MORPHOLOGICAL, PHENOLOGICAL, AND TRANSPIRATIONAL DIFFERENTIATION IN POPULATIONS OF *QUERCUS MACROCARPA* AND *QUERCUS RUBRA*

A Thesis

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Abstract

The characteristics and consequences of climate change are unfolding with unprecedented speed worldwide, and few places are changing faster than northern Minnesota. This study takes a unique approach to quantifying arboreal variation in physical plant traits and plant water use in the context of climate change. Two populations, northern and southern, of two species, *Quercus macrocarpa* and *Quercus rubra*, were grown in a common garden experiment at a northern field site and compared for climatic suitability. Traits that are known to have adaptive value, such as growth, leaf attributes, and phenological traits, were measured and compared. In addition, the transpiration rates of these populations were captured via portable rapid evapotranspiration chamber for comparison. Trees in a forested watershed can be responsible for up to 70% of the ecosystem's water loss. Population-level differentiation in transpiration has ramifications for water cycling and may be an important but understudied consideration in climate-forward forest management practices. Rather than struggling in an adverse environment, these two species maintained their cohorts well, indicating that climate shifts have created hospitable long-term conditions for these species. There was genetic differentiation between the two populations for each species for traits that are important for climate adaptation. These differences were especially strong for fall phenology traits and notable for physiological traits linked to water use adaptation, such as specific leaf area. Importantly, measured transpiration varied at a population level for both species. In late August 2020 and 2021, *Q. macrocarpa* populations significantly differed in their transpiration rate and therefore water use. Furthermore, in a year with more water stress, *Q. rubra* also demonstrated significant population differentiation in midsummer transpiration. This suggests true population differences, a pattern that may become more evident over time or if additional sites from the original experiment are measured. A model constructed using field precipitation, discharge, and transpiration measurements demonstrated that each population for each species would have a unique impact on the discharge of the Stewart River. This study is one of the known few to demonstrate population differentiation in tree transpiration rates, particularly in *Q. macrocarpa* and *Q. rubra*. Taken altogether, this study demonstrates that northern populations may be falling out of alignment with local climate, at the cost of lost growth opportunities and greater winter stresses at the margins of the growing season, and that tree cover can have distinct impacts on ecosystem water balances even at a population scale. This presents an opportunity to counteract the desynchronization of climate shifts with local adaptations, especially in heavily forested regions such as northern Minnesota, and highlights the need for further investigation of water use impacts of trees chosen for planting.

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Introduction

The characteristics and consequences of climate change are unfolding with unprecedented speed worldwide, and few places are changing faster than northern Minnesota (Baker *et al.* 1985, Bradbury *et al.* 1993, Dore 2005, MN DNR 2024a). Winters, in particular, are changing drastically in the northern latitudes (Johnson and Stefan 2006), with unknown effects on ecosystems within these regions. The state of Minnesota is a unique testing ground for the effects of climate change given that it contains at least two distinctive ecotones, a diverse ecosystem (including tallgrass prairie, northern coniferous forest, and eastern deciduous forest), and relatively large areas of minimally disturbed landscape (Martin *et al.* 1934, Geiss *et al.* 2003, Umbanhowar 2004, Novotny and Stefan 2007). In addition to a climatic transition zone that divides prairie, boreal forest and temperate forests, the state abuts Lake Superior, an enormous freshwater body that exerts well documented climate impacts (Scott and Huff 1996, Huff and Thomas 2014), colloquially called "lake effect." When cold air masses move over Lake Superior—often descending from Canada—the moist and contrastingly warm air hovering over the lake's waters is pushed to the lower atmosphere and spread along the margins of the lake basin, producing increased precipitation and warmer temperatures (Eichenlaub 1987). With the forest edge constrained by the lake and a sharp climate gradient moving inland, this region provides useful conditions to quantify climate change and its effects on natural processes such as water cycling and species migration (Person *et al.* 2007, McLauchlan *et al.* 2013, Muller *et al.* 2019).

This study takes a unique approach to quantifying arboreal variation in physical plant traits and plant water use in the context of climate change. Here, I not only compare two species that are predicted to thrive under climate change, but it also compare two populations of these species, one from a more southern origin than the other. In this experiment, these species and their populations were grown in a northern field site and compared for climatic suitability by measuring traits that are known to have adaptive value, such as growth, leaf attributes, and phenological traits. In addition, I measured the transpiration rates of these populations, an aspect of climate adaptation that, to the best of my knowledge, as never been studied at the intraspecific level in trees, although the related measurement of water-use efficiency been compared between tree populations using other methods (Zhang and Marshall 1994, Li 2000). Population-level differentiation in transpiration has ecosystem-wide ramifications for water cycling and may be an important but understudied consideration in climate-forward forest management practices.

Impact of Climate Change and Forests

It is likely that the speed of climate shifts is surpassing the migratory capabilities of plant species, particularly long-lived species such as trees (Sykes 2009, Shaw and Etterson 2012), including in Minnesota (Etterson *et al.* 2020). From 1899-2020, Minnesota warmed ~1.7°C, with especially severe warming in the winter. During that same time interval, precipitation increased ~8.6 cm annually (MN DNR 2024a). Precipitation patterns are also shifting towards heavy inundations punctuated by drought, especially in recent decades (MN DNR 2024a). This may affect how much water enters streams and rivers. Statewide, summer peak stream flows and high flow days have increased in frequency and magnitude since 1950, and stream baseflow is greater in both winter and summer (Novotny and Stefan 2007). Altered patterns of water availability in turn affect the health of watershed ecosystems, though the full extent of such effects remains unknown.

To date, Minnesota climates have shifted roughly 240 km to the northeast, introducing potential incompatibilities between local adaptations of forest tree species and novel climate conditions (Etterson *et al.* 2020, Reich *et al.* 2022). Climate stress also alters disturbance regimes such as fire (Clark 1988, Clark 1990) and drought, and increases forest susceptibility to further disturbances and stressors (Teshome *et al.* 2020). Resulting reductions in ecosystem drought resilience may cause declining forest health—climate-stressed boreal forests suffer biodiversity and productivity losses as well as increased mortality and susceptibility to disease and pests (Swanston *et al.* 2011, Duveneck *et al*. 2014). Ultimately, this may lead to widespread declines of common boreal tree species such as paper birch, quaking aspen, balsam fir, and white spruce and increases in species of the oak-hickory forest type (Duveneck *et al.* 2014). For example, species such as bur oak, *Quercus macrocarpa,* and northern red oak, *Quercus rubra,* are projected to increase in aboveground biomass in Minnesota (Duveneck *et al.* 2014) or at least remain present in the forest ecosystem in most modelled climate scenarios (Matthews *et al.* 2018).

Deteriorating forest health, in turn, impacts the health of forested watersheds. Climate-driven tree mortality reduces stand transpiration, with cascading impacts on overland flow, soil moisture, groundwater recharge, and streamflow (Adams *et al.* 2012). Tree loss can lead to increased nitrate concentrations in streams (Correll *et al.* 1997, Hanratty and Stefan 1998) and altered regimes of dissolved organic carbon, phosphorus, and total organic nitrogen (Garman and Moring 1991, Ohte *et al.* 2003). In this way, diminishing forest cover compounds environmental impacts on stream food webs, in addition to stressors such as drought (Ledger *et al.* 2013).

Migratory Response of Trees to Climate Change

As climate changes, trees are well known to expand and contract their geographic ranges (Davis and Shaw 2001). Species ranges contain the range of tolerable climate and available resources required by each species, and as the climate envelope shifts, mortality or recruitment occurs in response to changing conditions (Savolainen *et al.* 2007, Aitken *et al*. 2008). Trees may also evolve in response to migratory processes itself (Cwynar and MacDonald 1987). Pollen and other fossil evidence show that the historic ranges of tree species have expanded and contracted extensively in the past (Davis and Shaw 2001). At a molecular level, genetic markers indicating that a species may have once adapted substantially to different growing conditions in distant environments can still be detected in the genotypes of modern populations (Savolainen *et al.* 2007). However, historic variation is an imperfect analogue for ongoing climate change, which is currently the fastest in Earth's known history (Solomon *et al*. 2007). Models project unequal warming— 2-5°C on average in North America—with the greatest temperature increases at higher latitudes, whose winters may warm as much as 10°C (Solomon *et al*. 2007). The speed and magnitude of change are expected to drive many species to extinction (Davis and Shaw 2001). Biotic disruptions are already well underway, evidenced by environmental disturbances like drought and disease (Ste-Marie *et al.* 2011).

