, and its carbon sequestration is significant (Cavender-Bares and Bazzaz 2000). While *Q. rubra* is less vulnerable to climate change than many co-occurring hardwoods, it comprises a significant amount of biomass, with oak-hickory forests constituting 34 % of forested land in the eastern U.S. (Oswalt 2014).

Currently, *Q. rubra* distribution ranges from Quebec, Canada to Alabama and as far west as Oklahoma; however, this range is shifting with climate change. More extreme weather events, such as drought, frosts, and an increased mean annual temperature of 2 to 8 °C in the next century, are pushing trees north (Anderson and Tomlinson 1998, IPCC 2021). *Quercus rubra* is adapted to mesic sites, and the boundaries between mesic and xeric sites as well as the boundaries between prairie and forest are expected to shift northward as well (Anderson and Tomlinson 1998, Frelich and Reich 2010).

Quercus rubra is moving northward slower than the warming climate, causing concern, especially at northern range limits (Zhu et al. 2012, Sittaro et al. 2017). From 1970 to 2000, the northern range limit for *Q. rubra* adults and saplings shifted 0.036 and

0.531 km/yr⁻¹, respectively, at the 95th percentile latitudinal distribution difference (Sittaro et al. 2017). However, to keep pace with climate change, *Q. rubra* will need to migrate hundreds of km northward, as climates have already shifted more than 100 km during the 1900s in the Northeast and Upper Midwest U.S. (Zhu et al. 2012). Climate change projections predict *Q. rubra* will decline to between 60-80 % of its original population size under low emissions and 29-47 % under high emissions in Connecticut, Massachusetts, and Rhode Island, where *Q. rubra* is one of the top three most prevalent tree species (Iverson et al. 2008).

It is commonly believed that plants are adapted to their localized environments (Gunderson et al. 2012, Park and Talbot 2018). However, because *Q. rubra*'s environment is changing more quickly than it can migrate, *Q. rubra* is becoming maladapted to sites it used to be suited for (Park and Talbot 2018). One approach to combat climate change outpacing natural migration rates is forestry assisted migration, the practice of moving populations of a species to new areas in anticipation of how the species will be affected by climate change (Etterson et al. 2020).

Our study emulated assisted migration within the current species range, referred to as 'assisted population migration' (Williams and Dumroese 2013, Park and Talbot 2018). Assisted population migration is already practiced in Canada and widely studied (Park and Talbot 2018). Long term assisted migration studies in North America, such as the Assisted Migration Adaptation Trial funded by the British Columbia Ministry of Forests and the USDA, are being used to inform policy (Williams and Dumroese 2013). The US Forest Service plans to use assisted migration as a mitigation tool for climate

change (United States Forest Service 2008). However, foresters are reluctant to perform assisted migration without more evidence to determine if it is an appropriate strategy (Etterson et al. 2020). This study will join a growing body of work that experimentally simulates assisted migration in order to inform policy making (Etterson et al. 2020).

Although southern seed sources generally accumulate more biomass than northern seed sources in common garden and assisted migration studies (Abdala-Roberts et al. 2018, Etterson et al. 2020), other metrics such as phenology should also be considered when assessing which seed source is best suited for a site. For example, budburst is controlled by photoperiod, a minimum number of chilling days, and an accumulation of growing degree days (i.e., warm temperatures above a threshold), in long-lived late successional *Quercus rubra* and other *Quercus* species (Korner and Basler 2010, Gunderson et al. 2012). Senescence is controlled by photoperiod, temperature, and precipitation in deciduous tree species (Keenan and Richardson 2015). Because the mechanisms underpinning budburst and senescence are genetic, phenology can vary among geographically different seed sources, even when they are planted in the same location (e.g., in a common garden). Studying phenology across seed sources in a common garden environment allows us to determine the growing season length of seed sources and which is best suited for the site climate. For example, there is a known tradeoff between a longer growing season and risk of cold injury (Aitken et al. 2008).

Growing seasons are lengthening in many temperate zones due to climate change, and budburst is occurring earlier in the spring, making plants more prone to frost damage (Gunderson et al. 2012, Williams and Dumroese 2013, Parmesan and Hanley 2015).

Woody plants already show budburst 18 days earlier than they did in the 1850s in Massachusetts (Polgar et al. 2014). If the climate warms 4 °C, the growing season could extend by 5-30 days at both ends of the growing season, as reported in an Oak Ridge, Tennessee study on four deciduous species, including *Q. rubra* (Gunderson et al. 2012).

Arguably more important than a warming climate will be extreme weather events such as droughts and frosts, especially for seedlings (Zhu et al. 2012, Park and Talbot 2018). Hard frosts and cold variability are a large determining factor in predicting species range limits (Park and Talbot 2018). Also, seedlings, which are most likely to be transplanted using assisted migration, are less tolerant of frosts than adult trees due to their small size and their meristems being in the layer of cold air just above the soil (Howe et al. 2003, Hofmann et al. 2014). Past common garden studies have found cold hardiness correlates with seed source temperature in *P. menziesii* and *L. occidentalis* (Aitken et al. 2008). Plants from colder seed sources have historically gone into dormancy earlier, at the expense of aboveground growth, but achieving more cold hardiness, across multiple deciduous and coniferous species (Howe et al. 2003, Aitken et al. 2008).

We investigated the aboveground biomass and growth of *Q. rubra* seedlings from seed sources representing a latitudinal gradient of 10 degrees and a mean annual temperature (MAT) gradient of 10 °C in two common gardens in Michigan, U.S. for three years. The northern site, Ford common garden, is colder and drier, while the more southern site, Kellogg, is warmer and wetter. Additionally, in the more northern common

garden site, we measured phenology, including budburst, autumn senescence, leaf lifespan, and frost damage.

Our hypotheses for this study were as follows: *Hypothesis 1*: growth will increase with seed source mean annual temperature and precipitation in the common garden sites. We predict growth will be larger for warmer seed source plants because cold tolerant plants will grow more slowly, a known trade off (Rehfeldt et al. 1999). Plants at Kellogg will have a greater biomass than those at Ford because Kellogg receives warmer temperatures and more precipitation, resulting in a longer growing season. *Hypothesis 2*: Plants from a warmer seed source will have later budburst and senescence times than those from cold seed sources, making them more prone to frost damage in autumn. Because budburst is primarily controlled by an accumulation of growing degree days and northern seed sources have a lower threshold of accumulated growing degree days required to force budbreak, we predict colder seed sources will show earlier budburst in the northern site (Gunderson et al. 2012). Northern plants will senesce earlier than southern plants because they have an earlier photoperiod cue to senesce. Southern plants will be more prone to frost damage because a later budburst date is generally correlated with being less resistant to frost (Korner et al. 2016). *Hypothesis 3*: Leaf lifespan will be similar for all seed sources and warmer seed sources' growing season will be shifted later than cold seed source plants. Leaf lifespan is longer for plants growing in warmer environments when they are in their native environments (Wright et al. 2004). However, when warm seed source plants are transplanted to a colder, northern location, it may take them longer to accumulate the heat sum necessary for budburst than colder seed source plants, thus potentially shortening their leaf lifespan. We expect this to be offset by a later

senescence in southern seed source plants due to their later photoperiod cue, which would shift their leaf lifespan later in the season.

Methods

Common gardens: site description

The northern site, Ford Center common garden is located in Alberta, Baraga County, Michigan, USA (46.65°N, 88.48°W, 400 m elevation) near the northern range limit of *Q. rubra*. The mean annual temperature is 4.9 °C and mean annual precipitation is 879 mm, with 401 mm falling during the growing season months (Jarvi and Burton 2013) (Table 1). The soil is a Kallio cobbly silt loam (coarse-loamy, mixed, superactive, frigid Oxyaquic Fragiorthods) (Jarvi and Burton 2018). The southern site, the Kellogg Forest common garden, is located near the center of the *Q. rubra* range, near Augusta, Kalamazoo County, Michigan, USA (42.37°N, 85.35°W). It has an annual temperature of 9.9 °C and precipitation of 1027 mm, with 523 mm falling during the growing season (Abraha et al. 2020) (Table 2.1). The soil is classified as Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalf) (Crum and Collins 1995).

Experimental design

In fall 2018, over 1,000 *Q. rubra* acorns were collected from 4-6 mother trees at each of 12 locations, within the range *Q. rubra* (Figure 1, Table 2). The acorns were stratified at 4 °C after date of collection, ranging from September-November, until they were planted in 0.5 L pots on April 3-4, 2019 at the College of Forest Resources and Environmental Science at Michigan Technological University and placed in a

greenhouse. Conditions were maintained at 16 hours light, 8 hours dark with a daytime temperature of 23 °C and a nighttime temperature of 18 °C. Plants were watered by hand every 3 days.

Quercus rubra seedlings were planted at the Kellogg common garden site on June 10-12, 2019 and at the Ford common garden site on June 17-19, 2019. They were planted along with *Quercus ellipsoidalis* (northern pin oak) seedlings using an incomplete randomized block design. While part of the larger experiment, *Q. ellipsoidalis* seedlings were not assessed in this study.

In each common garden, there are 27 blocks, arranged in a 3 x 9 block structure, with a buffer row of plants between each row of three blocks (Figure 2.2). Each block contains 30 plants in a 3 x 10 formation where 3 columns are spaced 1.5 m apart, and 10 plants in each row are spaced 0.75 m apart (Figure 2.2). The buffer row has the same dimensions as a normal row. Each block represents, on average, one to two plants from each of 12 seed sources of *Q. rubra*, about 10 *Q. rubra* plants from seed sources not used in this analysis, and approximately 8 *Q. ellipsoidales*. The common gardens are surrounded by 10-foot fences to prevent herbivory and were regularly weeded by hand and with a glyphosate herbicide to prevent overcrowding and shading in 2019 and 2020. Plants at the Ford common garden were watered by hand every 3 days from June 17th to July 14th, 2019, and every 7 days from July 15th to September 2019. In 2020, plants at Ford were watered every 7 days from June 15th to July 12th, every 10 days from July 13th to July 26th, and every 14 days from July 27th to August 23rd. Iron Chelate was applied at Ford on August 12, 2019 and June 13, 2020. Plants were neither watered nor fertilized in

2021. Twelve seed source populations, containing approximately 16 seedlings at each of 54 blocks within the 2 sites, were used for growth and biomass measurements. In 2019, a total of 893 seedlings were assessed; in 2020, 850 were assessed due to mortality; and in 2021, 421 were assessed due to a concurrent drought experiment (which was beyond the scope of this study). At the Ford common garden only, approximately 450 seedlings were measured for budburst, frost damage, and senescence. Only 178 plants were measured for senescence in 2021 due to the drought treatment.

Environmental variables

Environmental variables for the twelve seed source populations of *Q. rubra* were collected from Worldclim version 2.0 with a spatial resolution of 30 seconds ($\sim 1 \text{ km}^2$) (Frick and Hijmans 2017) (Table 2.2). Two environmental variables were used: mean annual temperature (MAT; °C) and rain over active growth months of May to September (rain; mm). Common garden weather information is from weather stations located in Alberta and Battle Creek (NOAA 2022).

Growth measurements and Ford and Kellogg common gardens

Growth measurements taken at both common gardens included plant height, projected crown diameter, and stem diameter. In 2019, all growth measurements were taken five times at the Ford common garden (July 15-17, July 30-Aug 1, Aug 14-17, Aug 27-28, Sept 10-13) and once at the Kellogg common garden on August 19-20. In 2020, growth measurements were taken five times at Ford (June 15-19, June 29-July 3, July 13-17, July 27-31, August 24-28) and once at Kellogg (August 11-13). In 2021, growth

measurements were taken three times at Ford (June 21-25, July 19-23, August 16-20) and two times at Kellogg (July 12-16, August 9-13). Height was measured from the base of the plant to the terminal bud using a meter stick to the nearest cm. Projected crown diameter was measured in two directions (East to West = crown diameter EW; and North to South = crown diameter NS) using a meter stick to the nearest cm. Stem diameter was measured at the root collar of the plant using calipers to the nearest hundredth mm. Biomass index was calculated as $((\text{height} + \text{crown diameter EW} + \text{crown diameter NS})/3) * \text{stem diameter}$. Growth index represents growth across a season. Growth index was calculated as the difference in biomass index from one year to the next, as measured at the end of each growing season.

Frost damage and phenology at Ford common garden

Phenological measurements, including timing of budburst and senescence, as well as frost damage, were measured only at the Ford common garden for three years. Budburst was assessed weekly by visual observation from May 2nd through June 13th in 2020 (May 2, 9, 16, 23, 30 and June 6, 13) and from May 3rd through May 31st in 2021 (May 3, 10, 17, 24, 31). The progression of budburst was rated on a 6-stage scale where 0 represented buds still enveloped by scales and 6 represented fully expanded leaves (Chi-Wu 1962). The Julian date of budburst was noted when the plants progressed to a rating of 3, when buds were breaking, and leaves were just visible. Heat sum required for budburst at Ford in 2020 and 2021 was calculated using data from NOAA with a base temperature of 0 °C (NOAA 2022). The progression of senescence was assessed using a chlorophyll content meter, (CCM-220plus, Opti-Sciences, Hudson, NH, USA).

Chlorophyll measurements began in September and ended once leaves were either abscised or totally brown. In 2019, chlorophyll content was measured eight times, approximately weekly (September 16, 23, 30, October 7, 21, November 4, 11, 18). In 2020, chlorophyll content was measured four times, approximately biweekly (September 14, 28, October 12, 26). In 2021, chlorophyll content was measured eight times, weekly (September 17, 23, 30, October 7, 14, 21, 28, and November 7). The Julian dates of senescence in 2019, 2020, and 2021 were calculated based on date of the first chlorophyll reading that was 50% or less than the maximum chlorophyll reading. Frost damage (present or absent) was assessed visually on September 21-25, 2020 at the Ford common garden. Plants with necrosis on newly developing leaves were considered damaged. Leaf lifespan was calculated as the number of elapsed days between the Julian dates of budburst and senescence for 2020 and 2021.

Statistical analyses

Yearly biomass and growth index measurements were compared across common gardens and correlated with both seed source mean annual temperature (MAT) (°C) and rain over active growth months of May to September (mm) using analysis of covariance (ANCOVAs) in the package *rstatix* with site as a fixed factor (Kassambara 2020). The closest August measurement dates at Ford and Kellogg were used in these ANCOVAs.

Budburst and senescence measurements from Ford only were compared to seed source environmental variables using regression (R 2020). The first budburst value of 3 or higher was noted and the corresponding heat sum (with a base temperature of 0 °C)

was analyzed against seed source MAT and rain over growing season using regression (R 2020).

Regression was used to analyze the Julian date of senescence against seed source environmental variables. Frost damage was analyzed using a point biserial correlation in the R package graphics (R 2020). Leaf lifespan was analyzed against seed source environmental variables using a regression. All data analyses were conducted using R statistical software version 4.0.3 (R 2020).

Results

Biomass and growth at Ford and Kellogg

Biomass index and growth index were positively correlated with seed source mean annual temperature and rain over growing season in all three years. Biomass and growth indexes were greater at the more northern site, Ford, than the more southern site, Kellogg, in 2019 and 2020 (MAT Site and Rain Site $p < 0.001$ in all years) (Figure 2.3 and 2.4, Table 2.3). In 2021, growth index was greater at Kellogg than Ford (MAT Site and Rain Site $p < 0.001$) and site was not a significant factor in 2021 biomass index (MAT Site $p = 0.680$, Rain Site $p = 0.699$) (Figure 2.3 and 2.4, Table 2.3). The mean height of the trees at Kellogg in 2021 was 63.6 cm, while trees averaged 49.0 cm at Ford. For the 2021 biomass index, there were significant interactions between MAT with site and rain with site (MAT*Site $p = < 0.001$, Rain*Site $p = < 0.001$) (Figure 2.3 and 2.4, Table 2.3). These interaction effects represented biomass index increasing with MAT and rain at Kellogg, while biomass index was not correlated with MAT and rain at Ford.

Frost damage and phenology at Ford

Southern seed source plants had a later date of budburst and greater frost damage than northern seed source plants, while senescence timing varied for seed sources from year to year. In both years, 2020 and 2021, the heat sum required for budburst was positively correlated with seed source MAT and rain, indicating warmer seed source plants had later budburst later in the spring than cooler seed source plants (all regressions $p < 0.001$) (Figure 2.5, Table 2.4). The Julian date of senescence was positively correlated with MAT and rain in 2019 and 2021, where senescence occurred later in the season for warm seed source plants (all regressions $p < 0.001$) (Figure 2.6, Table 2.4). In 2020, however, senescence was negatively correlated with seed source MAT and rain (all regressions $p < 0.001$) (Figure 2.6, Table 2.4). It is important to note there are fewer senescence measurement dates in 2020. Frost damage was positively correlated with seed source MAT in fall 2020 ($p = 0.041$) but not rain ($p = 0.149$) indicating warmer, more southern seed source plants were more prone to frost damage (Figure 2.7, Table 2.4).

Leaf lifespan's correlation with seed source mean annual temperature and rain over growing season was negative in 2020 and positive in 2021. In 2020, leaf lifespan was negatively correlated with MAT and rain (all regressions $p < 0.001$) (Figure 2.8, Table 2.5). Warmer seed source plants had a shorter leaf lifespan than colder seed source plants. Budburst occurred later for warm seed source plants, and senescence occurred earlier, causing them to have a shorter leaf lifespan than cold seed source plants. In 2021, leaf lifespan was positively correlated with seed source MAT ($p = 0.013$) and rain ($p = 0.021$) with warmer, wetter seed source plants having a longer leaf lifespan than colder,

drier seed source plants (Figure 2.78 Table 2.5). Southern seed source plants had a later budburst and a later senescence, shifting and lengthening the growing season.

Discussion

Variation in growth across seed source climate at Ford and Kellogg

We expected growth to increase with increasing seed source mean annual temperature and precipitation and that plants at the southern common garden would have greater growth (H1). While we did find higher growth for plants from warmer and wetter seed sources at both common gardens for all years, which site showed more growth was not consistent from year-to-year. Rather, plants at the northern site had greater growth in 2019 and 2020, while plants at the southern site had greater growth in 2021.

The trend in higher biomass and growth with warmer, wetter seed source may be due to different growth strategies of seed sources. *Quercus rubra* is considered acquisitive on the plant economic spectrum (Wright et al. 2004, Abdala-Roberts et al. 2018). The plant economic spectrum spans from plants that are acquisitive and have a quick return on investment to plants that are conservative and have a slower return on investment in terms on leaf nutrient concentrations, gas exchange, leaf lifetimes, and leaf mass investments (Wright et al. 2004). However, how acquisitive populations of *Q. rubra* are likely varies based on seed source climate. Other common garden studies have shown wetter seed sources are more acquisitive than drier seed sources. In a study on 11 *Quercus* species, those from wetter climates had lower leaf mass per area, higher leaf nitrogen, and less condensed tannins, indicating more acquisitive strategies than those

from drier climates (Abdala-Roberts et al. 2018). In an assisted migration study where *Q. rubra* from central and northcentral U.S. seed sources were planted at the northern range limit of *Q. rubra*, more southern seed source seedlings had higher survival rate, grew faster, and had a longer growing season than their northern counterparts (Etterson et al. 2020). In another study, southern seed sources of *Q. macrocarpa* and *Picea glauca* had the largest height growth (Prud'homme et al. 2018, Etterson et al. 2020). These studies parallel our findings, where southern seed sources had a greater growth index.