Climate change is not novel to our planet; trees have migrated in response to shifting environmental conditions many times in the past. Species do not occupy every location within their known range; the range is simply the area in which the species, often established in concentrated populations, might be found. Migration occurs as populations of a species colonize into newly hospitable habitat at the leading range edge while populations on the trailing edge of the species range are steadily disconnected from their required growing conditions and die out (Aitken *et al*. 2008). Because trees have long lifespans, and therefore slower generational turnover, both range expansion and adaptive evolution proceed slowly (Amano *et al.* 2014). Migration is vital for species to adapt and adjust to climate change, but modern migration rates are impeded by anthropogenic obstacles on the landscape, which limits dispersal and increases risk of local extirpations (Davis and Shaw 2001, Aitken *et al*. 2008). At present, it appears that trees are not keeping pace with climate shifts via dispersal and natural geographic range shifts.

After the last ice age, most European broadleaf trees' ranges shifted about 50-500 m per year as they expanded into unoccupied land available because of the retreating ice sheets, while pines moved much faster—approximately 1,500 m per year (Hewitt 1999). Recent molecular studies have brought these estimated migration rates into question. For instance, chloroplast DNA surveys of American beech (*Fagus grandifolia*) and red maple (*Acer rubrum*) suggest that their historic range limits were much nearer to their modern expanse in North America than has previously determined by fossil pollen and modeling studies (McLachlan *et al.* 2005). Regardless of how far and fast each species historically moved; range adjustments likely occurred as a way to ensure a given species continued to grow in its ideal conditions. At present, it appears that trees are not keeping pace with climate shifts via dispersal and natural geographic range shifts.

Adaptive Response of Trees to Climate Change

Species often exhibit broad genetic differentiation and phenotypic plasticity (van Tienderen 1991, Joshi *et al.* 2001). Plasticity allows individual organisms to adjust to immediate environmental changes (Bradshaw 1984). However, there are limits to plastic responses or acclimation. Phenotypic plasticity cannot mediate long-term climate shifts in all species. For example, a British study on environmental warming found that annual plants are under strong selection because their reproductive fitness depends upon a single flowering season, but perennial plants like trees were not nearly as plastic (Amano *et al.* 2014).

Over time and under stable conditions, the need for plasticity lessens as relatively small genetic adjustments incorporate local conditions into a population's genotype, accumulating distinct local adaptations (Bradshaw 1984). These genetically based traits distinguish populations from one another, conferring site-specific advantages and often relative disadvantages when outside of their normal habitat, and the disadvantages may increase with distance (Sork *et al.* 1993, Aitken *et al.* 1996, Joshi *et al.* 2001, Hereford 2009, Smith *et al.* 2012). A reciprocal transplant of narrowleaf cottonwood (*Populus angustifolia*) genotypes across varied soil conditions in a single river drainage demonstrated strong patterns of local adaptation. When seedlings were grown in contrasting soils, they were 2.5 times more likely to survive in their "home" soil than seedlings grown in nonlocal soil; seedlings grown in local soil were approximately 17.5% bigger than seedlings grown in nonlocal soil (Smith *et al.* 2012).

Ecotypes evolve genetic specialties that can be differentiated from other ecotypes less than one degree of latitude away (Sork *et al.* 1993, Aitken *et al.* 1996, Savolainen *et al.* 2007). Though only miles apart, central and southern populations of sweet chestnut, *Castanea sativa*, had genetically based, earlier phenology than northern populations in response to differing drought regimes in Spain (Míguez-Soto *et al.* 2019). In California, populations of Ponderosa and Jeffrey pines were distinctly different in fitness and phenology despite being sourced from the same mountain range (Martínez-Berdeja *et al.* 2019). The degree of genetic variation in a species depends on the amount of gene flow among populations. Increased gene flow leads to greater genetic homogenization among populations, while isolation leads to population differentiation.

Climate Change, Forests, and Water Dynamics

Trees in a forested watershed are responsible for large portions of the ecosystem's water loss, up to 60-70% in some cases (van der Ent *et al*. 2010, Ellison *et al*. 2017). The term evapotranspiration describes the amount of water lost from a system via evaporation and transpiration. Evaporation is the phase change of water from a liquid to a gas and typically occurs off of wet surfaces such as soil and plants. Transpiration is water consumption via plant use, wherein water moves from soil reservoirs through the plant body, to be used for photosynthesis, and is subsequently released as water vapor. The physical process of transporting water into the air is driven by a vapor pressure deficit between the air and the leaves. Water loss at the leaf level leads to a gradient in water content that draws water up the vascular system as it flows from higher to lower concentrations. A small percentage of water drawn from the groundwater by plant roots is retained in the plant body, but most is released into the atmosphere as water vapor. Thus, plant transpiration is a major contributor to local water loss in forest ecosystems.

The total water transpired by a forest is dependent on its species composition and the water-use traits of the trees present (Bowden and Bauerle 2008). A tree's transpiration rate is also affected by environmental conditions (Zhu *et al.* 2022). It is well known that these traits differ between tree species (Matheny *et al*. 2016, Pappas *et al.* 2018). However, very little is known about *population differences within spec*ies in transpiration rates (but see Zhang and Marshall 1994, Li 2000). This is an important gap in our understanding given that many studies have demonstrated differences in local adaptation among tree populations at both large and small spatial scales (Zhang and Marshall 1994, Li 2000). Consequently, it is possible that variation in not just species, but also populations, has effects on water loss in forested ecosystems.

Work examining transpiration responses to changing environmental conditions and microclimates indicates a strong linkage between plant water use and ecosystem stresses such as climate change (Matheny 2021). Many transpiration studies take a top-down approach, focusing on differences by region and forest type and measuring total transpiration from a forest stand. Remote sensing studies can assess vegetation water content at a landscape scale (Konings *et al*. 2021) and may provide insights on whether ecosystems and tree species are matched to climatic conditions that best suit them, but only at a coarse scale. Nevertheless, these measurements are valuable in that they can contribute to immediate assessments for management. Data such as expected water yield and quality from catchments can inform management decisions across many disciplines, from fisheries management to stream restoration to municipal water needs. Other top-down study methods construct regional models to predict transpiration (Li *et al.* 2021). Oftentimes, the main goal of such investigations is to understand the effects of drought stress on well-understood tree stands (Li *et al.* 2021).

By contrast, bottom-up approaches to studying transpiration measure individual plants. Methods vary, often constrained by plant size, but include sap flow measurements (Smith and

Allen 1996, Poyatos *et al.* 2016, Sabater *et al.* 2023) or, if plants are small enough, contained chamber measurements (Stannard 1988). Bottom-up approaches to transpiration studies focus predominately on species differentiation and comparison rather than on populations within species (Thomsen *et al.* 2013, Matheny *et al.* 2014, Matheny *et al.* 2015, Matheny *et al.* 2016, Pappas *et al.* 2018). A few population-level transpiration studies do exist. For example, European beech (*Fagus sylvatica*) demonstrated population-level differentiation in photosynthetic rates and efficiency under water stress (Zhang and Marshall 1994, Li 2000, Bowden and Bauerle 2008, Sánchez-Gómez *et al.* 2013). Sánchez-Gómez (2013) completed their study under controlled settings in a greenhouse and specifically focused on drought responses across populations, covering a wide range of traits affected by water stress but not transpiration per se. In contrast, the experiment described herein allows comparisons to be made both between two species and between two populations that were sampled from each of these species. These trees were grown in a common garden, and this experimental design emphasizes tree water use when all measured individuals experience the same natural weather conditions, allowing for direct comparison of transpiration capacity.

How We Can Manage Forests to Facilitate Change

Emerging management solutions include the strategy of assisted migration, or the managed movement of species and ecotypes to facilitate or augment natural range expansion in response to climate change (Fig. 1A, Vitt *et al.* 2010). Assisted migration has been increasingly recommended in scientific literature for the rescue of ecological communities (Aitken *et al*. 2008, Ste-Marie *et al.* 2011, Leech *et al.* 2011, Way and Montgomery 2015).

Assisted migration is most often thought of in terms of assisted species migration: moving a species into an entirely novel location (Fig. 1A). However, assisted migration need not occur over vast distances. A less drastic approach is assisted range expansion, which establishes species just past present borders of their ranges in an effort to speed natural range shifts (Fig. 1B). Assisted migration may even be deployed within an active species range. Population relocation within species ranges (Fig. 1C) could maintain range expanses and preserve important genotypes by shifting populations from the trailing edges of ranges to locations within or just ahead of leading range boundaries, matching local adaptations back to the shifted climate conditions that suit them with relatively little ecological risk (Williams and Dumroese 2013). Most species cover broad geographical ranges and develop distinctive local adaptations in response to climate and many other factors (Hiesey and Nobs 1982, Kawecki and Ebert 2004, Savolainen *et al.* 2007, Alberto *et al.* 2013). Assisted population migration harnesses local adaptations of populations to climate and offers a management strategy to maintain forest cover on a changing planet.

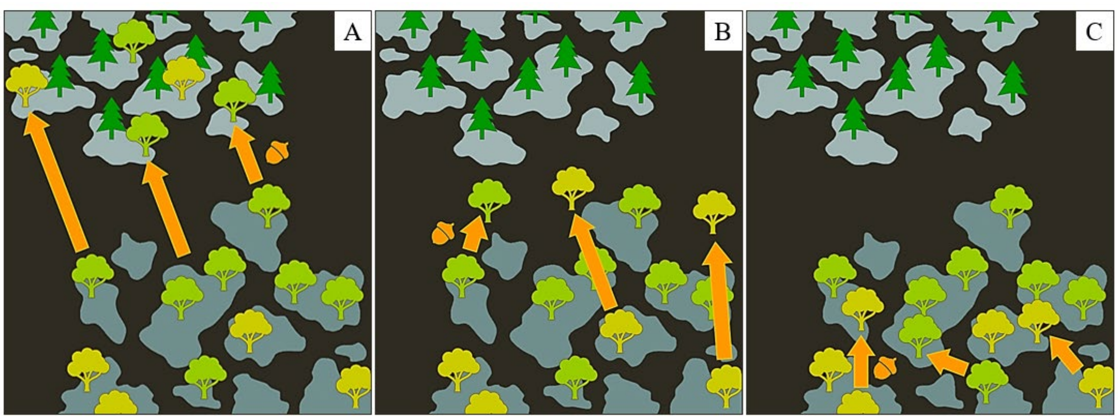

Figure 1. Modes of Assisted Migration. A: Assisted Species Migration, wherein a species is introduced into an entirely novel location. B: Assisted Range Expansion, wherein a species is introduced just past the borders of its historic or present range. C: Assisted Population Migration, wherein populations of a species are relocated within the species range.

Potential Consequences of Assisted Migration for Forest Water Dynamics

As a strategy for ecological management, assisted migration has inspired much debate. Much of the literature on assisted migration is framed as conceptual reviews (Sáenz-Romero *et al.* 2007, Pedlar *et al.* 2012, Williams and Dumroese 2013, Gömöry *et al.* 2020) and ethical analyses (Aubin *et al.* 2011, Palik *et al.* 2022). There are also proposed procedures and guidelines for when and how to use assisted migration in management (Beardmore and Winder 2011, Koralewski *et al.* 2015). For example, a decision framework that utilizes three conditions—a substantiated lag between the subject and climate, a record of proven biological impacts, and the existence of robust model projections—to assess whether assisted migration is an appropriate strategy for the problem at hand (Gray *et al.* 2011). The authors created a case study for *Populus tremuloides* in Canada that prescribed assisted migration based on field studies, vegetation indexes, and bioclimate modelling, with the caveat that their findings have a constrained effectiveness window of about 20 years.

In North America and Mexico, experiments in assisted forest migration have examined winter hardiness (Schreiber *et al.* 2013, Grady *et al.* 2015), elevation and moisture gradients (García-Hernández *et al.* 2019), maladaptations due to soil nutrients and fungal associations (Kranabetter *et al.* 2015), and many other responses of plants grown in novel locations and/or common garden experiments (Sáenz-Romero *et al.* 2021, Park and Rodgers 2023). Some previous experimental research on assisted migration in trees has focused on riparian or commercially valuable species with questions such as whether relocation can be successful or how to properly go about assisted migration (Beardmore and Winder 2011, Grady *et al.* 2011, Grady *et al.* 2015, Conroy *et al.* 2019, Zeng *et al.* 2022). However, few experimental studies focus on the watershed impacts of assisted migration of tree species.

To examine water resource impacts of climate shifts and forest disturbance, one study modeled the progression of four montane, forested watersheds in projected climate change scenarios (Buma and Livneh 2015). Accounting for species die-offs and range fluctuations, the simulations compared the effects of only natural migration responses versus active management that either replanted species that remained climatologically compatible or introduced novel tree species that suited the altered climate. This study showed that active management did significantly alter water resources to have increased water quality and yield in some cases, but the extent of its effectiveness varied across different types of hydrologic systems. This suggests that the use of assisted migration to change forest composition can affect evapotranspiration, an outcome that could inform water resource management (Buma and Livneh 2015). Putting assisted migration techniques into practice and testing the consequences for forest water dynamics will determine if tree water use should be considered when choosing tree stock to plant into forested watersheds.

Goals and Objectives

In this study, I investigated the climatic suitability and hydrologic differentiation of two populations from contrasting climates of two native tree species that are predicted to thrive in northern Minnesota with climate change, *Q. macrocarpa* and *Q. rubra* (Matthews *et al.* 2018, Moser *et al.* 2019, Reich *et al.* 2022, Faluyi and Irmak 2023). I measured and analyzed survival, growth traits, phenology, and transpiration in a common garden experiment that had been planted seven years before the start of my study. Each species was represented by two populations: a local, northern population in MN DNR seed zone 104 and a distal, southern population from MN DNR seed zone 105, directly south of zone 104 (Fig. 3). Climate shifts to date have resulted in a northeasterly shift of climate conditions once found 240 km to the south of the planting site.

With this experimental design, I developed the following four hypotheses.

H1) I hypothesized that a mismatch between local tree adaptations and climate that they currently experience in northern populations would confer survival and growth advantages to southern populations. High survivorship, advanced height and stem thickness, and low pest susceptibility are indicators of which population was better adapted to the shifted local climate.

H2) I hypothesized that the southern population phenology would be better aligned with seasonal climate compared to northern population phenology. The population with superior phenological suitability would take better advantage of the available growing season by developing leaves as soon as possible without incurring damage via lingering spring frosts and retaining them as long as possible without suffering overmuch from oncoming winter freezes.

H3) I expected that water use trends through the growing season would vary between species. Given that *Quercus macrocarpa* and *Quercus rubra* differ in their customary habitats and resource gathering strategies, they have distinct growth forms and biomass allocation (see the species entries in *Study Species*). Therefore, I anticipated variation in physiological traits linked to water use.

H4) I expected that local adaptations would lead to observable population differences in transpiration for both species. I could find no previous work on population-level differentiation in transpiration for my two species. I hypothesized that southern populations would transpire less, and thus use less water, than northern populations. If so, southern populations would demonstrate slower rates of water loss during field measurements of transpiration. In addition to direct comparisons, conducting three measurements per growing season would allow for the observation of transpiration trends over time.

Methods

Study Species

The species chosen for this study are *Quercus macrocarpa* (bur oak) and *Quercus rubra* (northern red oak). The common garden site falls within the northern reaches of the current geographic ranges for both species (Fig. 2). Their selection for these plantings is therefore considered assisted population migration (Ste-Marie *et al.* 2011). Climate and vegetation models indicate that these species will likely increase in abundance due to climate change (Duveneck *et al.* 2014).

Quercus macrocarpa (Michx.)

The current range of *Q. macrocarpa* is the broadest of North American *Quercus* species and extends north-south from the Gulf of Mexico in Texas into southern Canada and east-west from the Dakotas to Maine (Fig. 2A, Gucker 2011). However, it should be noted that *Q. macrocarpa's* range 50 years ago did not include the region directly beside the Great Lakes (Gucker 2011). *Q. macrocarpa* is considered a savannah species. It is drought and fire tolerant but inundation intolerant. Prior to European settlement, there were likely more than 16.5 million hectares (165,000 square kilometers) containing this species, but as of the year 2000, less than half of those areas remain (Gucker 2011). It tolerates a wide range of climatic and soil conditions including wet and dry habitat types. It is also one of the most cold-tolerant of the North American *Quercus* species. Some portions of its range receive as little as 380 millimeters of annual precipitation, while other areas may exceed 1,270 millimeters per year. Its growing season varies from 100-190 days. Its growth form varies based on its habitat. In alluvial floodplains, it may reach 50 meters in height, but in harsher areas, it may mature at only six meters. With space, it tends to spread its boughs horizontally. Its seedlings develop deep and extensive root systems quickly, and mature trees have widespread lateral roots and deep taproots, which has implications for soil water uptake and, by extension, transpiration. It is a long-lived species; some of the oldest known individuals are 300 to 400 years old, and its ideal seed-bearing age is 75 to 150 years old (Gucker 2001). According to the United States Forest Service's Climate Change Atlas (Matthews *et al.* 2018), *Q. macrocarpa* is predicted to be neutrally or somewhat beneficially affected by climate change. Several models predict that its range may expand, particularly in Minnesota (Matthews *et al.* 2018, Moser *et al.* 2019, Reich *et al.* 2022, Faluyi and Irmak 2023).