Warmer, wetter seed source plants may not allocate as much carbon to belowground biomass (Abdala-Roberts et al. 2018). Southern seed sources are not adapted to the harsh weather associated with the north, including early and late season frosts and summer droughts. Under drought conditions, seedlings often allocate more carbon to roots (Richter et al. 2012). Northern plants are adapted to less precipitation, so they may be allocating more carbon belowground.

Colder seed sources also tend to have greater cold hardiness (Howe et al. 2003) and may have greater biotic defenses. In a common garden study, *Q. petraea* height declined with increasing altitude of seed source, and higher altitude was correlated with greater cold tolerance (Vitasse et al. 2009). This tradeoff of higher growth and biomass vs. investing in cold tolerance and defenses may be of consequence for *Q. rubra* because it is predicted that *Q. rubra* will be one of the northern tree species most affected by insects and pathogens under climate change (Rogers et al. 2017). Southern seed sources have been browsed more frequently than local seed sources, possibly due to the larger biomass

of transplanted seedlings in another provenance study on nine northeastern U.S. tree species including *Q. rubra* (Clark et al. 2022).

Another explanation for greater biomass in southern seed source populations is that warmer, wetter seed source plants are adapted to a longer growing season and allocate carbon and nutrients belowground at the end of the growth season. However, when these warmer, wetter seed source seedlings were planted in the center and northern range they were likely frost damaged before they were able to remobilize carbon and nutrients belowground. A later senescence puts plants at risk of being unable to remobilize carbon and nutrients belowground as reported in *P. tremula* (Keskitalo et al. 2005). This is further supported by southern plants in our study being more frost damaged than northern plants when measured in fall 2020. Frost damage was also greater for southern seed source plants at Ford in May 2021 as shown in Rauschendorfer et al. (2021).

It is also important to note that juvenile growth may not predict which mature trees have greatest growth and survival. For example, another study also found Douglas-Fir seedlings from warmer seed sources also grew larger in the first few years of planting, but once trees matured the local seed sources were better adapted to the site, based on survival (St Clair et al. 2020). We also do not know the belowground biomass and how that will affect future growth of the respective populations. Colder, drier populations likely invest more carbon in root systems than warmer, wetter populations. Other studies have found Douglas-fir seedlings from drier regions have a greater root to shoot ratio (Howe et al. 2003). Greater belowground biomass may aid in greater aboveground growth later. The conservative strategies of northern seed source populations appear to be

better suited to the northern range limit, which may make them better candidates for assisted population migration at the northern range limit, even though they have less aboveground biomass in their juvenile years.

Difference between Ford and Kellogg common gardens

Contrary to hypothesis 1, growth was greater at the more northern site, Ford, than the more southern site, Kellogg, in 2019 and 2020. However, in year 3, this trend changed, and growth was greater at Kellogg than Ford in 2021. We expected greater biomass and growth at Kellogg due to warmer temperatures and more precipitation (Table 2). However, Ford plants received supplemental water and fertilizer in the first two years after planting, while Kellogg plants did not. Kellogg plants were also sun damaged when planted at Kellogg, which may have stunted their growth. Another reason for higher biomass at Ford in the first two years could be because Ford seedlings were grown in an open area, while Kellogg seedlings received some shade from a natural forest on the edge of the plot. In an assisted migration study, planted *Q. rubra* seedlings had higher growth and survival when grown in open conditions compared to closed canopy forests (Clark et al. 2022).

Growth at Kellogg outpacing that at Ford in 2021 is likely explained by a drought at Ford in summer 2021, where Ford received less than half the precipitation Kellogg did (Table 2.2). This is similar to a previous *Q. rubra* study, where intense heat waves coupled with lower soil moisture decreased biomass production (Bauweraerts et al. 2013). Drought frequency and severity, as well as heat waves, and are also expected to increase with climate change, and the negative effects on biomass may be more intense

than a uniform increase in temperature (Bauweraerts et al. 2013, Park and Talbot 2018). This is important to note because *Quercus* is generally found to be anisohydric; it maintains photosynthesis even during drought, risking cavitation when soil water potential is low (Park and Talbot 2018). Decreased growth at Ford in 2021 and less significant growth differences between populations at Ford is also likely the result of a strong frost in May 2021 that damaged plants, especially southern seed sources, stunting their growth. Based on water use strategies and cold hardiness, the more conservative northern *Q. rubra* populations used in this study may be more suitable for assisted migration at the northern range limit.

Budburst at Ford

In support of hypothesis 2, warmer seed source plants had later budburst dates in all observed years. This trend has been observed in other *Q. rubra* studies where latitude was the primary controlling component on *Q. rubra* budburst (Gerst et al. 2017). In provenance studies, in general, northern seed source plants have an earlier date of budburst (Howe et al. 2003). Elevation, used as a proxy for latitude, also correlates with heat sum, where there is a lower heat sum requirement for higher elevation *Fagus* in provenance studies (Basler and Korner 2012).

Earlier budburst may favor cold seed source plants because they can utilize early growing season conditions. This early season is considered a “luxury period” of moist soil, a more direct sun angle, and longer daylength, as opposed to the late fall when there are shorter days, less direct solar radiation, and potential declines in photosynthetic capacity (Gunderson et al. 2012). As such, having an earlier budburst, or extending the

growing season on the spring end, may have a stronger impact on carbon sequestration than extending the growing season on the autumn senescence end (Gunderson et al. 2012).

While northern plants have the advantage of an earlier budburst, which is correlated with increased growth for native plants, they also have an increased risk of springtime frost damage (Vitasse et al. 2009, Basler and Korner 2012, Korner et al. 2016, Iler et al. 2021). This is especially dangerous, as there is an increase in the frequency of frosts, extreme weather events, temperature variability, and new herbivores due to climate change (Augspurger 2013, Gerst et al. 2017, Iler et al. 2021). Earlier budburst may also expose the plant to reduced mobile soil nutrients (Iler et al. 2021). This may cause plants with earlier budburst dates to have a lower survival rate and less biomass than plants that have a later budburst (Augspurger 2013, Iler et al. 2021). Our results suggest that while northern seed source plants had an earlier budburst which may have exposed them to more hazards, they also were hardier than southern seed source plants and more equipped to handle those hazards, making it a tradeoff that appears to favor the earlier budburst.

While colder seed sources are often associated with earlier budburst, the mechanism that cues budburst in *Q. rubra* is not fully understood. The budburst signal may be a photoperiod cue or heat sum or a combination of factors. One study found that for late successional species such as *Quercus*, photoperiod was the primary budburst signal (Basler and Korner 2012). Another study where *Q. rubra* seedlings were experimentally warmed found heat sum was the driving force, not photoperiod

(Gunderson et al. 2012). Which cue is more strongly favored is heritable and varies for different seed sources (Morgenstern 1996). For example, *Quercus* from a climate with extreme wind and varying spring temperatures, including late frosts, was found to have a stronger photoperiod response than other seed sources (Basler and Korner 2012).

However, multiple studies agree that *Quercus* is in some way constrained by a photoperiod threshold, and the correlation between temperature and budburst is not linear (Korner and Basler 2010, Basler and Korner 2012). Using photoperiod as a cue is a more conservative strategy, while solely using heat sum as a cue for budburst is a common strategy for pioneer species and is risky due to unusual early warm periods followed by frosts (Basler and Korner 2012).

The abilities of trees to extend their growing seasons in response to climate change is important for carbon cycling, however we have a weak understanding of the mechanisms controlling phenological events (Olsen 2010, Way 2011). Climate change models predict and have observed earlier budbursts (Polgar and Primack 2011, Keenan and Richardson 2015). Despite warming air temperatures, day length will stay the same. Species controlled, or partially controlled, by photoperiod cues or unmet winter chilling requirements will not be able to extend their growing season (Polgar and Primack 2011, Way 2011, Polgar et al. 2014). This means, assuming *Q. rubra* budburst is at least partially controlled by photoperiod, that it will take generations for *Quercus* populations to select for earlier photoperiod cues associated with an earlier spring due to climate change (Basler and Korner 2012). This implies that northern seed sources, which showed earlier budburst dates than southern seed sources due to a lower heat sum requirement, photoperiod, or both, may be better able to capitalize on the luxury growing period of

early spring at the northern range limit. Another consequence of their earlier budburst is the ability to sequester more carbon as northern ecosystems are taking up more CO₂ during early spring and summer (Barlow et al. 2015).

Senescence at Ford

Contrary to hypothesis 2, timing of senescence varied from year to year at the Ford common garden. Senescence occurred later for warmer, wetter seed sources than colder, drier seed sources in 2019 and 2021, as predicted. However, in 2020 colder, drier seed sources senesced later. Warmer seed sources suffered more frost damage than colder seed sources in autumn 2020 and spring 2021, as predicted.

Northern seed source plants senesced earlier in 2019 and 2021, perhaps due to a longer photoperiod cue (Park and Talbot 2018). This reflects adaptation to their local seed source environment where temperatures are colder earlier in autumn compared to more southern regions. Conversely, southern seed source plants have a shorter photoperiod cue to senesce (senesce later in autumn) because they are adapted to warmer autumn temperatures which slow chlorophyll degradation (Way 2011). When southern seed source plants are transplanted north, a later senescence may not only cause frost damage, as we found, but can also put the plant at risk of not fully remobilizing nutrients (Keskitalo et al. 2005). In 2020, southern seed sources senesced earlier than northern seed sources. This may have been in response to a strong frost, measured as -1.7 °C, on September, 18 2020 (NOAA 2022). It may also be because senescence measurements were less frequent in autumn 2020, creating a less robust data set.

An earlier senescence decreases the risk of frost damage for plants in the northern range of *Q. rubra* (Park and Talbot 2018). These northern plants do not have to “cold acclimate” because they are already genetically suited for the climate, whereas warmer plants need to cold acclimate when transplanted north. Cold acclimation refers to morphological and physiological changes a plant undergoes to ready a plant for cold exposure (Ramírez-Valiente 2017). Lack of cold acclimation can be shown in lower photosynthetic capacity and lower photosystem II efficiency (Ramírez-Valiente 2017). The higher amount of frost damage on warmer seed source plants indicates they were not as cold adapted as colder seed source plants.

Leaf lifespan at Ford

We did not find evidence to support hypothesis 3, that leaf lifespan would be similar for all seed sources, with warmer seed sources having a leaf lifespan that is shifted later than cold seed source plants when planted in the northern common garden at Ford. Instead, in 2020 northern seed source plants had a longer leaf lifespan, while in 2021 warmer seed source plants had a longer leaf lifespan. Southern seed source plants had later budburst in both 2020 and 2021, as expected. However, in 2020 southern seed source plants unexpectedly senesced earlier than northern seed source plants, causing northern seed source plants to have a longer leaf lifespan, extending the growing season further into the fall. The early senescence of southern seed source plants was likely due to fall 2020 frosts. In 2021, southern seed source plants had a later and longer leaf lifespan, both budburst and senescence occurred later than northern seed source plants, as predicted.

Generally, leaf lifespan is shorter for plants from colder environments (Wright et al. 2004). This, however, is not true for plants transplanted into a common garden outside of their localized environment. When southern seed sources are transplanted to a colder location, it will take longer for them to accumulate the heat sum required for budburst and they will have a later budburst than in their local environment, shortening their leaf lifespan. If conditions allow, southern seed sources will also senesce later due to their later photoperiod cue, and their leaf lifespan will be shifted with later phenological events. This is the trend we saw in 2021. However, site conditions do not always allow southern seed source plants to have later phenological events. In 2020, an early frost stopped their growing season.

While the two years had differing leaf lifespan results, they both support the theory that leaf lifespan will vary based on how well adapted the seedlings are to site conditions. Northern seed source plants that are adapted to the climate had a consistent leaf lifespan. Southern seed source plants had more variable leaf lifespans due to not being adapted to the harsh weather at the northern range limit. This harsh weather that frost damaged southern seed source plants in 2020 is also predicted to increase with climate change (Anderson and Tomlinson 1998). Southern seed source populations will continue experiencing cold damage and abrupt endings to their leaf lifespans unless they acclimate to northern sites or climatic conditions change enough that they are better suited.

While climate change may increase leaf lifespan for plants adapted to a site, there are other limitations on leaf lifespan that minimize the effect climate change will have. Climate change is causing warming temperatures that are extending the growing season

through earlier budburst and later senescence (Gunderson et al. 2012). Even though longer growing seasons are predicted with warming climate, there may be other physiological limitations due to drought or increased evapotranspiration (Gunderson et al. 2012). Additionally, senescence and budburst are correlated in the eastern US, where a one-day earlier budburst is correlated with a 0.6 day earlier autumn senescence (Keenan and Richardson 2015). It is unknown why these phenological events are correlated. The mechanism could be indirect and related to soil water availability. For example, an earlier budburst may mean less water available at the end of the season and an earlier senescence, however, this depends on drought and its severity (Keenan and Richardson 2015). Other indirect mechanisms could be related to increased pest outbreaks or increased risk of frost damage with earlier budburst (Keenan and Richardson 2015). The correlation could also be due to a direct mechanism related to constraints on leaf lifespan such as programmed cell death (Lam 2004). Regardless of the mechanism, the positive correlation between dates of budburst and senescence (earlier budburst is correlated with earlier senescence) could minimize the impact of climate change on leaf lifespan.

Conclusion

Although warmer, wetter seed source plants had greater biomass and growth, they were also less equipped to deal with the harsh weather of a northern climate and were more prone to frost damage. Cold seed source plants accumulated less aboveground biomass in the first three years of growth but were better able to withstand environmental conditions in both the central range and at the northern range limit of *Q. rubra* due to their conservative growth strategy. Colder seed sources may be better suited for assisted

migration at this time because they can capitalize on early season snow melt and daylength and are not as prone to autumn frost damage. Local acclimation may not become apparent until *Q. rubra* are mature. By that time, colder seed sources, which are better adapted, may have higher survival and growth.

Table 2.1 Ford and Kellogg common gardens average temperatures, total precipitation, and number of frost days during growing season and winter from 2019-2021 (NOAA).

Site	Climate variable	May 2019-Sept. 2019	Oct. 2019-April 2020	May 2020-Sept. 2020	Oct. 2020-April 2021	May 2021-Sept. 2021
Ford	Average temp (°C)	14.3	-2.9	12.2	-2.7	11.9
Ford	Total precip (mm)	422	477	500	379	295
Ford	# of frost days (<0°C)	11	186	23	166	25
Kellogg	Average temp (°C)	21.5	3.5	21.8	4.4	22.6
Kellogg	Total precip (mm)	551	598	463	384	597
Kellogg	# of frost days (<0°C)	4	146	6	130	7

Table 2.2 Seed sources and environmental variables of *Quercus rubra* seedlings planted at the Ford and Kellogg common gardens. The environmental variables are mean annual temperature (MAT (°C), maximum temperature of warmest month (°C), minimum temperature of coldest month (°C), the range of temperature (max-min) (°C), and rain over active growth months of May to September (Rain) (mm).

Population name	Seed source	Lat.	Long.	Climate	MAT	Max	Min	Range	Rain
Rockwood Park, VA	29	37.45085	-77.58009	Warm	14.2	31.7	-3.9	35.6	493
Trail of Tears, IL	21	37.48017	-89.36005	Warm	13.6	31.8	-6.3	38.1	499
Hoosier, IN	18	38.05957	-86.66079	Warm	12.9	30.2	-6.6	36.8	521
Wartfordsberg, PA	27	39.73707	-78.18049	Warm	11.3	28.5	-8.6	37.1	437
Cygnat, OH	28	41.23797	-83.68050	Int.	9.8	27.6	-11.1	38.7	441
Big Bay de Noc, MI	16	45.86786	-86.51814	Int.	5.5	22.7	-15	37.7	396
Mouth of the Huron River, MI	12	46.90904	-88.03561	Int.	5.4	22.0	-15.4	37.4	385
Au Train, MI	15	46.43770	-86.87838	Int.	5.4	22.2	-14.9	37.1	404
Maastio Hihto, MI	4	47.13794	-88.61003	Cold	4.9	23.3	-16.9	40.2	376
Copper Harbor Sand dunes, MI	2	47.44013	-88.21871	Cold	4.5	21.0	-14.5	35.5	367
Cliff Drive, MI	3	47.35383	-88.35005	Cold	4.3	20.3	-15.9	36.2	371
Eagle Mine, MI	13	46.74914	-87.89082	Cold	4.1	22.10	-17.5	39.6	422

Table 2.3 Two-way ANCOVA results and degrees of freedom for common garden (CG) (Ford and Kellogg) and seed source climate variables of mean annual temperature (MAT) and rain over growing season (Rain) as well as climate* common garden interaction effects, for biomass index and growth index during summer 2019, 2020, and 2021.

Source	2019		2020		2021	
	Biomass index	Growth index	Biomass index	Growth index	Biomass index	Growth index
	DF P	DF P	DF P	DF P	DF P	DF P
MAT	893 <0.001	884 <0.001	850 <0.001	800 <0.001	421 <0.001	403 <0.001
CG	893 <0.001	884 <0.001	850 <0.001	800 <0.001	421 0.680	403 <0.001
MAT*CG	893 0.766	884 0.512	850 0.494	800 0.198	421 <0.001	403 0.649
Rain	893 <0.001	884 <0.001	850 <0.001	800 <0.001	421 0.026	403 <0.001
CG	893 <0.001	884 <0.001	850 <0.001	800 <0.001	421 0.699	403 <0.001
Rain*CG	893 0.440	884 0.219	850 0.487	800 0.0770	421 <0.001	403 0.241

Table 2.4 Regression results and degrees of freedom for senescence, budburst, and frost damage at Ford Center and seed source climate variables of mean annual temperature (MAT) and rain over growing season (Rain) for 2019, 2020, and 2021.

	2019		2020				2021			
Source	Senescence		Budburst		Senescence		Frost damage		Budburst	
	DF	P	DF	P	DF	P	DF	P	DF	P
MAT	446	<0.001	454	<0.001	252	<0.001	444	0.041	450	<0.001
Rain	446	<0.001	454	<0.001	252	<0.001	444	0.149	450	<0.001

Table 2.5 Regression results and degrees of freedom for leaf lifespan at Ford and seed source climate variables of mean annual temperature (MAT) and rain over growing season (Rain) for 2020 and 2021.