Quercus rubra (L.)

Q. rubra occurs across the eastern United states, extending north-south from Louisiana to southern Canada and east-west from eastern Kansas and Oklahoma to Maine (Fig. 2B, Tirmenstein 1991). It is considered a valuable hardwood species and is known for fire tolerance. It is not commonly associated with *Q. macrocarpa* in its vegetative communities. Its common habitat types include mesic forests, sandy and rocky open areas, and the edges of floodplains. The driest spots in its range have an average annual precipitation of 760 millimeters, and its wettest occupied areas receive an average annual precipitation of 2,030 millimeters. Its growing season varies across its range, from 100 days to 220 days, which is slightly longer than *Q. macrocarpa*. It is considered one of the tallest and fastest growing oaks and may be more than 50 meters tall. It has a more vertical growth form than *Q. macrocarpa*, and its root systems have deep lateral roots and a strong taproot. It is known for seedling dieback and vigorous resprouting. Seed production usually peaks after 50 years of age (Tirmenstein 1991). Like *Q. macrocarpa*, the Climate Change Atlas predicts that *Q. rubra* will thrive under climate conditions, as it is adaptable and hardy.

Population differentiation within species

Genetic studies have found low genetic diversification in *Q. macrocarpa* populations across a variety of landscapes, though there was significant, if not substantial, genetic differentiation in several cases where populations were isolated (Schnabel and Hamrick 1990, Craft and Ashley 2006, Craft and Ashley 2007, Kittelson *et al.* 2009). A study in the Minnesota River Valley found that younger cohorts of *Q. macrocarpa* had greater genetic diversity than older conspecific cohorts, indicating increased population isolation and diminished gene flow in the last 100 years (Kittelson *et al.* 2009).

The economic importance of *Q. rubra* has led to more intensive study and several introductions into nonnative territories around the world. Genetic studies in its natural range in North America have found relatively low genetic differentiation among populations (Magni *et al.* 2005, Borkowski *et al.* 2017), and studies of introduced populations seem to have stemmed from a narrow genotypic foundation that has since diversified in isolation (Daubree and Kremer 1993, Pettenkofer *et al.* 2019). In 2017, Khodwekar and Gailing found that gene introgression in *Q. rubra* is heavily impacted by environmental selection, particularly the proximity of conspecific populations, soil water holding capacity, and soil nutrient content (Khodwekar and Gailing 2017).

Despite low general genetic diversity in the study species, local adaptations at small geographical ranges are possible. In a reciprocal transplant study, *Q. rubra* seedlings demonstrated significant herbivory resistance when planted in the same location as their maternal line, indicating inherited, local adaptations (Sork *et al.* 1993). A 2023 study planted *Q. rubra* seedlings from twelve populations throughout the species range into two common gardens; southern populations grew at a faster rate than northern when planted in the central portion of the range, while the reverse was true in the northern planting site (Lindback *et al.* 2023). If similar population-level differentiation can be detected in plant growth traits for *Q. macrocarpa* and *Q.*

rubra in this study, there is possible that occurs in a broader array of species, indicating that assisted population migration is a viable forest management strategy in Minnesota forests.

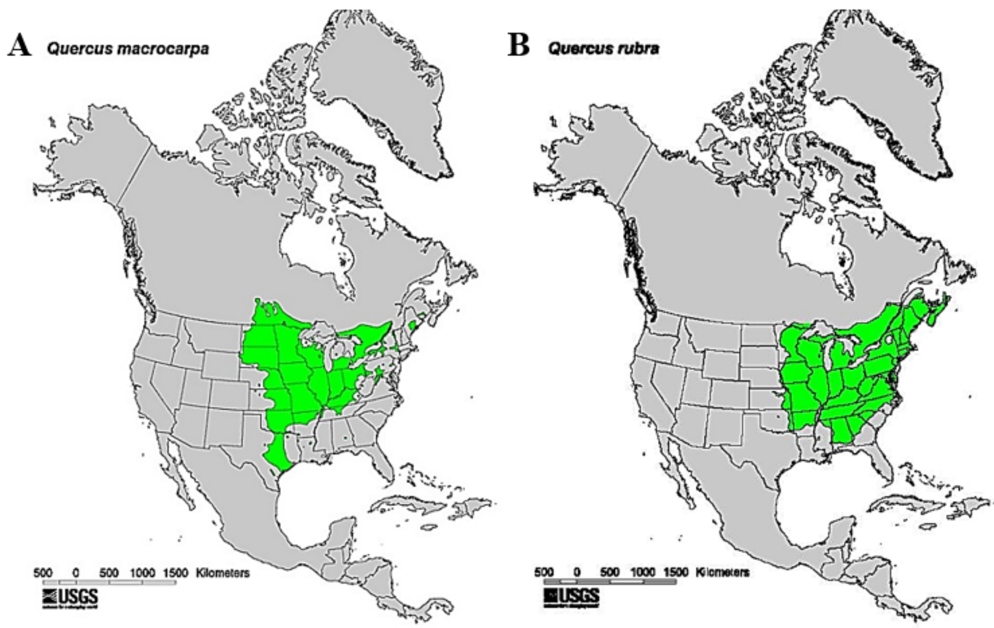

Figure 2. Geographic ranges for the two study species, 1971. **A:** range of *Q. macrocarpa* in North America. **B:** Range of *Q. rubra* in North America. © Elbert Little, United States Forest Service, 1971.

Study Area

This study was conducted in the Stewart River basin, which drains 68.7 km^2 into Lake Superior (Lake Co., MN) (Fig. 1). Land cover of the Stewart River is 83% deciduous forest, mixed forest, or forested wetlands, while less than 5% is used for agriculture or other human development (Axler *et al.* 2009). Soils are predominantly sandy loam Alfisols with abundant cobble sized clasts over basalt and basaltic andesite bedrock. The average slope for the basin is 9.5%. The forest cover is classified as boreal mixed (MN forest type FDn43), which is composed of mostly *Populus tremuloides, Betula papyrifera, Abies balsamea, Picea glauca, Pinus strobus,* and *Acer rubrum* (MN DNR 2024b). The average annual temperature is -4.5°C and mean annual precipitation is 734 mm (1895-2022) (MN DNR 2024a). Regional climate has warmed +1.0°C to 1.9°C in the last century (Melillo *et al.* 2014), particularly in fall and winter, and precipitation is shifting towards drought-and-deluge patterns with increasingly extreme events (Liess *et al.* 2022, MN DNR 2024a). Projections indicate that these trends will continue throughout the century (MN DNR 2024a).

This research was conducted on two adjacent harvested areas (considered blocks) that are part of a larger experiment by the Nature Conservancy and the University of Minnesota Duluth to evaluated assisted migration as a tool to mitigate the effects of climate change on forest communities (Etterson *et al.* 2020). In 2013, the blocks were harvested and planted with species predicted to thrive in future climates. This study focused on two of the three species that were planted at that time, *Quercus macrocarpa* and *Quercus rubra;* the trees were 10 years old at the time of this study. To test the potential effectiveness of assisted migration, roughly 160 seedlings of a local population (MN DNR Seed Zone 104, Fig. 3) and a more southern population (MN DNR Seed Zone 105) were planted into each block (2 blocks x 2 species x 2 population per species x 20 seedlings = approximately 160 total). Trees from seed zone 104 were considered the northern population, while trees from seed zone 105 were considered the southern population. With two species and two populations each, there were four total tree types compared (Fig. 4).

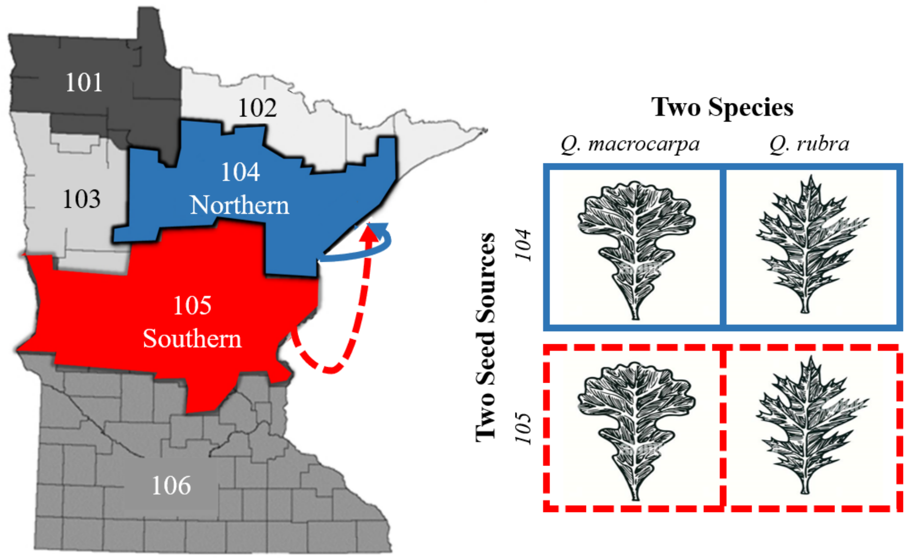

Figure 3. Planting design and tree type matrix. Two species, *Q. macrocarpa* (Bur oak) and *Q. rubra* (Northern red oak) and two seed sources (104 from the north, 105 from the south) combine to make four tree types. Seed zones were dictated by MN DNR policy. The study was designed and implemented in 2013 for the Adaptive Forestry in the Great Northwoods Project.

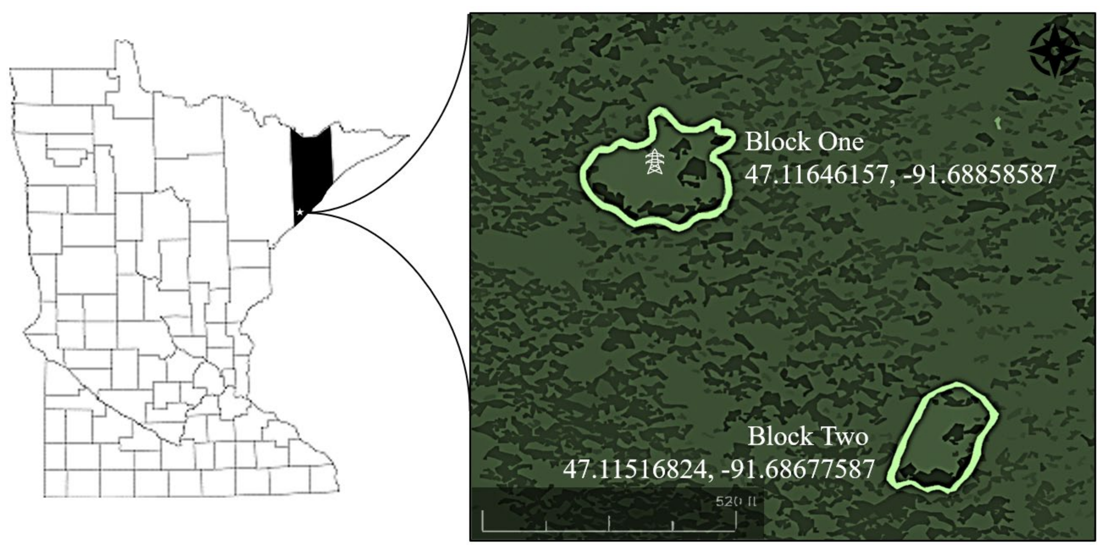

Figure 4. Locations and coordinates of the two study blocks. The weather station was located at Block 1 and is indicated by the tower symbol. Blocks were 10 km north of Two Harbors, MN.

Environmental Measurements

To monitor environmental conditions during the growing season, a weather station (HOBO RX3000 Remote Monitoring Station) was installed at Block 1 (Fig. 1, Appendix Fig. A3). Data were collected every 15 minutes between the first and last day of plant measurements and included: 1) air temperature and relative humidity (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger), 2) photosynthetically available light (Photosynthetic Light Smart Sensor), 3) wind speed and direction S-WSB-M003 Wind Speed Smart Sensor), and 4) rainfall (S-RGF-M002 Davis 0.02 mm Rain Gauge Smart Sensor) (all from Onset Computer Corporation, Bourne, MA, USA). The growing season was partitioned into three seasonal phases (Table 1) during the 2020 and 2021 growing seasons. For analysis, weather station data were downloaded and aggregated into daily values on a biweekly basis, except during transpiration measurements.

Table 1. Seasonal breakdown of weather station data. The summer period took place over the same dates each year. The spring and fall seasons were longer in 2021 than 2020.

Season	2020	2021
Spring	May 20th – June 20th	May 1st – June 20th
Summer	June 21st – Sept 20th	
Fall	Sept 21st – Nov 14th	Sept 21st – Dec 4th

Plant Measurements

Experimental plants were GPS tagged in 2020, and survival, height, and basal stem diameter were recorded at the start and end of the growing season thereafter. Height (cm) was measured as the distance from the soil line to the tree apical meristem and basal stem diameter (cm) was measured at the trunk-soil interface. Every ten days throughout the growing seasons, shoot growth was assessed by measuring the distance in millimeters from the current-year bud scar to the base of the apical bud on the same twig for each tree.

Phenological measurements were collected in spring and fall. Spring phenological stages were recorded every three to five days as follows: 1) all buds tightly closed, 2) first bud scales separating, 3) first immature leaf or leaves visible, 4) first mature leaf or leaves present, 5) all leaves mature leaves, or full canopy attainment (Appendix Fig. A1, Rich *et al.* 2015, Sendall *et al.* 2015, Etterson *et al.* 2020). Fall phenology was tracked using three stages, where leaf senescence means that the leaves are abscised or no longer green: 1) 33% senescence, 2) 66% senescence, and 3) 100% senescence. In 2020, the date of the final field site visit occurred before all trees were 100% senesced and was declared the 100% senescence date for all trees. In 2021, field site visitations continued until all trees were 100% senesced. The days of canopy retention was calculated as the number of days between budburst and 100% senescence. Acorn development was noted, but no seeds were collected.

After transpiration measurements, the number of leaves was counted for each tree and the uppermost fully expanded (or second highest in case of damage) leaf was collected to measure for specific leaf area (SLA, cm^2/g). Leaves were pressed in the field, dried, weighed, scanned, and measured for area (cm^2) using WinFOLIA PRO (Regent Instruments, Inc.).

There was significant insect parasitism on the study trees. Most notably, gall-forming wasps of the family *Cynipidae* infested both study blocks. In both years of monitoring, there was springtime and end-of-summer gall formation in leaf tissues and buds. These predominately took the form of 2.5 to 5 cm diameter spheres, commonly called oak apple galls. There were also rare occasions of pocket gall formation credited to larval flies of the family *Cecidomyiidae*. The presence and number of galls were recorded on each survey visit.

Transpiration Measurements

For two field seasons, transpiration was measured on 139 trees three times per year (early July, late July, and early August). Each round of measurements occurred over a seven-day period (Table 2). Transpiration measurements were conducted using a portable rapid evapotranspiration chamber that was modified from the method developed by Stannard (1988).

The chamber was built with two configurations to accommodate trees of different heights ("tall," 3-m, $V = 0.839$ m^3; "short," 1.75-m, $V = 0.543$ m^3**,** Fig. 4). It had PVC framing and was skinned with four-year ultraviolet-treated, anti-condensation, heavy-duty polyethylene. Within the chamber, airflow was maintained using two (short) or four (tall) brushless fans. Temperature, relative humidity (RH), and light intensity were logged every 10 seconds within the chamber (HOBO Pro v2 Temperature/Relative Humidity data logger, HOBO Pendant Temperature/Light sensor, Onset Computer Corporation, Bourne, MA, USA).

Table 2. Transpiration sampling schedule for 2020 and 2021.