Source	Leaf lifespan 2020		Leaf lifespan 2021	
	DF	P	DF	P
MAT	251	<0.001	175	0.013
Rain	251	<0.001	175	0.021

Figures

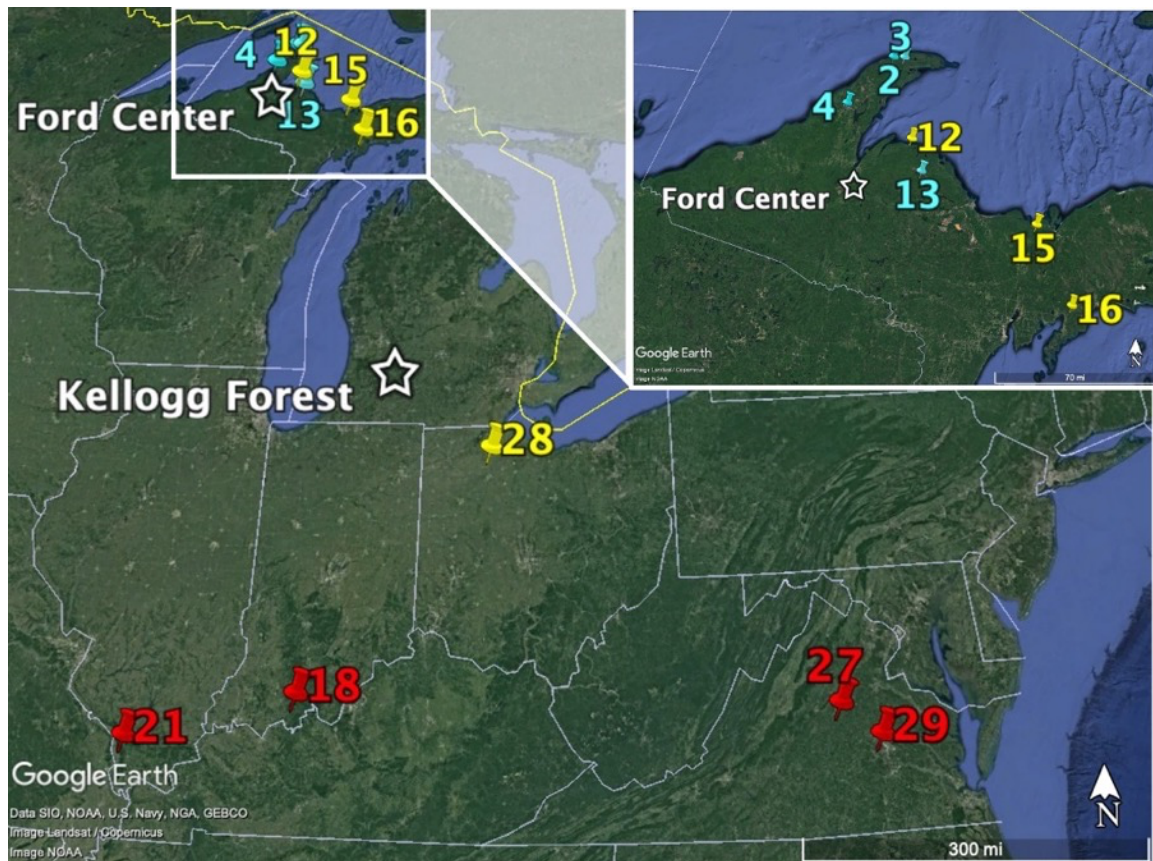


Figure 2.1 Map of *Quercus rubra* seed sources, four cold (blue), four intermediate (yellow), and four warm (red), Ford and Kellogg common gardens are represented with white stars.

A	B	C
D	E	F
G	H	I
J	K	L
M	N	O
P	Q	R
S	T	U
V	W	X
Y	Z	ZZ

buffer	buffer	buffer
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
<i>Qe</i>	<i>Qr</i>	<i>Qr</i>
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
<i>Qr</i>	<i>Qr</i>	<i>Qe</i>
<i>Qr</i>	<i>Qe</i>	<i>Qe</i>
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
<i>Qr</i>	<i>Qe</i>	<i>Qr</i>
<i>Qr</i>	<i>Qe</i>	<i>Qe</i>
<i>Qr</i>	<i>Qr</i>	<i>Qe</i>
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
buffer	buffer	buffer

Figure 2.2 Common garden design where each letter is a block with 30 plants in 3 columns x 10 rows followed by a row of buffer plants. In the enlarged figure of block C, *Qe* denotes *Q. ellipsoidalis* and *Qr* denotes *Q. rubra*. Colored plants are *Qr* plants used in the study. Red plants are from warm seed source sites, yellow plants are from intermediate seed source sites, and blue plants are from cold seed source sites.

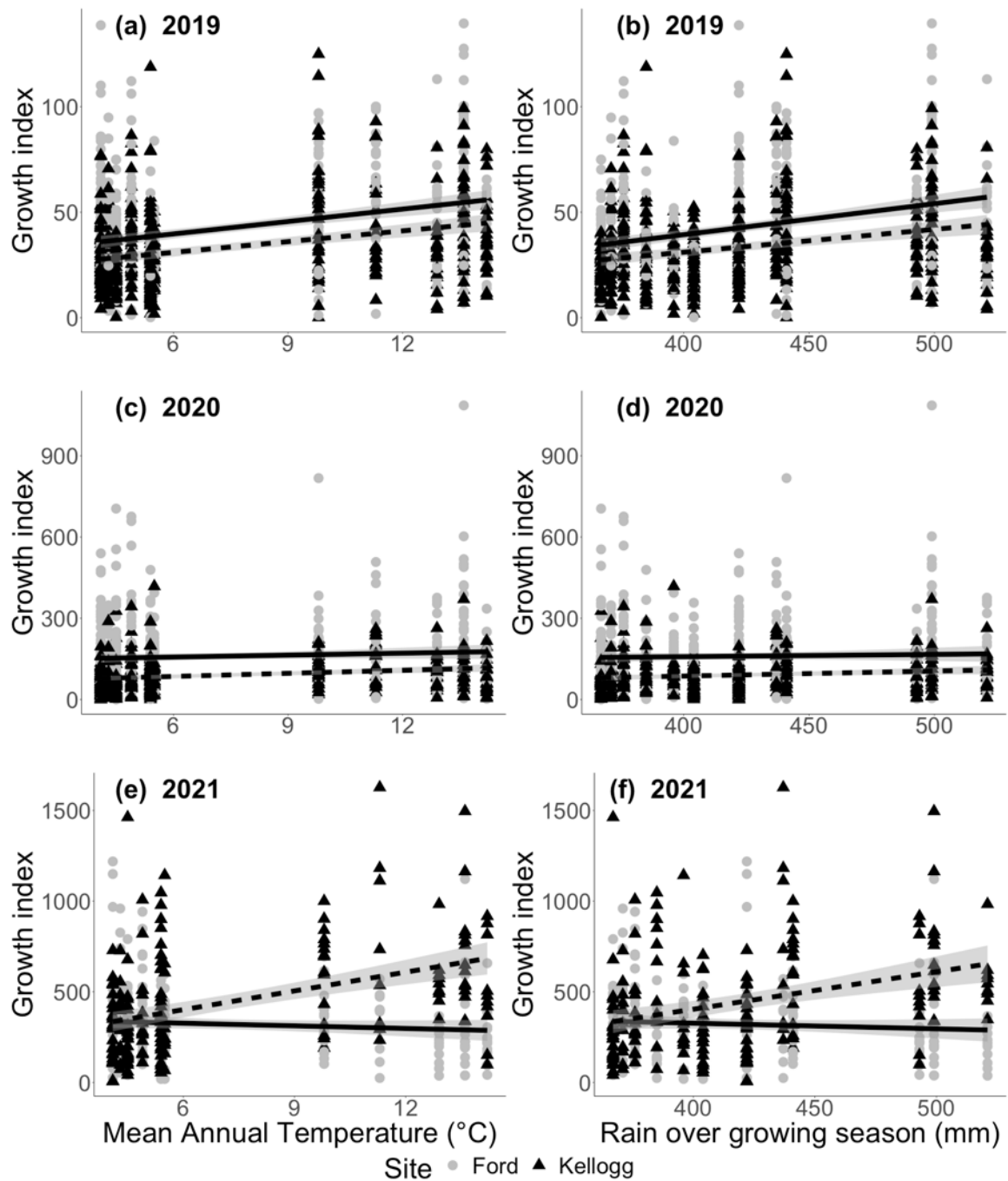


Figure 2.3 Interaction plots of growth index (biomass accumulated over a growing season) with seed source mean annual temperature and rain over growing season for 2019, 2020, and 2021 growing season, Kellogg depicted by dashed line and black triangles, Ford depicted by solid line and gray circles, shading = \pm 1 SE.

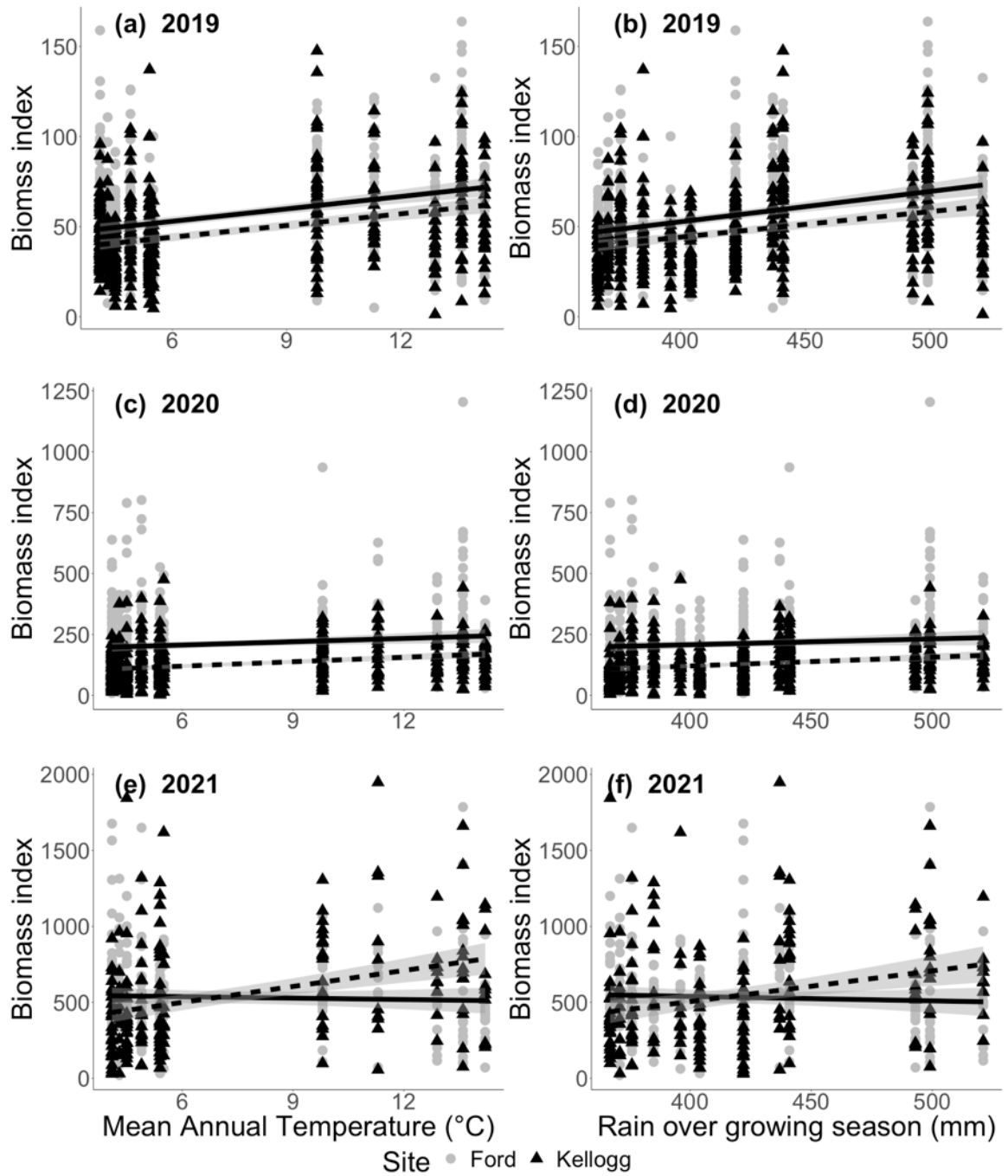


Figure 2.4 Interaction plots of biomass index with seed source mean annual temperature and rain over growing season for 2019, 2020 and 2021 season, Kellogg depicted by dashed line and black triangles, Ford depicted by solid line and gray circles, shading = \pm 1 SE.

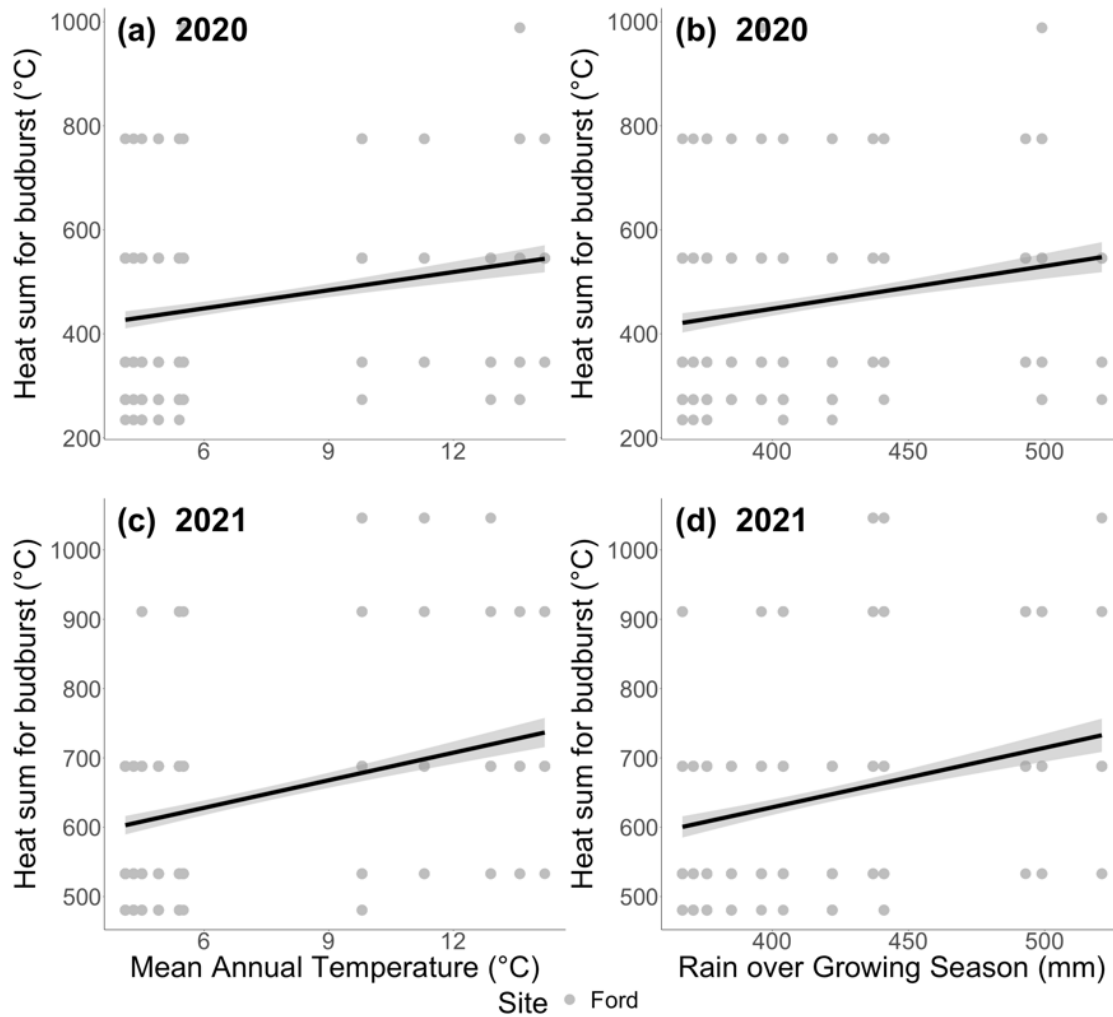


Figure 2.5 Regression plots of heat sum required for budburst with seed source mean annual temperature and rain over growing season for 2020 and 2021 season at Ford, shading = \pm 1 SE.

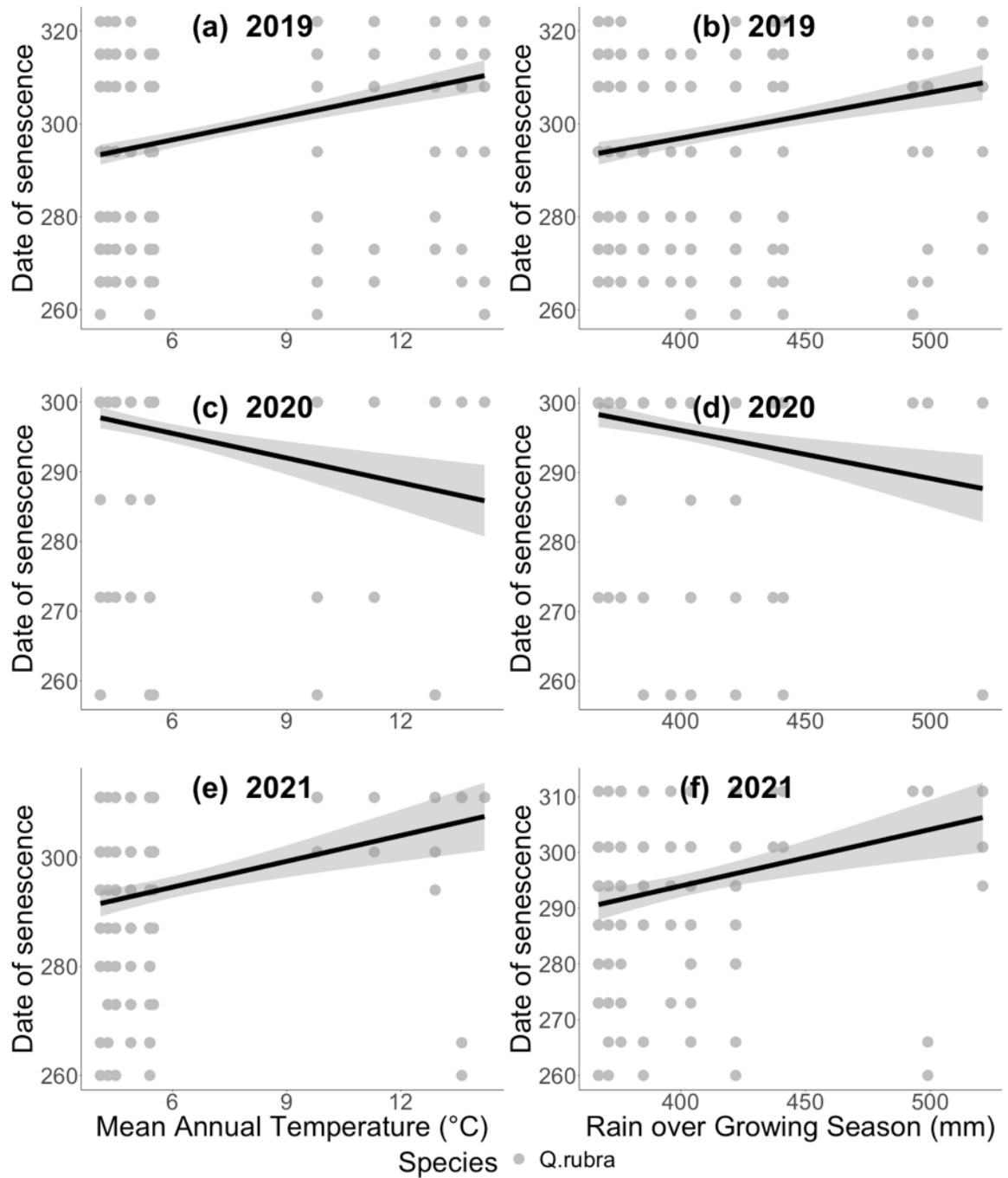


Figure 2.6 Regression plots of Julian date of senescence with seed source mean annual temperature and rain over growing season for 2019, 2020 and 2021 season at Ford, shading = ± 1 SE.