Round	**Start Date**	**End Date**	**Mean Air Temperature (°C) (prior 7 days)**	**Total Precipitation (mm) (prior 7 days)**	**Sample Size**
1	7/20/2020	7/29/2020	20.06	88.11	139
2	8/5/2020	8/12/2020	17.25	0.25	139
3	8/19/2020	8/25/2020	18.30	3.29	139
1	7/21/2021	8/4/2021	19.91	0.00	139
2	8/9/2021	8/17/2021	19.81	26.63	139
3	8/21/2021	9/4/2021	22.37	0.00	139

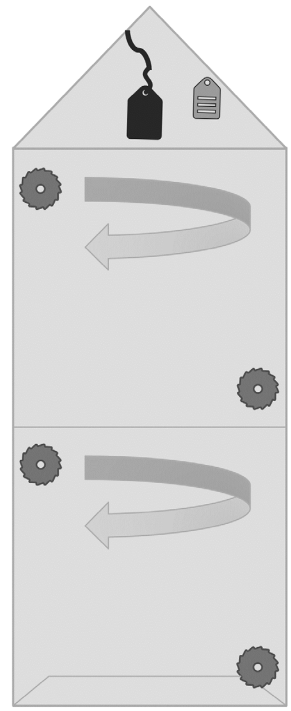

Figure 3. Diagram of the portable rapid evapotranspiration chamber. Chamber components include PVC framing, polyethylene skinning, four brushless fans in opposite corners for airflow, and two sensors to track environmental conditions inside. The base was a ground tarp fitted with a collar for tree trunks and Velcro to attach to the chamber's PVC frame. The chamber skin had flaps that extended to the sides on the ground, which were weighted against the wide margin of the ground tarp to form an airtight seal.

Trees were sorted by height to determine the appropriate chamber size and then measured in a consistent randomized order. All "tall" chamber measurements were collected before making "short" chamber measurements. Measurements were conducted between 12:00 and 16:00 on non-rainy days after >90% of the study trees had attained full canopies. A ground tarp was fitted around the base of each tree to eliminate evaporation from nontarget vegetation (Appendix Fig. A4). The chamber was positioned over the tree and quickly closed to an airtight position. Internal water vapor concentrations were monitored in 10 second intervals until RH stabilized (approximately five minutes per tree, maximum 31 minutes). Additional two-minute increments of measurement were added as needed until RH stabilized.

Percent volumetric soil moisture was measured directly after each transpiration measurement in four cardinal directions beneath the tree canopy (HydroSense TDR probe, Campbell Scientific, Inc., Logan, UT, USA). Soil moisture measurements were attempted directly adjacent to tree trunks initially and adjusted outwards if the soil was too rocky by the tree. The four soil moisture measurements per tree were averaged for subsequent analyses.

Transpiration Rate Conversions

Transpiration conversions (Stannard 1988) began by calculating saturation vapor pressure (e^*, Pa) for each timestep, then converting to absolute humidity (Eqn. 1), and finally to instantaneous transpiration rate (Eqn. 2):

$$AH = \left(\frac{1000 \times RH \times e^*}{461.5 \times (T + 273.15)}\right) \qquad \text{(Eqn. 1)}$$

where AH is absolute humidity (g m^{-3}), RH is relative humidity, e^* is the saturation vapor pressure (Pa), and T is air temperature (°C). The gas constant for water vapor is 461.5 J kg K^{-1}.

AH was plotted against time (10 second increments, Fig. 6) to identify the timestep in which the greatest change in absolute humidity occurred (M, g m^{-3} s^{-1}), which corresponded with the tree's instantaneous transpiration rate. M was then converted to the representative transpiration rate (T, mm h^{-1}) (Eqn. 2):

$$T = 86.4 \times \left(\frac{MVC}{A}\right) \qquad \text{(Eqn. 2)}$$

where V is the chamber volume (m^3), A is the chamber basal area (m^2), C is a unitless chamber calibration factor for chamber hydrophobicity (assumed to be completely hydrophobic, C=1), and 86.4 is a unitless conversion factor.

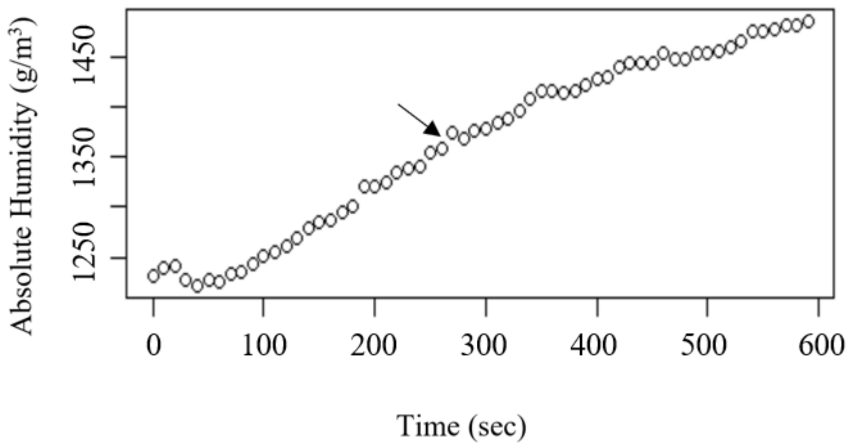

Figure 4. An example plot of absolute humidity vs. time. Used to calculate the instantaneous transpiration rates of each tree. The arrow indicates the 10-second segment where the greatest change in absolute humidity, analogous to the fastest transpiration rate, occurred.

Stream Measurements

To characterize streamflow for subsequent modeling exercises, a stream gauging station was maintained downstream of the study field site in the Stewart River (47° 5'52.66" N, 91°42'54.45" W) following United States Geological Survey protocols (USGS, Stone 2012). From early summer (May-June) to late autumn (October-December), the water level was measured every 15 minutes (Onset HOBO U20-001-01 Water Level Data Logger, Bourne, MA, USA).

To capture the range of discharge values, cross sections were taken of the river at the stream gauge point at least ten times during each growing season. Rebar segments on the channel banks were used as reference points. Cross section measurements were derived from the USGS's procedures for the velocity-area method (Rantz 1982, Turnipseed and Sauer 2010). The river's cross section was divided into 30 fixed segments, each sized to hold no more than 5% of the river's total discharge in any subsection. The area of the stream channel that was deepest and had the fastest flow velocity had the narrowest segments.

To conduct a cross section, a meter tape was drawn across the channel and anchored on the rebar posts. First, depths of the bank profile and water were recorded at each segment. Then water velocity was measured at each segment using a Swoffer Model 2100 Flowmeter. The turbine was held in place at 60% depth for three 40 second rounds to get the average velocity for each segment. After the fact, the area of each subsection was calculated and multiplied by the average velocity to get the volume of water passing through that segment at the time of measurement. The volume of all segments was summed to get the total discharge in cubic meters per second. Publicly available records from the weather station at the airport in Two Harbors, MN were used to correct for barometric pressure, and total discharge was then plotted against water depth, or stage. The relationship was characterized with a best-fit logarithmic function to create a stream rating curve (Appendix Fig. A2).

Data Analysis

To determine if the plant traits of the species and their northern and southern populations differed, I analyzed the following data: survival, height and basal diameter at the beginning and end of growing season, the date of budburst, the date of full canopy, and the date of each senescence stage, the duration of leaf retention, number of leaves, presence of acorns, and presence of leaf and bud galls (JMP Pro 16.2.0 by SAS Institute, Inc., 2021). Survival and presence of galls were analyzed with a Generalized Linear Model using a logit link assuming a binomial distribution. All continuous data were analyzed to detect population and block effects by Analysis of Variance with variables block and population and block x population. Species effects were analyzed by Analysis of Variance with variables year, block, species, and the combined effects of year x block, year x species, and block x species. Transpiration rates were also analyzed by round with this analysis.

Differences in transpiration rates between species and populations were tested using a mixed model with an unstructured repeated measures matrix, with measurement rounds as the repeated effect. Transpiration rates were then analyzed for each round by year and species with a generalized linear mixed model. These models included a several covariates. *Number of leaves* was used to represent the canopy fullness, and by extension, the overall size, of each of the trees. In absence of height and other growth trait factors, this provided a comparison of stature. *Specific leaf area (SLA)* is the ratio of surface area to mass of leaves and indicates leaf thickness. This trait has implications for tree water use and plasticity in response to drought. Since field measurement rounds took multiple days to complete each time, the *day of the year* was used to account for variation from day to day. Field measurements also took place over the course of afternoons, so *time of day* accounted for any resulting variation. Trees draw water through their roots to conduct transpiration, so the *average volumetric soil moisture* was measured to quantify the amount of water available for uptake. Transpiration and photosynthesis are intrinsically linked processes, so the amount of *photosynthetically available radiation (LUX)* was measured as light at the start of a measurement. The *vapor pressure deficit (VPD)* at the start of each measurement was also measured, as it creates the saturation gradient that moves water from the soil, through a transpiring plant, and into the air.

Data were transformed as necessary to meet the assumptions of constant variance. Log log-transformed plant trait data included: number of leaves (2021), SLA (2020), date of budburst, and date of full canopy (2021). Square-root-transformed data included: date of full canopy (2020). The 2020 measurement of SLA for tree 332 was excluded as an outlier by error. Transpiration rates for both the repeated measures analysis and the by-round analysis were log-transformed. Transformations were only used if they significantly improved the normality of the residuals. Outliers were only excluded if they resulted from measurement error rather than natural variation.

Modeling Potential Impacts of Tree Type on Forest Hydrology

I used a hydrologic mass-balance approach to model potential watershed-scale hydrologic impacts of evapotranspiration differences between the four tree types (Eqn. 3). For this analysis, I made the following simplifying assumptions: 100% of the watershed area was forested with one of the four tree types, precipitation was the primary input of water to the watershed, and stream discharge and forest transpiration were the primary water exports.

$$P = Q + ET \qquad \text{(Eqn. 3)}$$

where P is precipitation, Q is discharge, and ET is evapotranspiration, all in mm.

Precipitation for each year was the total amount recorded by the weather station during the period of measurements (2020: May to November; 2021: April to December). Discharge records from the stream gauging station were taken for the same measurement period in each year, but sensor error prevented accurate measurements in 2021. The 2020 discharge records were converted from cubic meters per second to total area-weighted flow (mm). The potential evapotranspiration (PET) was first calculated using the water balance method (Eqn. 5).

$$PET = P - Q \qquad \text{(Eqn. 4)}$$

The PET determined via Eqn. 5 was 338 mm. This value was compared to PET calculated via the Thornthwaite method (Thornthwaite 1948; Eqn. 6) as a test on the effectiveness of the water balance approach:

$$PET = 1.6 \times \frac{(10 \times T_a)^a}{I} \qquad \text{(Eqn. 5)}$$

where PET is in centimeters over a month, T_a is the mean monthly air temperature (°C), I is an annual heat index based on a 30-day month and 12-hour day, and a is calculated based off of I (Eqn. 7).

$$a = 0.49 + (0.00179 \times I) + (0.000077 \times I^2) + (0.000000675 \times I^3) \qquad \text{(Eqn. 6)}$$

PET was calculated monthly and summed across the measurement period. The cumulative PET determined by the Thornthwaite method and the water balance method were considered congruent (267 mm).

For modeling purposes, transpiration measurements for each tree type were scaled up to encompass the full area of the Stewart River watershed and used to model potential total discharge for the stream per year. The modelled area-weighted discharge was compared to the observed area-weighted discharge measured in the Stewart River. Departures in the modelled discharge from the observed discharge were used to infer differences watershed-level water use across the four study tree types. Predicted changes in discharge were based on the following hydrologic mass balance model:

$$Predicted\ Q = P - Predicted\ ET \qquad \text{(Eqn. 7)}$$

The *Predicted ET* was scaled from the instantaneous transpiration measurements as:

$$Predicted\ ET = ET_x\ \times 10\ \times CR_x \quad \text{(Eqn. 8)}$$

where ET_x is a tree type's average, minimum, or maximum ET rate (mm h^{-1}) as measured in the field during a transpiration measurement round or for the year, 10 is the average number of assumed daylight hours during the study period, and CR_x is the average days of canopy retention for that tree type that year (Fig. 10).

The predicted discharges (Eqn. 7) were compared to the measured discharge of the Stewart River to quantitate the difference between modelled and observed discharges:

$$Predicted\ Q - Measured\ Q = Discharge\ Departures \quad \text{(Eqn. 9)}$$

Differences in potential discharge were calculated for the average, minimum, and maximum transpiration rates for each tree type. Models were constructed separately for each round of measurements and for the average transpiration rates of each tree type over all three rounds. Negative departure values indicate that more water would be lost than is available at scale. Forest cover with that transpiration rate would create a water deficit. Positive departure values indicate that there would be a surplus of water with that level of water use.

The model was applied to 2020 data to showcase differences in potential watershed hydrologic impacts by population and species. These potential impacts were displayed by predicted discharge departure (Table 9), calculated from the difference in annual precipitation (364 mm in 2020) and predicted transpiration (Table 8).

Results

Climate Differences Between Years

During this two-year study, climate variables differed between years (Table 3; Fig. 7). Compared to 2020, there were more weather extremes in 2021, as indicated by higher daily maximum and lower minimum temperatures (2020: 34.7 °C maximum, -13.4 °C minimum; 2021: 38.4 °C maximum, -16.3 °C minimum), and more seasonal precipitation (2020: 364 mm; 2021: 421 mm). Overall, there was almost 16% more growing season precipitation in 2021 compared to 2020, but it occurred at more irregular intervals. In 2020, only one eight-day stretch separated rainfall events all season. In 2021, three periods without rain exceeded ten days, and two of those lasted 15 days. Additionally, in 2021, there were several spring overnight freezing incidents in late May and early June (Fig. 7 B, see arrows) that caused damage to nascent canopy formation (*personal observation*).

Table 3. Average temperature and precipitation measurements at the field site. Monitored by a weather station at Block 1, and measurement periods lasted from the first day of phenology observations (tracking bud burst) to the last (tracking 100% senescence), covering the length of the growing season.

Climate Variable	2020	2021
Minimum Temperature (°C)	-13.4	-16.3
Maximum Temperature (°C)	34.7	38.4
Mean Annual Temperature (°C)	13.2	13
Spring Precipitation (mm)	48.2	82.9
Summer Precipitation (mm)	265.4	228.8
Fall Precipitation (mm)	51.1	109.6
Total Precipitation (mm)	364	421

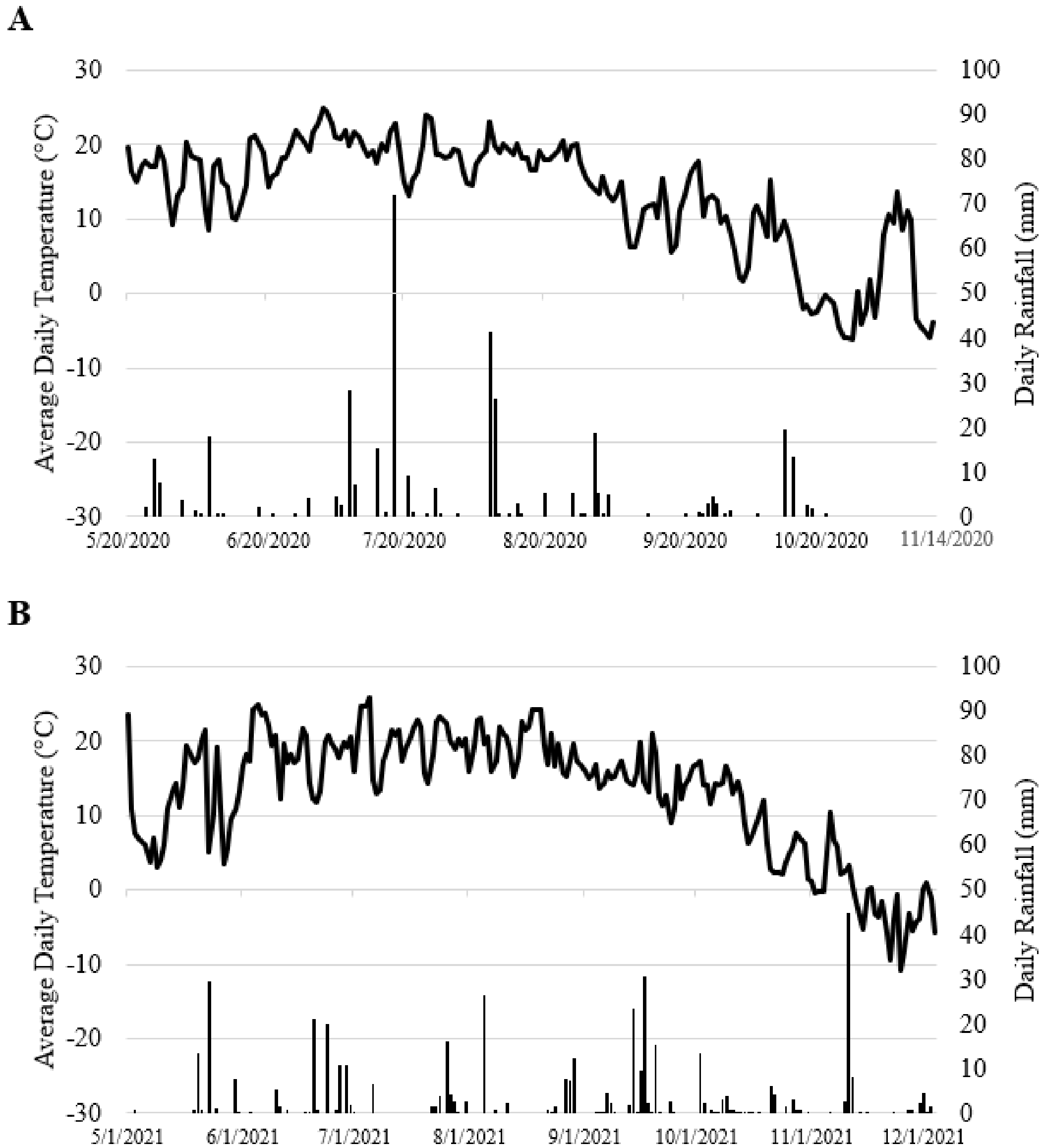

Figure 5. Average daily temperatures (C) and cumulative daily rainfall (mm). **A)** 2020 and **B)** 2021. Temperatures are shown by the upper, continuous line with the corresponding left axis. Cumulative precipitation is shown in the lower hyetograph with the corresponding right axis. The timing of the two spring overnight freezing events in 2021 are marked with arrows.