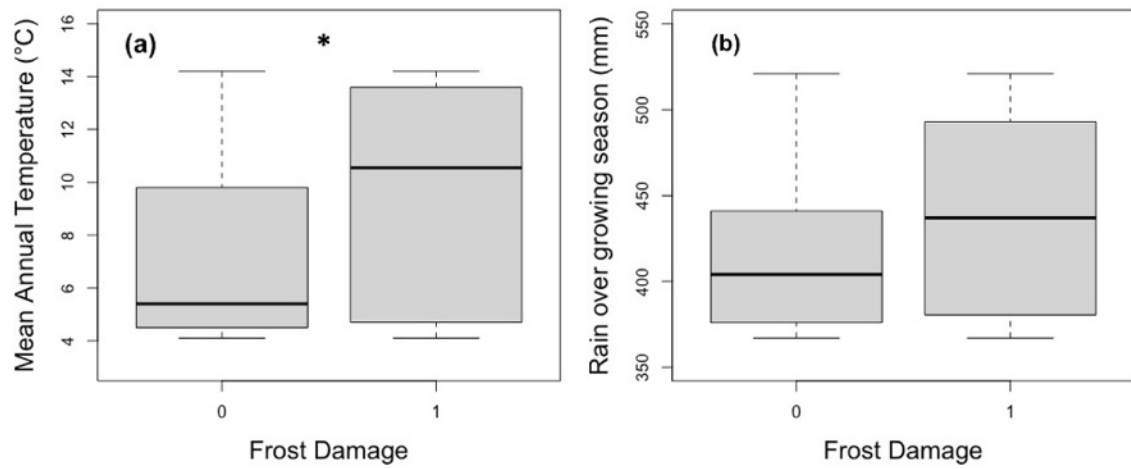


Figure 2.7 Regression plots of frost damage in September 2020 with seed source mean annual temperature (a) and rain over growing season (b) at Ford, dark center line is median, shaded area represents upper and lower quartile, whiskers represent minimum and maximum.

* p-value=0.041

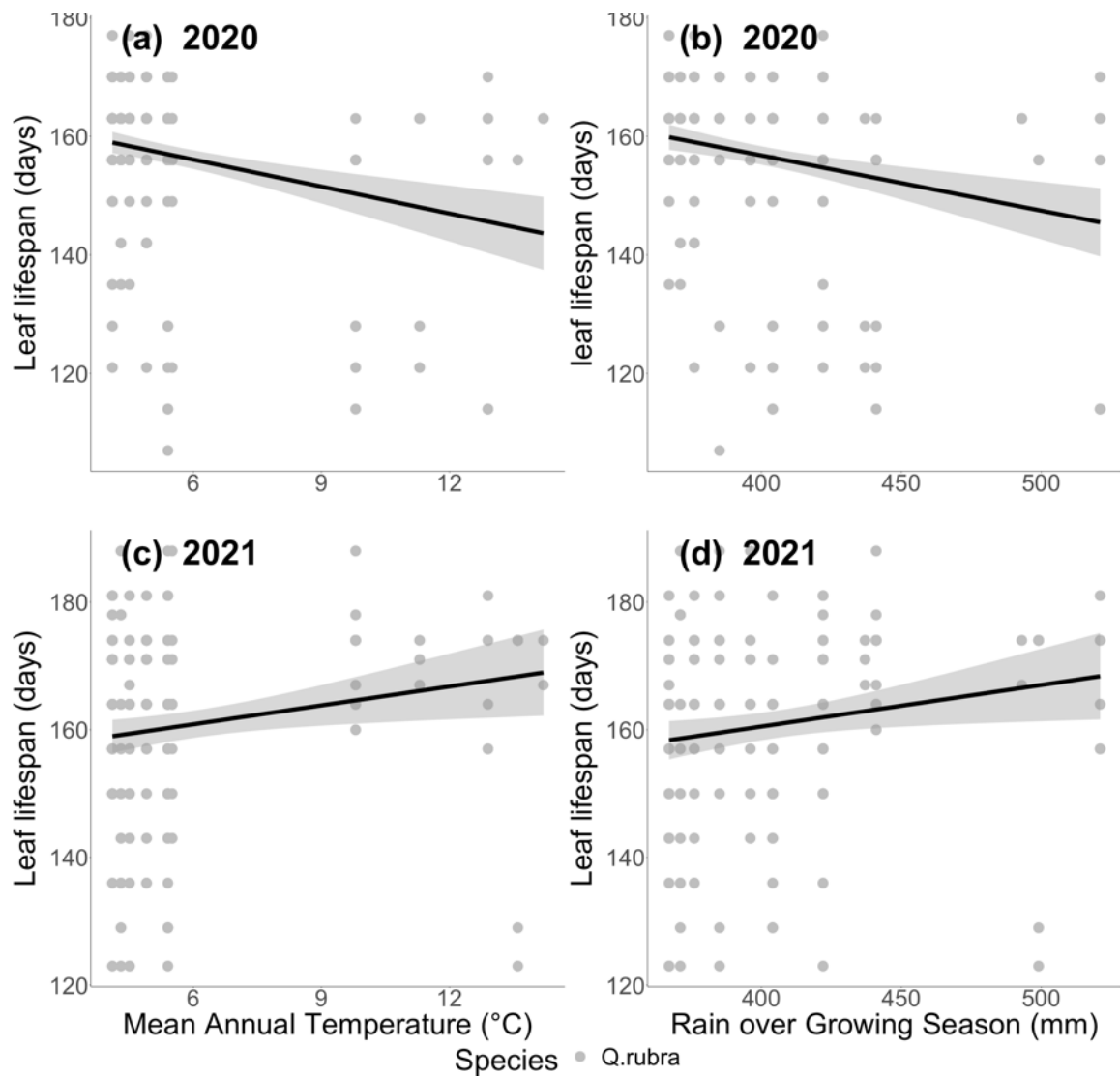


Figure 2.8 Regression plots of leaf lifespan with seed source mean annual temperature and rain over growing season for 2020 and 2021 season at Ford, shading = ± 1 SE.

Chapter 3. Northern populations of *Quercus rubra* are best suited for expanding range due to wider photosynthetic thermal niche and greater stress tolerance

Introduction

Climate change is affecting forests of the eastern North America, changing environmental conditions and therefore species potential ranges and realized niches. The two principle drivers of species distribution are means and extremes of both temperature and precipitation (Korner et al. 2016). Climate change observations and models predict increased temperatures of 2 to 8 °C this century in eastern North American forests, with very large increases in mean temperature in the winter (Anderson and Tomlinson 1998, IPCC 2021). There will also be increased mean and extreme precipitation and more extreme weather events (IPCC 2021).

This is concerning for northern red oak (*Quercus rubra* L.), a common tree species that comprises a large amount of the biomass in forests of eastern North America (Rogers et al. 2017). In Connecticut, Massachusetts, and Rhode Island, *Q. rubra* is one of the top three most prevalent species (Iverson et al. 2008). *Quercus rubra* provides habitat for mammals, birds, rare species of insects, and other wildlife (Nagel 2015). It also provides ecosystem services such as soil stabilization, windbreak, and can serve as a fire-resistant shelterbelt species (Nagel 2015). *Quercus rubra* is considered a versatile and valuable coarse-grained wood (Nicolescu et al. 2020). Not only is *Q. rubra* ecologically and economically important, but it is also culturally important to North America (Leroy

et al. 2020). Oaks have been used as a symbol of strength and longevity in cultures, religions, and politics for centuries (Leroy et al. 2020).

However, *Q. rubra* is one of many tree species that have not been able to migrate quickly enough to keep pace with climate change, causing adaptation lags (Frelich and Reich 2010, Duveneck et al. 2014). The edge of the northern range limit, specifically the 95th percentile latitudinal distribution difference, of *Q. rubra* adults and saplings has shifted north at a rate of 0.036 and 0.531 km/yr⁻¹, respectively, from 1970-2000 (Sittaro et al. 2017). Climates have already shifted over 100 km northward, causing a gap at the northern range limit where *Q. rubra* is not migrating northward fast enough (Zhu et al. 2012). One way to mitigate adaptation lags is to assist tree populations through human-facilitated migration in a process called assisted migration. Assisted population migration is the practice of moving source populations of a species within its native range in anticipation of where future suitable climate will exist (Etterson et al. 2020). This is practiced in Canada and studied across the world (Park and Talbot 2018). Long term studies on assisted migration are being performed in North America, such as the Assisted Migration Adaptation Trial funded by the British Columbia Ministry of Forests and the United States Department of Agriculture (Williams and Dumroese 2013). These investigations will help inform the US Forest Service as they plan to use assisted migration as a mitigation tool for climate change (United States Forest Service 2008).

Currently, *Q. rubra* is occupying climates that expose it to temperatures outside of its thermal optima, which may decrease carbon assimilation or potentially cause local extinctions (Gunderson et al. 2010). Eastern North American forest canopies may already

have declines in carbon assimilation due to high temperatures. In one study, under current climate conditions both temperate and tropical canopy leaves saw maximum temperatures higher than their optimum temperatures, decreasing photosynthesis and potentially damaging photosystems (Mau et al. 2018). By 2100, many temperate deciduous forest canopies of North America may see temperatures above their thermal optima, leading to overall decreased carbon sequestration (Mau et al. 2018).

To understand which populations of *Q. rubra* are best suited for the changing climate and potentially human mitigated northward expansion (i.e., assisted migration), we created two common garden sites. The Kellogg common garden, located in Lower Michigan, represents the central range of *Q. rubra* and has a mean annual temperature (MAT) of 9.9 °C (Jarvi and Burton 2013) (Figure 3.1). The Ford common garden, located in the Upper Peninsula of Michigan, is near the northern range limit for *Q. rubra* and has a MAT of 4.9 °C (Abraha et al. 2020) (Figure 3.1). The gardens house over 800 *Q. rubra* seedlings from 12 seed source populations representing their natural geographic range (Figure 3.1, Table 3.1). Common gardens allow us to observe if transplanted seedlings are acclimating to local environmental conditions. Within our common gardens, we monitored *Q. rubra* photosynthetic capacity, stress response, photosynthetic temperature response, and water use efficiency for three years to understand the physiological limits of the populations and how they responded to the environmental conditions of the common garden.

The response of net photosynthesis to increasing measurement temperatures is parabolic, where temperature at the highest rate of photosynthesis (A_{opt}) is called T_{opt}

(Yamori et al. 2010, Yamori et al. 2014). A parameter describing the width of the curve, Ω , defines the photosynthetic range of the plant or the photosynthetic thermal breadth (Berry and Bjorkman 1980, June et al. 2004, Yamori et al. 2014, Perez et al. 2021).

Plants from southern seed sources are adapted to a warmer climate and may have a higher T_{opt} . However, they may have a lower assimilation at T_{opt} because they evolved in an area with a longer growing season, providing a longer window to gain carbon. Photosynthetic thermal breadth may be greatest for plants from northern seed sources because of their conservative, generalist strategy which results from fluctuating temperatures (Perez et al. 2021).

A plant's photosynthetic stress tolerance may be estimated using a measurement of chlorophyll fluorescence. The ratio of variable chlorophyll fluorescence : maximum fluorescence (F_v/F_m), or the "optimal quantum efficiency" of a plant indicates how photosystem II (PSII) is functioning (Ritchie 2006). It is a useful indicator of stress because PSII is the most stress-sensitive component involved in photosynthesis (Havaux 1996, Marias et al. 2017). A high F_v/F_m value, typically a maximum value of ~ 0.8 , indicates a high PSII stress tolerance (Ritchie 2006). In our study, this measurement was taken during periods of stress for the plants, such as drought, to see which populations were more stressed by suboptimal conditions. Northern seed sources have a generalist strategy suited to their harsh native environment with fluctuating temperatures, so PSII may be less sensitive to drought and heat stress (Perez et al. 2021).

Gas exchange parameters and stable isotopes of carbon in *Q. rubra* leaves can be used to estimate water use efficiency (WUE) of the plants, or the ratio of carbon gained

to water lost via stomata. Instantaneous WUE can be quantified as assimilation/stomatal conductance (A/g_s) using an infrared gas analyzer (Farquhar et al. 1982). The stable isotope of carbon, ^{13}C , is a more integrated estimate of WUE over the lifetime of the leaf. Values of foliar $\delta^{13}\text{C}$ are assumed to be positively correlated with WUE because the enzyme rubisco discriminates against heavier $^{13}\text{CO}_2$, preferring $^{12}\text{CO}_2$ (Farquhar et al. 1982, Verlinden et al. 2015). ^{13}C enrichment occurs when the stomata are closed, and less CO_2 is available, where a higher or less negative value of $\delta^{13}\text{C}$ represents greater WUE. *Quercus rubra* from northern seed sources may have greater WUE because they are better adapted to less growing season precipitation.

Our overarching hypothesis for this study is that plants from northern seed sources grown in common garden sites will be more physiologically tolerant of heat and water stress compared to plants from more southern seed sources, making them be better suited to the climate at the northern range limit. Specifically, we expect: (1) *Q. rubra* from northern seed sources will have greater photosynthetic capacity compared to southern seed source plants when grown at a common garden site, due to the shorter growing seasons of seed source climates. (2) *Quercus rubra* from northern seed sources will be less vulnerable to stress as measured by F_v/F_m , have higher overall photosystem functioning, whereas all seed sources will be more vulnerable to stress, at the northern common garden compared to the southern site. (3) *Quercus rubra* from southern seed sources will have narrower photosynthetic temperature ranges with higher optimum temperatures, and growth environment will not affect temperature response. Finally, we hypothesize that (4) *Quercus rubra* from northern seed sources will have greater water use efficiency.

Methods

Site description

The northern common garden site, Ford Center, is in Alberta, Baraga County, Michigan, USA (46.65 °N, 88.48 °W, 400 m elevation). The mean annual temperature at Ford common garden is 4.9 °C and it receives 879 mm of mean annual precipitation, with 401 mm of this precipitation falling during the growing season months (Jarvi and Burton 2013). The soil is classified as Kallio cobbly silt loam (coarse-loamy, mixed, superactive, frigid Oxyaquic Fragiorthods) (Jarvi and Burton 2018). The central range site, Kellogg Forest, is in Augusta, Kalamazoo County, Michigan, USA (42.37 °N, 85.35 °W). This site receives 1027 mm of precipitation annually, with 523 mm falling during the growing season (Abraha et al. 2020). The mean annual temperature at Kellogg is 9.9 °C (Abraha et al. 2020), and the soil type is Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalf) (Crum and Collins 1995).

Experimental design

Over 800 acorns from 4-6 mother trees at 12 locations across the range of *Q. rubra* range were collected in autumn 2018 (Figure 3.1, Table 3.1). Acorns were collected from September - November 2018 and stratified at 4 °C in the greenhouse at the College of Forest Resources and Environmental Science at Michigan Technological University from collection date until they were planted in 0.5 L pots on April 3-4, 2019. Greenhouse conditions were maintained at 16 hours light, 8 hours dark. The daytime temperature was 23 °C and nighttime temperature was 18 °C. Plants were watered by hand every 3 days. *Q. rubra* seedlings were planted at the Kellogg site on June 10-12,

2019 and at the Ford site on June 17-19, 2019. Seedlings were planted along with *Q. ellipsoidalis* and other *Q. rubra* not used in this study in an incomplete randomized block design (Figure 3.2, Table 3.1).

There are 27 blocks at each common garden, arranged in a 3 x 9 block structure, with a buffer row of plants between each row of three blocks (Figure 3.2). Within each block there are 30 plants in a 3 x 10 formation with 3 columns spaced 1.5 m apart and 10 plants in each row which are spaced 0.75 m apart (Figure 3.2). The buffer row has the same dimensions. Each block contains one to two plants from each of 12 seed sources of *Q. rubra* used in this study, about 10 *Q. rubra* plants from seed sources not used in this analysis, and approximately 8 *Q. ellipsoidales*. The common gardens are both protected from herbivory by 10-foot fences and were sprayed with a glyphosate herbicide and regularly weeded by hand to prevent overcrowding and shading in 2019 and 2020. Plants were watered by hand at the Ford site every 3 days from June 17th to July 14th, 2019, and every 7 days from July 15th to September 2019. From June 15th to July 12th 2020 plants at the Ford site were watered every 7 days, from July 13th to July 26th they were watered every 10 days and from July 27th to August 23rd every 14 days. Iron Chelate was applied at the Ford site on August 12, 2019 and June 13, 2020. In 2021, plants were neither watered nor fertilized.

In 2019, there were 893 seedlings total in the two common gardens. In 2020 there were 850 due to mortality, and in 2021, only 421 were available for this study, with the remainder surviving seedlings assigned to a concurrent drought experiment.

Photosynthetic capacity, A/g_s , and stress response were measured on all 12 *Q. rubra*

populations while foliar N, photosynthetic temperature response, and ^{13}C were measured on a subset of populations, called the core populations (Table 3.1). The core populations included 6 populations of seedlings at both common gardens that represented two populations from cold seed sources ($\text{MAT} < 5^\circ\text{C}$), two populations from intermediate seed sources ($\text{MAT } 5\text{-}10^\circ\text{C}$), and two populations from warm seed sources ($\text{MAT} > 10^\circ\text{C}$) (Table 3.1).

Photosynthetic capacity

Photosynthetic capacity (A_{max}) was measured twice per year for two years at the Ford common garden (July 10-12 and August 6-8, 2019; June 29-July 2 and July 27-30, 2020) and once in 2021 (August 16-20). At the Kellogg common garden, A_{max} was measured once per year for three years (August 19-20, 2019, August 12-13, 2020, and August 9-13, 2021). A LI-COR 6400 (LI-COR, Inc, Lincoln, NE) was used for 2019 measurements and June 29-July 2, 2020 measurements and a LI-COR 6800 (LI-COR, Inc, Lincoln, NE) was used for the July 27-30, 2020, August 12-13, 2020, and all 2021 measurements. Measurements were taken on non-water stressed plants, based on visual observation and stomatal conductance, from approximately 9:00 to 16:00. Chamber settings were maintained at 400 ppm CO_2 with a flow rate of $400 \mu\text{mol m}^{-2}\text{s}^{-1}$ and block temperature of 25°C . Optimum photosynthetic photon flux density was determined by preliminary light response curves to be $800 \mu\text{mol m}^{-2}\text{s}^{-1}$ (data not shown). Vapor pressure deficit was maintained between 1-2 kPa when possible, and did not exceed 3 kPa. The healthiest fully developed terminal leaf was used for A_{max} measurements. A subset of randomly selected plants representing all seed sources were used for A_{max} measurements

at Ford and Kellogg common garden in all years. In 2019, 212 were used, in 2020, 147 were used and in 2021, 155 were used. Approximately half of the measured plants were at Ford and approximately half were at Kellogg each year.

Leaf mass per area (LMA) and foliar nitrogen concentration were measured differently in 2020 and 2021. On September 21-25, 2020, the healthiest fully developed terminal leaf was collected from each plant in the core population at the Ford common garden, totaling 270 leaves. Leaf surface area was measured by laying leaves flat and using the app Leafscan (Anderson and Rosas-Anderson 2017). The leaves were kept on ice until later in the day when they were frozen in liquid nitrogen and stored in a -80°C freezer. Leaves were freeze dried with the VirTis Advantage EL-85 Freezer Dryer for 48 hours (SP Scientific, Stone Ridge, NY), and dry weight was measured using a balance to ± 0.0000 g. LMA was calculated as dry mass (g) divided by leaf area (m^2). For N analysis, after freeze drying, the samples were stored in sealed 15 ml falcon tubes to ensure they did not gain more water. They were ground into a fine powder using a mixer mill (Retsch MM400, Verder Scientific, Haan, Germany) and weighed (3.00 mg \pm 0.5 mg) into tin capsules for elemental concentration of N using a PDZ Europa ANCA-GSL elemental analyzer (University of California Davis Stable Isotope Facility). Nitrogen per unit mass (N_{mass}) was calculated as (total N (mg)/sample mass (g)), and N per unit leaf area (N_{area}) was calculated as $((\text{LMA (g/m}^2)) * (N_{\text{mass (mg/g))}))$.

In 2021, after gas exchange measurements (A_{max} and temperature curve) were completed, the leaf used for gas exchange was cut at the base of the petiole and put in plastic bag inside a cooler. There were 255 leaves total. Each night, fresh weight was

measured to the nearest 0.00 g using a precision balance. Leaf area was measured by taking a photograph of leaves on graph paper next to a ruler with a plastic sheet to flatten the leaves. Leaf area was analyzed using imageJ v1.50 image analysis software (Rasband 1998-2018). Each leaf was put in a brown paper bag and dried in an oven at 60°C for 48-72 hours. The dried leaf samples were used for calculating LMA as (dry mass (g)/area (m²)). In 2021, leaf nitrogen was measured on the 38 leaves used for temperature curves plus 6 leaves that did not meet quality standards for temperature curves, totaling 44 leaves. The dried leaves were turned into a fine powder using a mixer mill (Retsch MM400, Verder Scientific, Haan, Germany) and weighed (4.000 mg +/- 0.500 mg) for leaf N analysis using a combustion analyzer (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA, USA). N_{mass} was calculated as (total N (mg)/sample mass (g)). N_{area} was calculated as ((LMA (g/m²)) * (N_{mass} (mg/g))).

Stress response

Maximum quantum yield of Photosystem II (Fv/Fm) was assessed by measuring chlorophyll fluorescence of dark-adapted leaves between the hours of 2:00 and 5:00 am. Fv/Fm was measured once per year at Ford (August 9, 2019, July 31, 2020, August 4, 2021) and once only at Kellogg (August 21-22, 2019). Fv/Fm was measured using a handheld portable fluorometer (FluorPen FP Max, Photo Systems Instruments, Drasov, Czech Republic). The healthiest fully developed terminal leaf was used for Fv/Fm measurements. In 2019, all seedlings at both common gardens, totaling 865 were used for Fv/Fm measurements. In 2020, all seedlings at Ford, 453, were used in Fv/Fm

measurements. While in 2021, 227 seedlings at Ford were used in Fv/Fm measurements due to a concurrent drought experiment.

Photosynthetic temperature response curves

Photosynthetic temperature response curves were measured once at Kellogg and once at Ford in 2021 (July 13-16 and July 19-22, 2021) using a LI-COR 6800 (LI-COR, Inc, Lincoln, NE). Measurements were taken on non-water stressed plants from approximately 6:00 to 20:00, when temperature was conducive (i.e., not more than 10 °C below minimum set point or above maximum set point of temperature curve). Chamber settings were the same for A_{\max} measurements. Temperature settings were 17, 20, 24, 27, 30, 33, 35, 37 °C with approximately 6 minutes at each temperature or until stabilization was reached based on H₂O, CO₂, and assimilation slopes of 0.30. Temperature response curves were measured on 3-4 randomly selected plants within the 6 core populations at each site. There were 38 total plants measured at the sites in 2021. The healthiest fully developed terminal leaf was used for measurements.

Parameters were extracted using the equation of June et al. (2004):

$$A_{net} = A_{opt} \times e^{-\left(\frac{T_{leaf} - T_{opt}}{\Omega}\right)}$$

Equation 1

Where A_{net} is net assimilation and A_{opt} is the maximum rate of assimilation. T_{leaf} is the instantaneous leaf temperature and T_{opt} is the temperature at A_{opt} . Ω is the width of the temperature response curve, measured as the difference between T_{opt} and the temperature at which assimilation is 37% of A_{opt} , representing the photosynthetic thermal niche (June

et al. 2004, Carter et al. 2020). To ensure quality, if T_{opt} was <15 , that curve was dropped for T_{opt} analysis. If Ω was <0 it was dropped from Ω analysis.

Water use efficiency

Water use efficiency (WUE) was measured using both gas exchange and stable isotope techniques. Instantaneous WUE was calculated as photosynthetic capacity divided by stomatal conductance (A_{max}/g_s) using data from A_{max} campaigns. The same leaves, 270, used for LMA and foliar nutrient analysis in 2020 were used for ^{13}C isotopic analysis, see above for sample preparation methods. Samples were analyzed for ‰ ^{13}C at the University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Leaf water content (LWC) was measured on the same leaves, 255, that were used for LMA and gas exchange in 2021. The leaves were dried as described above. Leaf water content, LWC (%) was calculated as ((fresh mass-dry mass)/fresh mass) *100).

Statistical Analyses

Linear regressions were used to compare 2020 LMA, N_{mass} and N_{area} as well as 2020 and 2021 Fv/Fm measurements and 2020 $\delta^{13}C$ values to seed source mean annual temperature (MAT; °C) and seed source precipitation over the active growth months of May to September (Rain; mm) in R studio (R 2020). Yearly A_{max} and A/g_s measurements, 2021 LMA, N_{mass} , N_{area} , and LWC as well as 2019 Fv/Fm measurements and extracted photosynthetic temperature curve parameters were compared to the two seed source environmental variables, MAT and Rain, using analysis of covariance

(ANCOVAs) in the package *rstatix* (R 2020) with common garden as a fixed factor. Late season A_{\max} measurement dates, in August of each year, at Ford and Kellogg common gardens were used in the ANCOVAs.

Results

Photosynthetic capacity

Photosynthetic capacity was higher for northern seed source plants than southern seed source plants. A_{\max} was negatively correlated with climate variables in all years, where colder, drier seed sources had higher A_{\max} values than warmer, wetter seed sources (Figure 3.3, Table 3.3). Site was significant in 2019, with Kellogg plants having a greater A_{\max} than those at Ford ($p < 0.001$), but there were no differences across site in 2020 or 2021. There were no significant interactions in any year.

Northern seed source plants generally had higher leaf nitrogen at Ford, but patterns at Kellogg were the opposite. In fall 2020, at Ford both mass- and area-based leaf nitrogen was greater in plants from colder, drier seed sources (Figure 3.4, Table 3.4). In 2021, we found significant interactions between MAT and common garden for both N_{mass} and N_{area} , where nitrogen increased with MAT at Kellogg, but decreased with MAT at Ford (Figure 3.5, Table 3.4). Seed source Rain had no effect on foliar nitrogen, either on a mass- or area- basis in 2021, but N_{area} was significantly higher at Ford compared to Kellogg (Figure 3.5, Table 3.4).

Stress response

Northern seed source plants were generally less vulnerable to stress with a higher Fv/Fm representing less stress on photosystem II during harsh conditions. Fv/Fm was negatively correlated with seed source MAT and precipitation in 2019 and 2020 (Figure 3.6, Table 3.3). Plants from warmer, wetter seed sources had a lower Fv/Fm. In 2019, the only year both sites were measured, there were MAT*common garden and Rain*common garden interaction effects. Plants at both common gardens had negative correlations between seed source environmental variables and Fv/Fm, however the trend was stronger at Ford. In 2021, Fv/Fm of Ford plants was neither correlated with seed source MAT nor Rain (Figure 3.6, Table 3.3).

Photosynthetic temperature response

Northern seed sources had a larger photosynthetic temperature range and higher assimilation at optimum temperature than southern seed sources, while optimum photosynthetic temperature did not vary between seed sources. T_{opt} , extracted from temperature curves, was neither correlated with seed source environmental variables nor common garden site (Figure 3.7, Table 3.5). A_{opt} and Ω from temperature curves were both negatively correlated with seed source MAT and Rain, and there were no significant differences across common garden sites nor interactions (Figure 3.7, Table 3.5). Plants from colder, drier seed sources had a greater temperature range for photosynthesis and a greater A_{opt} than those from warmer seed sources.

Water use efficiency

There were conflicting water use efficiency trends for seed sources based on year and variable measured. Using gas exchange measurements, southern plants had higher instantaneous water use efficiency in 2021, while northern plants had integrated higher water use efficiency based on stable carbon isotopes in 2020. In 2019 and 2020, A/g_s was not correlated with seed source climate variables (Figure 3.8, Table 3.6). In 2021, however, A/g_s increased with both MAT and Rain at both sites, where plants from warmer, wetter seed sources had higher A/g_s (Figure 3.8, Table 3.6). Ford plants had a higher A/g_s than Kellogg plants in all years (Figure 3.8, Table 3.6). Carbon isotope analysis of leaves from Ford only show $\delta^{13}C$ was negatively correlated with seed source climate variables, indicating higher WUE in colder, drier seed source plants in 2020 (Figure 3.9, Table 3.6). LWC was not significantly correlated to seed source environmental variables or common garden sites in 2021 (Figure 3.9, Table 3.6).

Discussion

Photosynthetic capacity

Our findings reveal northern seed source plants have a more generalist strategy compared to southern seed source plants. Northern seed source plants had a higher photosynthetic capacity and temperature range, higher stress tolerance and greater water use efficiency. However, it is important to note that there is large variability both between and within populations.

In support of hypothesis 1, A_{max} was higher for colder, drier seed sources in all years, and foliar N was greater in 2020. We hypothesized that northern plants have

evolved greater photosynthetic capacity to compensate for shorter growing seasons. Another study found cold grown plants, *Arabidopsis thaliana* grown at 5 °C had higher photosynthetic and respiration capacities than their warm-grown counterparts (Aitken et al. 2008). Southern seed source plants may have not acclimated to the shorter growing season at the common gardens and increased A_{\max} to a rate comparable to northern seed sources. In general, A_{\max} is a genetically conserved trait (Gunderson et al. 2010), and while some acclimation can occur, it will likely take generations for southern plants to increase A_{\max} . Northern seed source plants also had a larger photosynthetic temperature range, Ω . These two photosynthetic parameters are often positively correlated with each other (Perez et al. 2021). The higher A_{\max} of northern seed source plants coupled with a larger photosynthetic temperature range make them better adapted to the short growing season and large temperature fluctuations at the northern range limit.

In support of hypothesis 1, colder, drier seed source plants at Ford had greater foliar nitrogen than warmer, wetter seed sources in 2020. However, in 2021, there was no correlation between foliar nitrogen with seed source environmental variables. Higher foliar nitrogen in northern seed source plants at Ford in 2020 may be a metabolic adaptation to the cold (Tjoelker et al. 1999). Colder ecotype plants often have higher foliar nitrogen than their warmer ecotypes counterparts (Tjoelker et al. 1999). This is further supported by higher N_{area} of plants at Ford, where the MAT is 5°C colder than at Kellogg. All populations may be increasing foliar nitrogen in response to the colder environment. It is also supported by the stronger negative trend of foliar nitrogen with seed source MAT and rain at Ford. Another reason for higher foliar nitrogen in northern seed sources may be because there is higher soil organic matter carbon and nitrogen in

colder locations (Craine et al. 2015). However, we have not sampled soil at the seed source locations to verify if this trend applies to our study.

Stress Response

In support of hypothesis 2, Fv/Fm was greater in northern seed source plants in 2019 and 2020. Fv/Fm, variable chlorophyll fluorescence/ maximum fluorescence, indicates how photosystem II (PSII) is functioning. A higher Fv/Fm value is expected in more conservative, generalist populations that invest in the metabolic cost of PSII stress tolerance (Perez et al. 2021). Northern seed sources may have a higher PSII stress tolerance because they are adapted to less precipitation. This is supported by a study on tropical trees that reported drought tolerance and thermotolerance were positively correlated (Sastry et al. 2018).

Fv/Fm measurements in conjunction with temperature response curves measure the thermotolerance of *Q. rubra*. Northern seed sources had a wider temperature range for photosynthesis and a higher Fv/Fm, showing they invest more in PSII stress tolerance. Our results are supported by another study that found a larger photosynthetic temperature range is generally correlated with higher PSII stress tolerance (Perez et al. 2021). This is indicative of thermal generalists (Perez et al. 2021). A generalist strategy with high stress tolerance is especially important, as *Q. rubra* is particularly sensitive to heat and cold, and fluctuating temperatures are expected to increase with climate change. *Quercus rubra* are prone to heat stress, and in one study they had the largest reduction in Fv/Fm of three oak species when exposed to heat (Percival 2005). In our study, southern seed source plants were more prone to frost damage than northern seed source plants at Ford, likely

because northern seed source plants are adapted to the harsh climate at the northern common garden. This further supports the idea that northern seed source plants have a higher stress tolerance and are better suited for the climate at the northern range limit.

Photosynthetic temperature response

Contrary to hypothesis 3, T_{opt} was not correlated with seed source environmental variables. In support of hypothesis 3, A_{opt} and Ω were negatively correlated with seed source MAT and rain. Northern seed sources had a higher maximum rate of photosynthesis and also maintained high rates of carbon assimilation across a wider range of temperatures than warmer, wetter seed sources.

T_{opt} was not correlated with seed source environmental variables, possibly because the plants have acclimated to the temperatures at the common gardens. Photosynthetic acclimation to temperature occurs when a plant adjusts its physiological and biochemical mechanisms to photosynthesize more efficiently at growth temperatures (Yamori et al. 2014). While this is not always found in all trees (Ow et al. 2008), *Q. rubra* has been found to thermally acclimate (Gunderson et al. 2010). Thermal acclimation is commonly seen as a shift in T_{opt} , A_{opt} , or Ω under warmer growth temperatures (Yamori et al. 2014, Carter and Cavaleri 2018). Other biochemical responses to cold temperatures may include increasing Rubisco and other enzymes used in the carbon cycle to compensate for lower activity at colder temperatures (Yamori et al. 2014). Cold acclimated plants may also increase membrane fluidity by increasing unsaturated: saturated fatty acids (Yamori et al. 2014). Plants acclimated to high temperatures need greater thylakoid membrane stability and heat tolerance, as proton

leakiness can occur (Bukhov et al. 2000, Yamori et al. 2014). Heat shock proteins may also be expressed in response to high temperatures (Vierling 1991, Yamori et al. 2014).

Our speculation of thermal acclimation in all populations is supported by experiments where T_{opt} shifts with growth temperature (Berry and Bjorkman 1980, Cunningham and Read 2002, Gunderson et al. 2010, Slot and Winter 2017). In one study, the average air temperature from only two days prior was used as a predictor of T_{opt} (Gunderson et al. 2010). However, in one *Q. rubra* study, no temperature acclimation occurred and colder seed source plants had lower T_{opt} than warmer seed source plants, even after being exposed to the same environmental conditions in a common garden for twelve years (Robakowski et al. 2012). We hypothesize that similar T_{opt} across populations in our study is evidence of acclimation, however we do not know T_{opt} of the plants in their native environments. Because of this, we cannot rule out that the similar T_{opt} among populations is simply due to no genetic differences in T_{opt} among seed source populations. However, acclimation is supported by the nearly significant trend of higher T_{opt} in Kellogg plants compared to Ford plants ($p = 0.071$; $p = 0.080$; Table 3.5). While our study may showed acclimation in all populations, northern populations generally have greater acclimation potential than southern populations (Sage and Kubien 2007). This may only be apparent at more extreme temperatures.

All *Q. rubra* populations were grown in common gardens with the same environmental conditions, yet colder seed sources had a larger photosynthetic temperature range, which implies Ω is a genetically conserved trait. In another *Q. rubra* study, under experimentally warmed conditions, Ω did not change, supporting this theory

(Gunderson et al. 2010). A greater Ω as seen in colder seed source plants is indicative of a generalist strategy (Perez et al. 2021). One reason northern seed source plants have a larger photosynthetic range may be because they are subject to more fluctuating temperatures during the growing season. While all seed source locations have a similar range of temperatures (Table 3.2), northern seed sources endure more frost and drought events. Fluctuating temperatures have been found to correlate with higher A_{\max} (Cunningham and Read 2003), which is supported by our results.

A_{opt} was greater for northern seed sources. This was expected as A_{opt} is a genetically conserved trait (Gunderson et al. 2010). In a warming experiment, A_{opt} was not correlated with temperature history or treatment (Gunderson et al. 2010). However, some acclimation may occur. For example, temperature acclimation can be seen as a shift in T_{opt} or a higher A_{opt} (Carter and Cavaleri 2018). We cannot determine definitively if any of our populations acclimated to site conditions because we do not have baseline temperature curve measurements of them at their original site.

In our study, larger photosynthetic ranges and higher A_{opt} were both negatively correlated with seed source temperature and precipitation over growing season. Both a higher A_{opt} and wider Ω are marks of generalist strategies more common of northern plants. As temperature fluctuations are predicted to increase with climate change, generalist strategies are favored as being able to withstand a wider range of temperatures is important.

Water use efficiency

Contrary to our hypothesis, instantaneous WUE, estimated as assimilation / stomatal conductance (A/g_s), was not correlated with seed source climate variables in 2019 and 2020. In 2021, A/g_s was positively correlated with seed source MAT and rain, opposite of the hypothesized trend. The lower A/g_s of northern seed source plants in 2021 may be attributed to evaporative cooling. Evaporative cooling allows a plant to “sweat” in response to high temperatures by increasing stomatal conductance (Marias et al. 2017). Physiological responses such as evaporative cooling and heat shock proteins prevent damage to PSII photochemistry (Marias et al. 2017). If there is adequate water available, evaporative cooling may be a strategy for the plant to cool itself without risking cavitation. The theory that northern seed source populations of *Q. rubra* are evaporative cooling is further supported by our results at Kellogg. *Quercus rubra* plants at Kellogg (all populations) had lower A/g_s than Ford plants. Kellogg receives more precipitation and has higher temperatures, providing conditions that make evaporative cooling an effective strategy without risk of cavitation. Evaporative cooling during heat waves with well-watered conditions was also seen in loblolly pine (Ameye et al. 2012).

Higher concentrations of $\delta^{13}\text{C}$, indicating higher integrated WUE, were present in the foliar tissue of northern seed sources when sampled at Ford in September 2020, as predicted. Foliar ^{13}C is representative of WUE across the entire growing season (Weiwei et al. 2018) meaning *Q. rubra* from colder, drier seed sources had overall higher WUE at Ford for the 2020 season. Higher $\delta^{13}\text{C}$ and greater drought resistance in drier ecotype populations has been found in other studies on *Pinus* (Kerr et al. 2015, Marias et al. 2017).

We found colder, drier seed sources also have greater A_{\max} , meaning the difference in A/g_s between seed source populations is likely due to a lower stomatal conductance in northern seed source *Q. rubra*. This is further supported by a study on *Q. robur* that found WUE and $\delta^{13}\text{C}$ were related to differences in stomatal conductance and not photosynthetic capacity (Roussel et al. 2009). Another study found that at drier sites, *Quercus* species maintain high $\delta^{13}\text{C}$ by reducing intercellular to ambient CO_2 partial pressures (C_i/C_a) (Turnbull et al. 2002).

Foliar $\delta^{13}\text{C}$ measurements found higher water use efficiency in northern seed sources, which we did not see in gas exchange measurements. This may be due to harsh site conditions at Ford in summer 2020. Plants at Ford were given some supplemental water, however, there were frequent summer droughts, creating conditions that required high WUE and did not allow the *Q. rubra* to cool themselves by increasing stomatal conductance without risk of cavitation. When measuring A/g_s , we used healthy well-watered plants during peak photosynthetic hours. However, *Quercus* been found to close their stomata earlier in the day when water stressed (Cavender-Bares and Bazzaz 2000). When this midday depression occurs, we did not measure gas exchange. During this time the plant has closed its stomata and is lowering its internal CO_2 , C_i , and cannot discriminate against the heavier ^{13}C , contributing to a higher concentration of ^{13}C in the leaf.

Quercus species generally have high WUE compared to other deciduous tree species, due in part to thick leaves, small stomata, and deep roots which allow them to maintain high water potential even in dry conditions (Abrams 1990). However,

transplanted *Q. rubra* are likely to be seedlings and do not have deep roots that can access groundwater, making them more vulnerable to heat stress and drought (Cavender-Bares and Bazzaz 2000, Marias et al. 2017). Higher $\delta^{13}\text{C}$ enrichment under drought conditions and the ability to evaporative cool under conditions with adequate water supply show acclimation and drought resistance in colder, drier seed sources. Stress responses (T_{opt} , F_v/F_m) and drought resistance (A/g_s , $\delta^{13}\text{C}$) will be important in determining how species will respond to changing climatic conditions and how this will affect species distribution (Marias, 2016). We predicted lower LWC for drought adapted northern seed sources, however LWC was not correlated with seed source. LWC did not vary based on treatment in another *Q. rubra* study (Rodgers et al. 2018). This provides evidence that *Q. rubra* may not regulate leaf water content based on moisture availability or is able to maintain constant LWC even in stressful conditions.

Conclusions

Northern seed source populations appear to be better suited for current and predicted climate conditions at the northern range limit of *Q. rubra*. Northern populations have a conservative, generalist strategy as evidenced by higher photosynthetic capacity and wider photosynthetic temperature range than southern populations. As temperature, drought and extreme weather events are expected to increase with climate change, a conservative, generalist strategy is favored. These findings are consistent with Chapter 1, where northern seed sources had greater cold tolerance, and provide further evidence that northern seed sources may be the most well suited for assisted migration of *Q. rubra* at the northern range limit.

Table 3.1 Seed sources and environmental variables of *Quercus rubra* seedlings planted at the Ford and Kellogg common gardens. The environmental variables are mean annual temperature (MAT (°C), maximum temperature of warmest month (°C), minimum temperature of coldest month (°C), the range of temperature (max-min) (°C), and rain over active growth months of May to September (Rain) (mm), shaded seed sources represent the core populations.

Population name	Seed source	Lat.	Long.	Climate	MAT	Max	Min	Range	Rain
Rockwood Park, VA	29	37.45085	-77.58009	Warm	14.2	31.7	-3.9	35.6	493
Trail of Tears, IL	21	37.48017	-89.36005	Warm	13.6	31.8	-6.3	38.1	499
Hoosier, IN	18	38.05957	-86.66079	Warm	12.9	30.2	-6.6	36.8	521
Warfordsberg, PA	27	39.73707	-78.18049	Warm	11.3	28.5	-8.6	37.1	437
Cygnét, OH	28	41.23797	-83.68050	Int.	9.8	27.6	-11.1	38.7	441
Big Bay de Noc, MI	16	45.86786	-86.51814	Int.	5.5	22.7	-1.5	37.7	396
Mouth of the Huron River, MI	12	46.90904	-88.03561	Int.	5.4	22.0	-15.4	37.4	385
Au Train, MI	15	46.43770	-86.87838	Int.	5.4	22.2	-14.9	37.1	404
Maasto Hihto, MI	4	47.13794	-88.61003	Cold	4.9	23.3	-16.9	40.2	376
Copper Harbor Sand dunes, MI	2	47.44013	-88.21871	Cold	4.5	21.0	-14.5	35.5	367
Cliff Drive, MI	3	47.35383	-88.35005	Cold	4.3	20.3	-15.9	36.2	371
Eagle Mine, MI	13	46.74914	-87.89082	Cold	4.1	22.10	-17.5	39.6	422

Table 3.2 Ford and Kellogg common gardens average temperatures, total precipitation, and number of frost days during growing season and winter from 2019-2021 (NOAA).

Site	Climate variable	May 2019- Sept. 2019	Oct. 2019- April 2020	May 2020- Sept. 2020	Oct. 2020- April 2021	May 2021- Sept. 2021
Ford	Average temp (°C)	14.3	-2.9	12.2	-2.7	11.9
Ford	Total precip (mm)	422	477	500	379	295
Ford	# of frost days (<0°C)	11	186	23	166	25
Kellogg	Average temp (°C)	21.5	3.5	21.8	4.4	22.6
Kellogg	Total precip (mm)	551	598	463	384	597
Kellogg	# of frost days (<0°C)	4	146	6	130	7

Table 3.3. Two-way ANCOVA results and degrees of freedom for common garden (CG) (Ford and Kellogg) and seed source climate variables of mean annual temperature (MAT) and rain over growing season (Rain) as well as climate*common garden interaction effects for A_{max} in 2019-2021 and Fv/Fm in 2019. Regression results and degrees of freedom for Fv/Fm of plants at Ford Center in 2020, 2021.

Source	A _{max}						Fv/Fm					
	2019			2020			2021			2019		
	DF	P		DF	P		DF	P		DF	P	
MAT	212	0.013		147	0.001		155	<0.001		865	<0.001	453
CG	212	<0.001		147	0.287		155	0.661		865	<0.001	NA
MAT*CG	212	0.467		147	0.078		155	0.163		865	0.011	NA
Rain	212	0.046		147	<0.001		155	0.002		865	<0.001	453
CG	212	<0.001		147	0.321		155	0.784		865	<0.001	NA
Rain*CG	212	0.418		147	0.151		155	0.212		865	0.006	NA

bolded values denote significance (<0.05)

Table 3.4 Two-way ANCOVA results and degrees of freedom for common garden (CG) (Ford and Kellogg) and seed source climate variables of mean annual temperature (MAT) and rain over growing season (Rain) as well as climate*common garden interaction effects for N_{area} and N_{mass} in 2021. Regression results and degrees of freedom for N_{area} and N_{mass} of plants at Ford in 2020.

Source	N_{area}					N_{mass}				
	2020		2021			2020		2021		
	DF	P	DF	P		DF	P	DF	P	
MAT	268	<0.001	44	0.286		268	<0.001	44	0.266	
CG	NA	NA	44	0.008		NA	NA	44	0.480	
MAT*CG	NA	NA	44	0.005		NA	NA	44	0.019	
Rain	268	<0.001	44	0.241		268	<0.001	44	0.403	
CG	NA	NA	44	0.013		NA	NA	44	0.511	
Rain*CG	NA	NA	44	0.140		NA	NA	44	0.172	

Table 3.5 Two-way ANCOVA results and degrees of freedom for common garden (CG) (Ford and Kellogg) and seed source climate variables of mean annual temperature (MAT) and rain over growing season (Rain) as well as climate*common garden interaction effects for temperature curve parameters T_{opt} , A_{opt} , and Ω during summer 2021.

Source	T_{opt}		A_{opt}		Ω	
	DF	P	DF	P	DF	P
MAT	37	0.199	37	0.013	36	0.006
CG	37	0.071	37	0.857	36	0.104
MAT*CG	37	0.636	37	0.216	36	0.210
Rain	37	0.397	37	0.004	36	0.001
CG	37	0.080	37	0.797	36	0.078
Rain*CG	37	0.652	37	0.954	36	0.122

bolded values denote significance (<0.05)

Table 3.6 Two-way ANCOVA p-value and degrees of freedom for common garden (CG) (Ford and Kellogg) and seed source climate variables of mean annual temperature (MAT) and rain over growing season (Rain) as well as climate*common garden interaction effects for A/g_s in 2019-2021 and LWC in 2021. Regression results and degrees of freedom for ¹³C of plants at Ford Center in 2020.

Source	A/g _s		¹³ C		LWC	
	2019		2020		2021	
	DF	P	DF	P	DF	P
MAT	212	0.977	147	0.848	155	0.014
CG	212	<0.001	147	0.010	155	<0.001
MAT*CG	212	0.113	147	0.056	155	0.167
Rain	212	0.880	147	0.880	155	0.039
CG	212	<0.001	147	<0.001	155	<0.001
Rain*CG	212	0.225	147	0.225	155	0.557
					268	0.019
					NA	NA
					NA	NA
					257	0.125
					257	0.125
					257	0.460
					257	0.223
					257	0.125
					257	0.679

bolded values denote significance (<0.05)

Figures

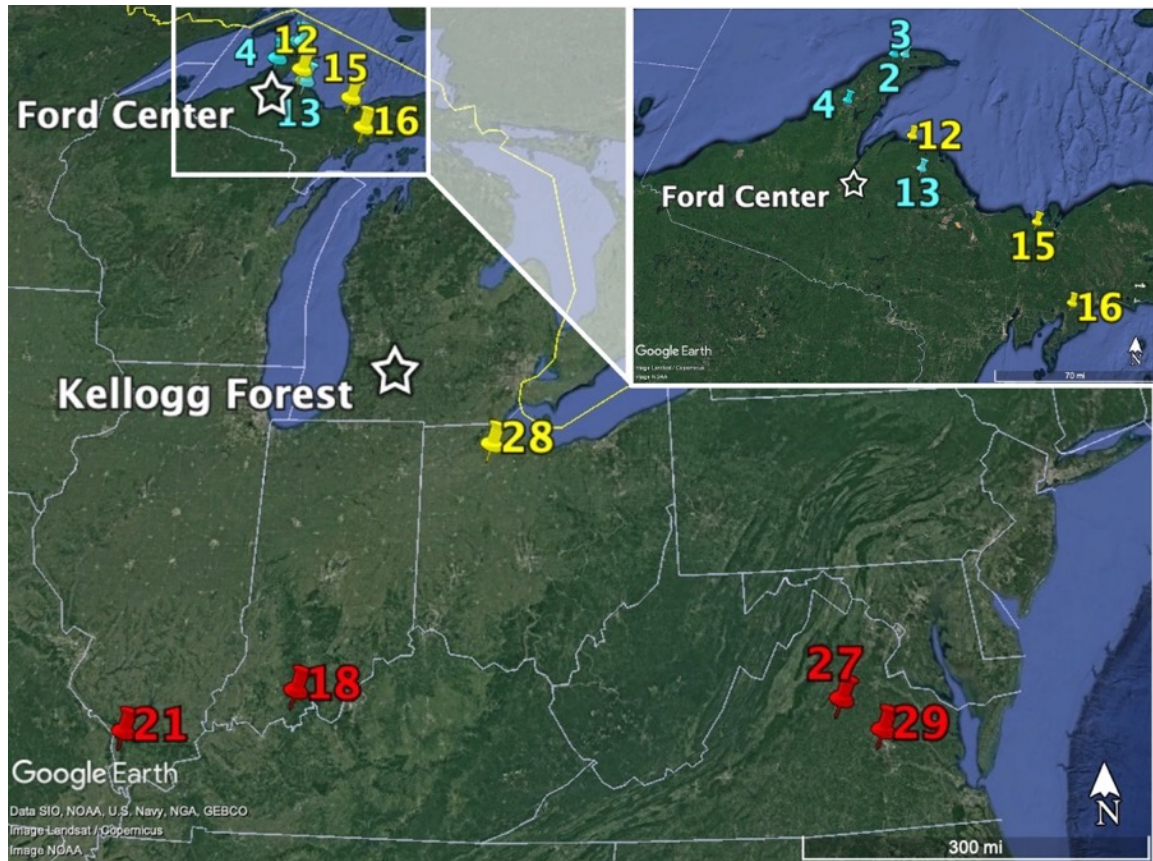


Figure 3.1 Map of *Quercus rubra* seed sources, four cold (blue), four intermediate (yellow), and four warm (red), Ford and Kellogg common gardens are represented with white stars.

A	B	C
D	E	F
G	H	I
J	K	L
M	N	O
P	Q	R
S	T	U
V	W	X
Y	Z	ZZ

buffer	buffer	buffer
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
<i>Qe</i>	<i>Qr</i>	<i>Qr</i>
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
<i>Qr</i>	<i>Qr</i>	<i>Qe</i>
<i>Qr</i>	<i>Qe</i>	<i>Qe</i>
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
<i>Qr</i>	<i>Qe</i>	<i>Qr</i>
<i>Qr</i>	<i>Qe</i>	<i>Qe</i>
<i>Qr</i>	<i>Qr</i>	<i>Qe</i>
<i>Qr</i>	<i>Qr</i>	<i>Qr</i>
buffer	buffer	buffer

Figure 3.2 Common garden design where each letter is a block with 30 plants in 3 columns x 10 rows followed by a row of buffer plants. In the enlarged figure of block C, *Qe* denotes *Q. ellipsoidalis* and *Qr* denotes *Q. rubra*. Colored plants are *Qr* plants used in the study. Red plants are from warm seed source sites, yellow plants are from intermediate seed source sites, and blue plants are from cold seed source sites.

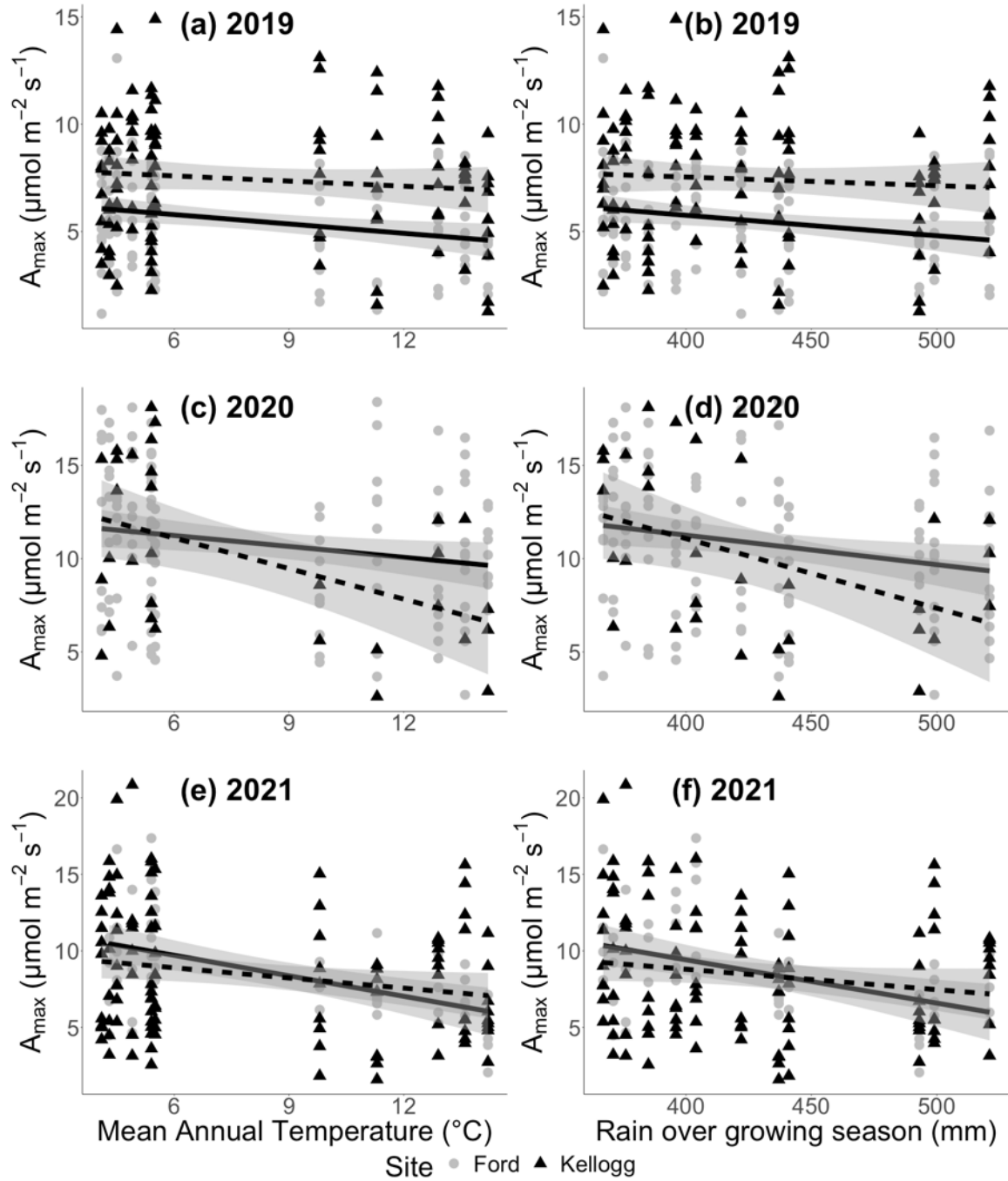


Figure 3.3 Interaction plots of A_{\max} with seed source mean annual temperature and rain over growing season for 2019 season (a), (b), 2020 season (c), (d), and 2021 season (e), (f), Kellogg depicted by dashed line, Ford depicted by solid line, shading = ± 1 SE.

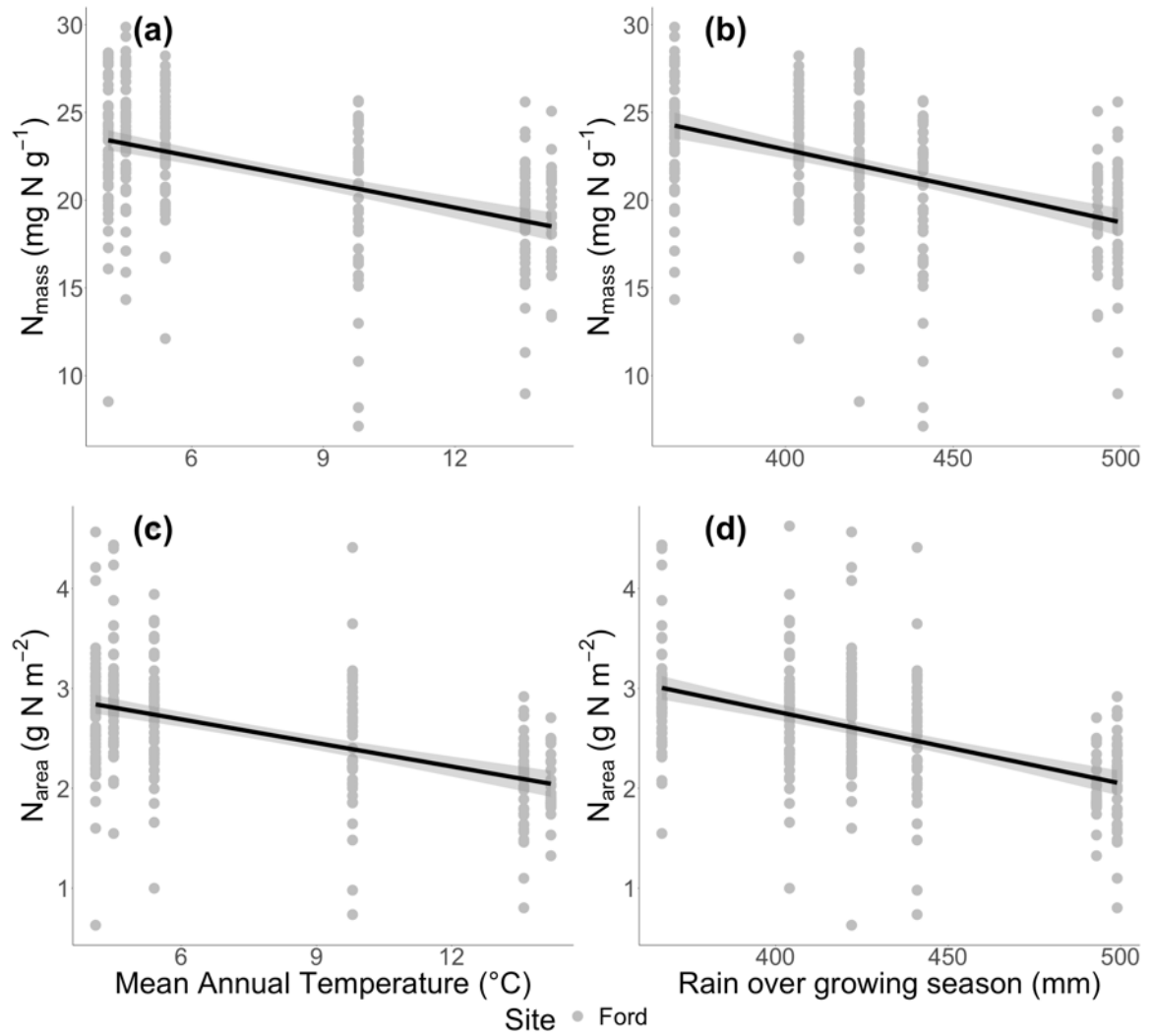


Figure 3.4 Regression plots of N_{mass} (a) (b) and N_{area} (c) (d) with seed source mean annual temperature and rain over growing season for core population plants during 2020 season at the Ford common garden, shading = ± 1 SE.

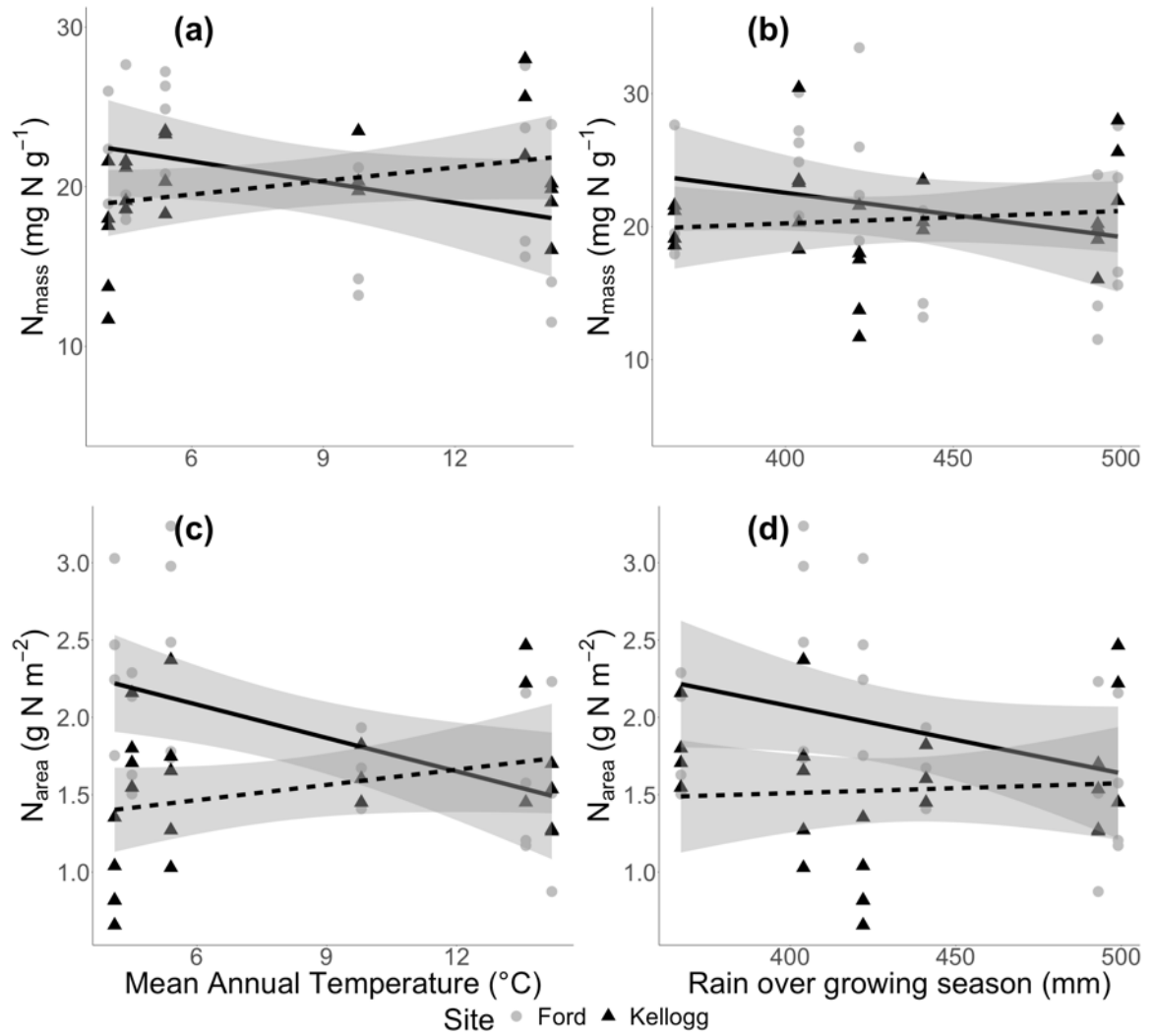


Figure 3.5 Interaction plots of N_{mass} (a), (b), and N_{area} (c), (d) with seed source mean annual temperature and rain over growing season during 2021 season at the Ford common garden, shading = ± 1 SE.

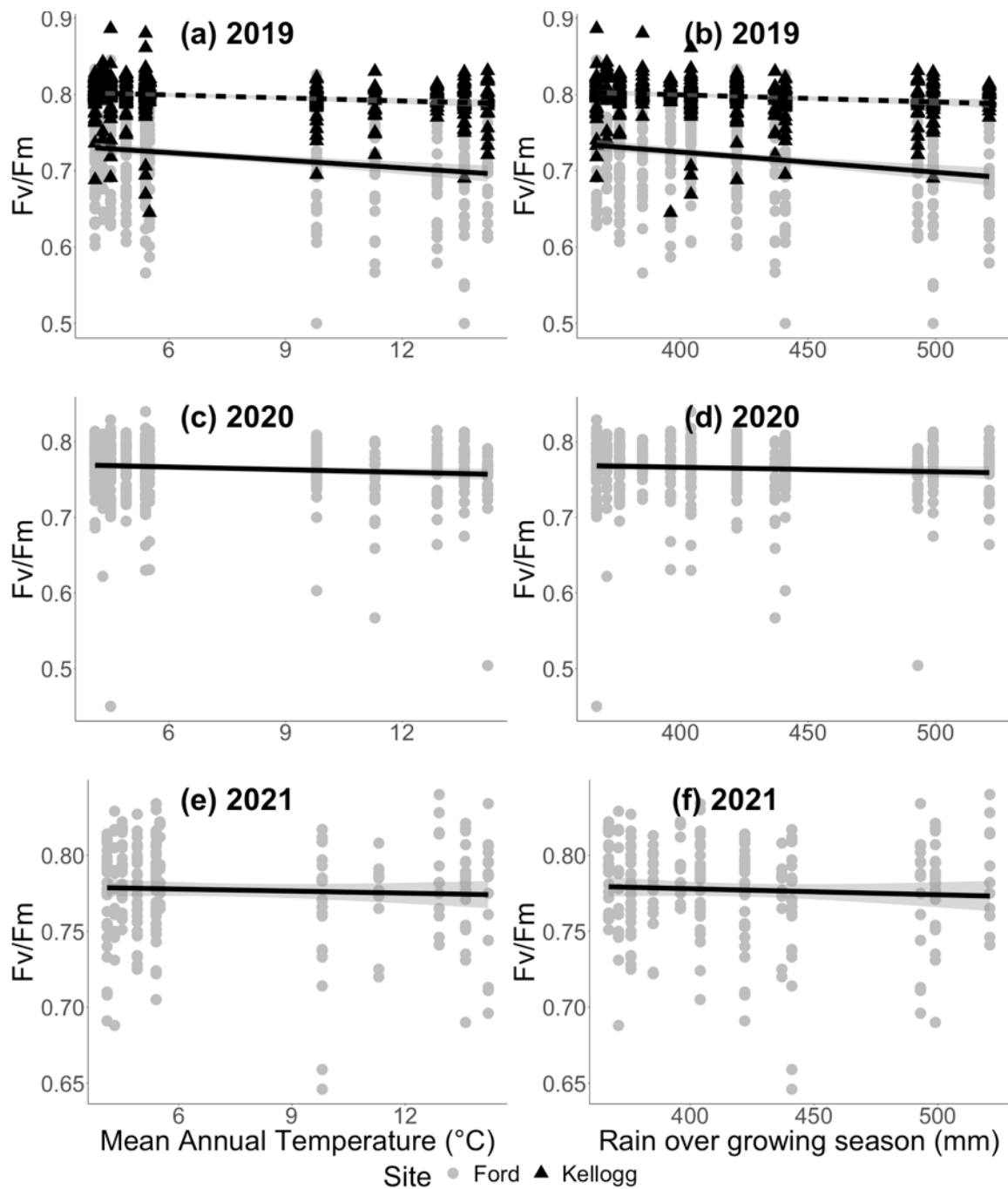


Figure 3.6 Interaction and regression plots of F_v/F_m with seed source mean annual temperature and rain over growing season for 2019 season (a), (b), 2020 season (c), (d), and 2021 season (e), (f), Kellogg depicted by dashed line, Ford depicted by solid line, shading = ± 1 SE.

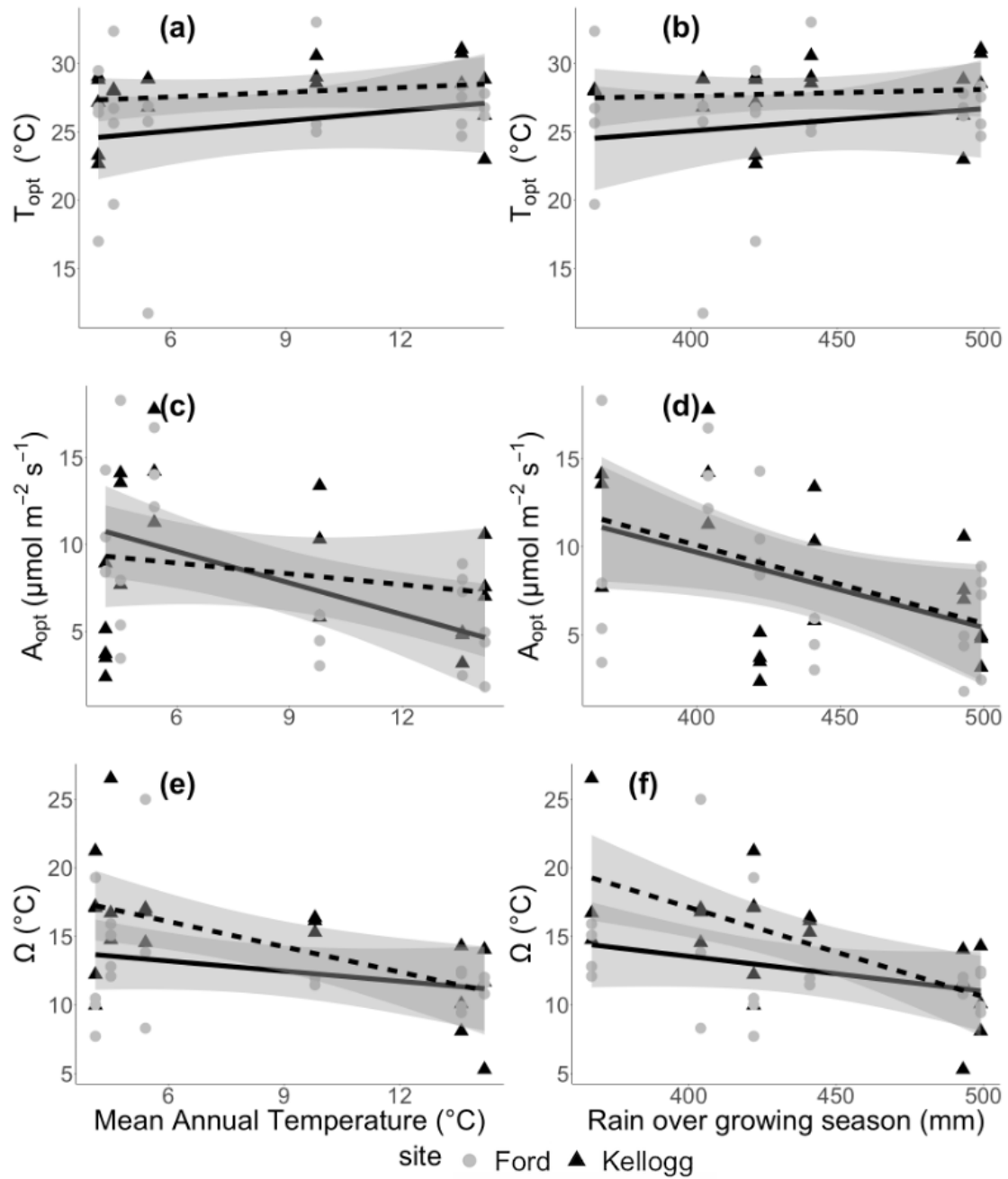


Figure 3.7 Interaction plots of temperature curve parameters T_{opt} (a), (b), A_{opt} (c), (d), and Ω (e), (f) with seed source mean annual temperature and rain over growing season for 2021 season, Kellogg depicted by dashed line, Ford depicted by solid line, shading = ± 1 SE.

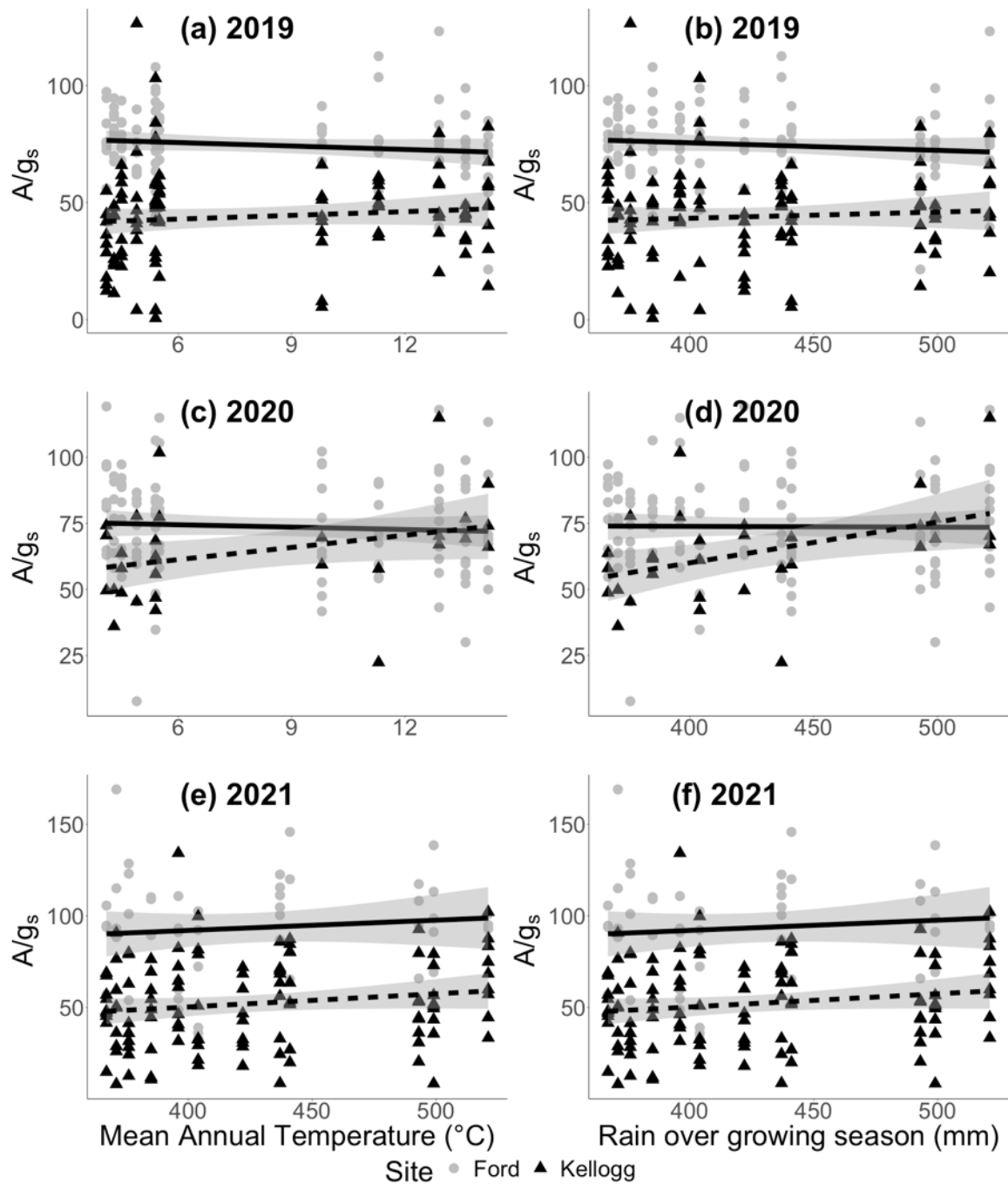


Figure 3.8 Interaction plots of A/g_s (A_{\max} /stomatal conductance) with seed source mean annual temperature and rain over growing season for 2019 season (a), (b), 2020 season (c), (d), and 2021 season (e), (f), Kellogg depicted by dashed line, Ford depicted by solid line, shading = ± 1 SE.

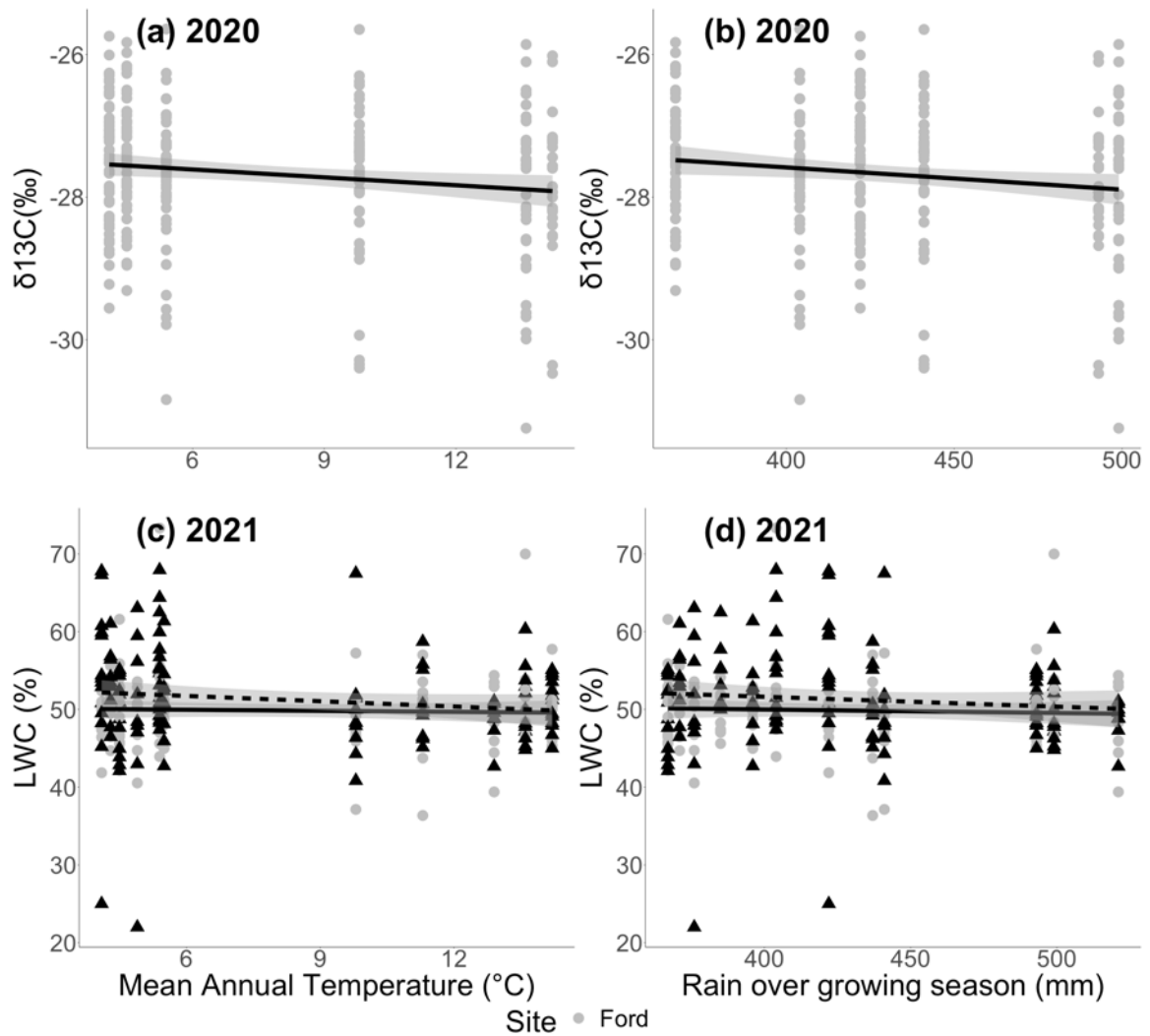


Figure 3.9 Regression plots of $\delta^{13}\text{CVPDB}$ (‰) at the Ford common garden during the 2020 season (a) (b) and interaction plots of LWC (c) (d) with seed source mean annual temperature and rain over growing season during 2021 season, Kellogg depicted by dashed line, Ford depicted by solid line, shading = ± 1 SE.

Citations

- Abdala-Roberts, L., A. Galman, W. K. Petry, F. Covelo, M. de la Fuente, G. Glauser, and X. Moreira. 2018. Interspecific variation in leaf functional and defensive traits in oak species and its underlying climatic drivers. *Plos One* **13**.
- Abraha, M., J. Q. Chen, S. K. Hamilton, and G. P. Robertson. 2020. Long-term evapotranspiration rates for rainfed corn versus perennial bioenergy crops in a mesic landscape. *Hydrological Processes* **34**:810-822.
- Abrams, M. D. 1990. Adaptations and responses to drought in *Quercus* species of North-America. *Tree Physiology* **7**:227-238.
- Aitken, S. N., S. Yeaman, J. A. Holliday, T. L. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* **1**:95-111.
- Ameye, M., T. M. Wertin, I. Bauweraerts, M. A. McGuire, R. O. Teskey, and K. Steppe. 2012. The effect of induced heat waves on *Pinus taeda* and *Quercus rubra* seedlings in ambient and elevated CO₂ atmospheres. *New Phytologist* **196**:448-461.
- Anderson, C. J. R., and P. J. Rosas-Anderson. 2017. Leafscan (Version 1.3.21) [Mobile application software].
- Anderson, P. D., and P. T. Tomlinson. 1998. Ontogeny affects response of northern red oak seedlings to elevated CO₂ and water stress - I. Carbon assimilation and biomass production. *New Phytologist* **140**:477-491.

- Augspurger, C. K. 2013. Reconstructing patterns of temperature, phenology, and frost damage over 124 years: Spring damage risk is increasing. *Ecology* **94**:41-50.
- Barlow, J. M., P. I. Palmer, L. M. Bruhwiler, and P. Tans. 2015. Analysis of CO₂ mole fraction data: first evidence of large-scale changes in CO₂ uptake at high northern latitudes. *Atmospheric Chemistry and Physics* **15**:13739-13758.
- Basler, D., and C. Korner. 2012. Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology* **165**:73-81.
- Bauweraerts, I., T. M. Wertin, M. Ameye, M. A. McGuire, R. O. Teskey, and K. Steppe. 2013. The effect of heat waves, elevated CO₂ and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. *Global Change Biology* **19**:517-528.
- Berry, J., and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher-plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **31**:491-543.
- Bukhov, N. G., G. Samson, and R. Carpentier. 2000. Nonphotosynthetic reduction of the intersystem electron transport chain of chloroplasts following heat stress. Steady-state rate. *Photochemistry and Photobiology* **72**:351-357.
- Carter, K. R., and M. A. Cavaleri. 2018. Within-Canopy Experimental Leaf Warming Induces Photosynthetic Decline Instead of Acclimation in Two Northern Hardwood Species. *Frontiers in Forests and Global Change* **1**.
- Carter, K. R., T. E. Wood, S. C. Reed, E. C. Schwartz, M. B. Reinsel, X. Yang, and M. A. Cavaleri. 2020. Photosynthetic and Respiratory Acclimation of Understory

- Shrubs in Response to in situ Experimental Warming of a Wet Tropical Forest. *Frontiers in Forests and Global Change* **3**.
- Cavender-Bares, J., and F. A. Bazzaz. 2000. Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia* **124**:8-18.
- Chi-Wu, H. B. K. a. W. 1962. The Interaction Between Provenance and Degree of Chilling in Bud-Break of Sugar Maple. *Silvae Genetica* **11**.
- Clark, P. W., A. W. D'Amato, K. S. Evans, P. G. Schaberg, and C. W. Woodall. 2022. Ecological memory and regional context influence performance of adaptation plantings in northeastern US temperate forests. *Journal of Applied Ecology* **59**:314-329.
- Craine, J. M., E. N. J. Brookshire, M. D. Cramer, N. J. Hasselquist, K. Koba, E. Marin-Spiotta, and L. X. Wang. 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant and Soil* **396**:1-26.
- Crum, J. R., and H. P. Collins. 1995. KBS Soils.
- Cunningham, S. C., and J. Read. 2002. Comparison of temperate and tropical rainforest tree species: photosynthetic responses to growth temperature. *Oecologia* **133**:112-119.
- Cunningham, S. C., and J. Read. 2003. Do temperate rainforest trees have a greater ability to acclimate to changing temperatures than tropical rainforest trees? *New Phytologist* **157**:55-64.

- Duveneck, M. J., R. M. Scheller, M. A. White, S. D. Handler, and C. Ravenscroft. 2014. Climate change effects on northern Great Lake (USA) forests: A case for preserving diversity. *Ecosphere* **5**.
- Etterson, J. R., M. W. Cornett, M. A. White, and L. C. Kavajecz. 2020. Assisted migration across fixed seed zones detects adaptation lags in two major North American tree species. *Ecological Applications* **30**.
- Farquhar, G. D., M. H. O'Leary, and J. A. Berry. 1982. On the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves *Australian Journal of Plant Physiology* **9**:121-137.
- Frelich, L. E., and P. B. Reich. 2010. Will environmental changes reinforce the impact of global warming on the prairie-forest border of central North America? *Frontiers in Ecology and the Environment* **8**:371-378.
- Frick, S. E., and R. J. Hijmans. 2017. Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*.
- Gerst, K. L., N. L. Rossington, and S. J. Mazer. 2017. Phenological responsiveness to climate differs among four species of *Quercus* in North America. *Journal of Ecology* **105**:1610-1622.
- Gunderson, C. A., N. T. Edwards, A. V. Walker, K. H. O'Hara, C. M. Campion, and P. J. Hanson. 2012. Forest phenology and a warmer climate - growing season extension in relation to climatic provenance. *Global Change Biology* **18**:2008-2025.

- Gunderson, C. A., K. H. O'Hara, C. M. Campion, A. V. Walker, and N. T. Edwards. 2010. Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate. *Global Change Biology* **16**:2272-2286.
- Havaux, M. 1996. Short-term responses of photosystem I to heat stress - Induction of a PS II-independent electron transport through PS I fed by stromal components. *Photosynthesis Research* **47**:85-97.
- Hofmann, M., M. Jager, and H. Bruelheide. 2014. Relationship between frost hardiness of adults and seedlings of different tree species. *Iforest-Biogeosciences and Forestry* **7**:282-288.
- Howe, G. T., S. N. Aitken, D. B. Neale, K. D. Jermstad, N. C. Wheeler, and T. H. H. Chen. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany-Revue Canadienne De Botanique* **81**:1247-1266.
- Iler, A. M., P. J. CaraDonna, J. R. K. Forrest, and E. Post. 2021. Demographic Consequences of Phenological Shifts in Response to Climate Change. Pages 221-245 *in* D. J. Futuyma, editor. *Annual Review of Ecology, Evolution, and Systematics*, Vol 52, 2021.
- IPCC. 2021. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.
- Iverson, L. R., A. M. Prasad, S. N. Matthews, and M. Peters. 2008. Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *Forest Ecology and Management* **254**:390-406.

- Jarvi, M. P., and A. J. Burton. 2013. Acclimation and soil moisture constrain sugar maple root respiration in experimentally warmed soil. *Tree Physiology* **33**:949-959.
- Jarvi, M. P., and A. J. Burton. 2018. Adenylate control contributes to thermal acclimation of sugar maple fine-root respiration in experimentally warmed soil. *Plant Cell and Environment* **41**:504-516.
- June, T., J. R. Evans, and G. D. Farquhar. 2004. A simple new equation for the reversible temperature dependence of photosynthetic electron transport: a study on soybean leaf. *Functional Plant Biology* **31**:275-283.
- Kassambara, A. 2020. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.6.0.
- Keenan, T. F., and A. D. Richardson. 2015. The timing of autumn senescence is affected by the timing of spring phenology: implications for predictive models. *Global Change Biology* **21**:2634-2641.
- Kerr, K. L., F. C. Meinzer, K. A. McCulloh, D. R. Woodruff, and D. E. Marias. 2015. Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. *Tree Physiology* **35**:535-548.
- Keskitalo, J., G. Bergquist, P. Gardestrom, and S. Jansson. 2005. A cellular timetable of autumn senescence. *Plant Physiology* **139**:1635-1648.
- Korner, C., and D. Basler. 2010. Phenology Under Global Warming. *Science* **327**:1461-1462.
- Korner, C., D. Basler, G. Hoch, C. Kollas, A. Lenz, C. F. Randin, Y. Vitasse, and N. E. Zimmermann. 2016. Where, why and how? Explaining the low-temperature range limits of temperate tree species. *Journal of Ecology* **104**:1076-1088.

- Lam, E. 2004. Controlled cell death, plant survival and development. *Nature Reviews Molecular Cell Biology* **5**:305-315.
- Leroy, T., C. Plomion, and A. Kremer. 2020. Oak symbolism in the light of genomics. *New Phytologist* **226**:1012-1017.
- Marias, D. E., F. C. Meinzer, D. R. Woodruff, and K. A. McCulloh. 2017. Thermotolerance and heat stress responses of Douglas-fir and ponderosa pine seedling populations from contrasting climates. *Tree Physiology* **37**:301-315.
- Mau, A. C., S. C. Reed, T. E. Wood, and M. A. Cavaleri. 2018. Temperate and Tropical Forest Canopies are Already Functioning beyond Their Thermal Thresholds for Photosynthesis. *Forests* **9**.
- Morgenstern, E. K. 1996. Geographic variation in forest trees : genetic basis and application of knowledge in silviculture. UBC Press, Vancouver.
- Nagel, R.-V. 2015. Northern red oak. In *Potentials and risks of introduced tree species*. *Göttinger Forstwissenschaften* **7**:219-267.
- Nicolescu, V. N., T. Vor, W. L. Mason, J. C. Bastien, R. Brus, J. M. Henin, I. Kupka, V. Lavnyy, N. La Porta, F. Mohren, K. Petkova, K. Redei, I. Stefancik, R. Wasik, S. Peric, and C. Hernea. 2020. Ecology and management of northern red oak (*Quercus rubra* L. syn. *Q. borealis* F. Michx.) in Europe: a review. *Forestry* **93**:481-494.
- NOAA, National Oceanic and Atmospheric Administration. 2022. Climate Data Online Search.
- Olsen, J. E. 2010. Light and temperature sensing and signaling in induction of bud dormancy in woody plants. *Plant Molecular Biology* **73**:37-47.

- Oswalt, S. N. S., W. Brad; Miles, Patrick D.; Pugh, Scott A. 2014. Forest Resources of the United States, 2012: a technical document supporting the Forest Service 2015 update of the RPA Assessment. . Gen. Tech. Rep. WO-91. Washington, DC: U.S. Department of Agriculture, Forest Service, Washington Office. 218 p. .
- Ow, L. F., K. L. Griffin, D. Whitehead, A. S. Walcroft, and M. H. Turnbull. 2008. Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides* x *nigra*. *New Phytologist* **178**:123-134.
- Park, A., and C. Talbot. 2018. Information Underload: Ecological Complexity, Incomplete Knowledge, and Data Deficits Create Challenges for the Assisted Migration of Forest Trees. *Bioscience* **68**:251-263.
- Parmesan, C., and M. E. Hanley. 2015. Plants and climate change: complexities and surprises. *Annals of Botany* **116**:849-864.
- Percival, G. C. 2005. The use of chlorophyll fluorescence to identify chemical and environmental stress in leaf tissue of three oak (*Quercus*) species *Journal of Arboriculture* **31 (5)**:215-227.
- Perez, T. M., A. Socha, O. Tserej, and K. J. Feeley. 2021. Photosystem II heat tolerances characterize thermal generalists and the upper limit of carbon assimilation. *Plant Cell and Environment* **44**:2321-2330.
- Polgar, C., A. Gallinat, and R. B. Primack. 2014. Drivers of leaf-out phenology and their implications for species invasions: insights from Thoreau's Concord. *New Phytologist* **202**:106-115.
- Polgar, C. A., and R. B. Primack. 2011. Leaf-out phenology of temperate woody plants: from trees to ecosystems. *New Phytologist* **191**:926-941.

- Prud'homme, G. O., M. S. Lamhamedi, L. Benomar, A. Rainville, J. DeBlois, J. Bousquet, and J. Beaulieu. 2018. Ecophysiology and Growth of White Spruce Seedlings from Various Seed Sources along a Climatic Gradient Support the Need for Assisted Migration. *Frontiers in Plant Science* **8**.
- R Studio Team. 2020. R Studio: Integrated Development for R. RStudio, PBC, Boston, MA.
- Ramírez-Valiente, J. C.-B. a. J. 2017. *Physiological Evidence from Common Garden Experiments for Local Adaptation and Adaptive Plasticity to Climate in American Live Oaks (Quercus Section Virentes): Implications for Conservation Under Global Change*. In: Gil-Pelegrín E., Peguero-Pina J., Sancho-Knapik D. (eds) *Oaks Physiological Ecology. Exploring the Functional Diversity of Genus Quercus L.* Springer Cham.
- Rasband, W. S. 1998-2018. ImageJ.in W. S. Rasband, editor. <https://imagej.nih.gov/ij/>, U. S. National Institutes of Health, Bethesda, Maryland, USA.
- Rauschendorfer, J., E. Lindback, R. Rooney, S. Frantti, V. Peck, M. Cavaleri, and C. Kulheim. 2021. Quercus rubra seedling biomass response related to mean annual temperature conditions of associated provenance. Proceedings of the Northern Hardwoods Conference **IN PRESS**.
- Rehfeldt, G. E., C. C. Ying, D. L. Spittlehouse, and D. A. Hamilton. 1999. Genetic responses to climate in Pinus contorta: Niche breadth, climate change, and reforestation. *Ecological Monographs* **69**:375-407.

- Richter, S., T. Kipfer, T. Wohlgemuth, C. C. Guerrero, J. Ghazoul, and B. Moser. 2012. Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia* **169**:269-279.
- Ritchie, G. A. 2006. Chlorophyll Fluorescence: What is it and what do the numbers mean? USDA Forest Service Proceedings.
- Robakowski, P., Y. Li, and P. B. Reich. 2012. Local ecotypic and species range-related adaptation influence photosynthetic temperature optima in deciduous broadleaved trees. *Plant Ecology* **213**:113-125.
- Rodgers, V. L., N. G. Smith, S. S. Hoeppner, and J. S. Dukes. 2018. Warming increases the sensitivity of seedling growth capacity to rainfall in six temperate deciduous tree species. *Aob Plants* **10**.
- Rogers, B. M., P. Jantz, and S. J. Goetz. 2017. Vulnerability of eastern US tree species to climate change. *Global Change Biology* **23**:3302-3320.
- Roussel, M., E. Dreyer, P. Montpied, G. Le-Provost, J.-M. Guehl, and O. Brendel. 2009. The diversity of ^{13}C isotope discrimination in a *Quercus robur* full-sib family is associated with differences in intrinsic water use efficiency, transpiration efficiency, and stomatal conductance. *Journal of Experimental Botany* **60**:2419-2431.
- Sage, R. F., and D. S. Kubien. 2007. The temperature response of C-3 and C-4 photosynthesis. *Plant Cell and Environment* **30**:1086-1106.
- Sastry, A., A. Guha, and D. Barua. 2018. Leaf thermotolerance in dry tropical forest tree species: relationships with leaf traits and effects of drought. *Aob Plants* **10**.

- Sittaro, F., A. Paquette, C. Messier, and C. A. Nock. 2017. Tree range expansion in eastern North America fails to keep pace with climate warming at northern range limits. *Global Change Biology* **23**:3292-3301.
- Slot, M., and K. Winter. 2017. Photosynthetic acclimation to warming in tropical forest tree seedlings. *Journal of Experimental Botany* **68**:2275-2284.
- St Clair, J. B., G. T. Howe, and J. G. Kling. 2020. The 1912 Douglas-Fir Heredity Study: Long-Term Effects of Climatic Transfer Distance on Growth and Survival. *Journal of Forestry* **118**:1-13.
- Tjoelker, M. G., P. B. Reich, and J. Oleksyn. 1999. Changes in leaf nitrogen and carbohydrates underlie temperature and CO₂ acclimation of dark respiration in five boreal tree species. *Plant Cell and Environment* **22**:767-778.
- Turnbull, M. H., R. Murthy, and K. L. Griffin. 2002. The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. *Plant Cell and Environment* **25**:1729-1737.
- United States Forest Service, U. 2008. Forest service strategic framework for responding to climate change.
- Verlinden, M. S., R. Fichot, L. S. Broeckx, B. Vanholme, W. Boerjan, and R. Ceulemans. 2015. Carbon isotope compositions ($\delta^{13}\text{C}$) of leaf, wood and holocellulose differ among genotypes of poplar and between previous land uses in a short-rotation biomass plantation. *Plant Cell and Environment* **38**:144-156.
- Vierling, E. 1991. THE ROLES OF HEAT-SHOCK PROTEINS IN PLANTS. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**:579-620.

- Vitasse, Y., S. Delzon, C. C. Bresson, R. Michalet, and A. Kremer. 2009. Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. *Canadian Journal of Forest Research* **39**:1259-1269.
- Way, D. A. 2011. Tree phenology responses to warming: spring forward, fall back? *Tree Physiology* **31**:469-471.
- Weiwei, L. U., Y. U. Xinxiao, J. I. A. Guodong, L. I. Hanzhi, and L. I. U. Ziqiang. 2018. Responses of Intrinsic Water-use Efficiency and Tree Growth to Climate Change in Semi-Arid Areas of North China. *Scientific Reports* **8**.
- Williams, M. I., and R. K. Dumroese. 2013. Preparing for Climate Change: Forestry and Assisted Migration. *Journal of Forestry* **111**:287-297.
- Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. *Nature* **428**:821-827.
- Yamori, W., K. Hikosaka, and D. A. Way. 2014. Temperature response of photosynthesis in C-3, C-4, and CAM plants: temperature acclimation and temperature adaptation. *Photosynthesis Research* **119**:101-117.

- Yamori, W., K. Noguchi, K. Hikosaka, and I. Terashima. 2010. Phenotypic Plasticity in Photosynthetic Temperature Acclimation among Crop Species with Different Cold Tolerances. *Plant Physiology* **152**:388-399.
- Zhu, K., C. W. Woodall, and J. S. Clark. 2012. Failure to migrate: lack of tree range expansion in response to climate change. *Global Change Biology* **18**:1042-1052.