Model with Two-Way Interactions

To determine whether the full data set should be analyzed in subsets by species and year, trait data was analyzed using a model that included the main effects of year, species, block, and their two-way interactions, as well as population nested within species (Table 4). Overall, this analysis showed that more than half of the plant traits differed between years (65% of traits differed), species (53%), and blocks. Moreover, numerous two-way interactions were significant between year and species (29%), year and block (47%) and species and block (24%). Population differences within species were also frequently significant (71%). Thus, given the complexity of the interactions, the data were broken into subsets by year and species to better comprehend population differences and their interaction with the blocks (see *Population Differences*, below). Differences between years, species, and blocks are briefly described below (Table 4).

Year, Species, and Block Differences

Survival only differed marginally between species. It did not differ between years, but it differed between blocks (Table 4). In terms growth traits, *Quercus rubra* was taller than *Quercus macrocarpa* in both years. The plants grew between 2020-2021 as is evident by significant year effects for spring stem diameter and seasonal leaf production (Fig. 8 Ai). On average, *Q. rubra* produced ~25% more leaves than *Q. macrocarpa*. SLA differed significantly between species and was also significant for all pair-wise interactions between year, species, and block. *Q. rubra* produced leaves that were ~14% thinner (higher SLA) compared to *Q. macrocarpa* (Fig. 8 Bi), although this difference also depended upon the year and the block in complex ways. The presence of galls also differed between years, species, and blocks. Galls were 1.7-fold more abundant for *Q. rubra* than *Q. macrocarpa* (Fig. 8 Ci).

Both spring and fall phenology differed substantially between years, species, blocks, and the interactions between these factors (Table 4). Bud burst was eleven days earlier in 2021 compared to 2022; *Q. macrocarpa* was earlier than *Q. rubra* but the magnitude of the difference depended upon year. Correspondingly, the date that the plants obtained a full mature canopy also differed significantly by year, species, block and the interaction between block and year. Full canopy was attained earlier in 2020 compared to 2021. The phenology of canopy development of *Q. rubra* was slightly more rapid (3%) than that of *Q, macrocarpa.* In the fall, the timing of canopy loss also differed between years, species, and blocks—yearly differences were significant at 33% and 100% senescence, species differences were significant at 100% senescence (Fig. 8Di), and block differences were significant at all three stages of fall phenology. The fall phenological differences were also affected by the interaction between year and block. Taking both spring and fall phenology into account, the total number of days that the saplings had leaves during the growing season also differed between years, species, and blocks. The number of days of leaf retention was 26 days longer in 2021 compared to 2020. *Q. rubra* maintained its leaves for 2.8 more days than *Q. macrocarpa* (Fig. 8Fi).

Annual differences in transpiration were highly significant for each of the three timeframes during the growing season (Round One: July; Round Two: early August; Round Three: late August) (Table 3). Species, however, did not differ as a main effect at any timeframe. Transpiration measurements in rounds one and three were affected by the interactions between block and both year and species. All three two-way interactions were significant for the round two measurement.

Population Differences - Quercus macrocarpa

Fitness: Survival of the northern and southern populations of *Q. macrocarpa* did not differ in either year (Table 5A). Survival also did not differ between blocks on average. However, there was a significant block by population interaction in both years. In 2020, there was no difference in survival between populations in Block 1, but the northern population had higher survival than the southern population in Block 2. In 2021, the opposite was true. Survival was the same for both populations in Block 2 but was higher for the southern population in Block 1 compared to the northern population.

Morphology: There were no significant differences of any kind in either year for measurements for *Q. macrocarpa* spring and fall height, and spring and fall stem diameter (Table 5A). However, the southern population produced significantly more leaves than the northern population in both years (Fig. 8Aii). In 2020 and 2021 there were differences between the blocks with respect to SLA. In addition, in 2021, southern *Q. macrocarpa* had significantly lower SLA (thicker leaves) than northern populations (Fig. 8Bii). There were very low rates of gall infestation in either year, and there were no significant differences for this trait of any kind (Fig. 8Cii).

Phenology: In 2020, the northern population burst its buds approximately 2.4 days earlier than the southern population but there was no difference in 2021 (Table 5A). Bud burst did not differ significantly for any other factor in the model. The time at which the populations obtained full canopy also did not differ between populations in either year but was significantly different between blocks in 2020 (Fig. 8Dii). In contrast, all measurements of fall phenology were significantly different between the northern and southern populations in both years. The timing at which the trees lost 33%, 66%, and 100% of their leaves was consistently earlier for the northern population compared to the southern population (Fig. 8Eii). Considering both the timing of bud burst and the timing of leaf senescence, populations differed with respect to the total number of days when they had green leaves. In both years, the southern population retained its canopy for more days than the northern population (Fig. 8Fii). There were also block differences for this trait in 2020 but not in 2021, and there were no significant interactions between block and population.

Population Differences - Quercus rubra

Overall, the northern and southern populations of *Q. rubra* were more highly differentiated compared to the two populations of *Q. macrocarpa* (Table 5B).

Fitness: The populations did not differ in survival, nor was survival influenced by an interaction between block and population. However, in both years, *Q. rubra* did have significantly higher survival at Block 1 than at Block 2.

Morphology: Populations did not differ in height for either year. In 2021, southern *Q. rubra* had significantly greater basal diameter than all other tree types in the spring, and this significance persisted weakly in the ensuing fall. In 2021, the northern *Q. rubra* population had marginally more leaves than the southern *Q. rubra* population. Significant differentiation in number of leaves was based on block; Block 1 had significantly more leaves than Block 2. Southern *Q. rubra* had significantly lower SLA than northern trees in 2020. In 2021, the SLA of *Q. rubra* populations differed by block instead, with significantly lower SLA at Block 2 than Block 1 (Table 5B, Fig. 8Biii).

Phenology: In 2020, northern *Q. rubra* budburst before southern, but there were no other instances of differentiation by population or any other factor. Northern populations also reached full canopy significantly before southern in both 2020 and 2021, and full canopy arrived significantly sooner for *Q. rubra* at Block 1 than at Block 2 (Fig. 8Diii). In both years, northern populations preceded southern through 33%, 66%, and 100% senescence stages, and block had no significant impact (Fig. 8Eiii). As a result of the strong differentiation at the start and end of the growing season, populations differed significantly in the time spent with green leaves. Northern populations gained leaves first, but they lost leaves much sooner, leaving them with a shorter period of canopy retention overall (Fig. 8Fiii).

Differences in Parasitism by Gall-Forming Insects

Gall-forming insects significantly favored *Q. rubra* over *Q. macrocarpa* in both years (Table 4, Fig. 8). 2021 had significantly more galls than 2020, and Block 2 had significantly more galls than Block 1 (Table 4). In 2020, northern *Q. rubra* were parasitized the most heavily, but in 2021, southern *Q. rubra* had the most galls (Table 5, Fig. 8). Galls on *Q. rubra* were almost exclusively caused by parasitoid wasps: disruptive, roughly one-inch spheres to form in leaf and bud tissue commonly known as oak-apple galls (Appendix Fig. A5 A). The relatively few galls found on *Q. macrocarpa* leaves were small, pocket-vein galls caused by other varieties of insect (Appendix Fig. A5 B).

Differences in Reproduction

Acorn production occurred in both years of the study. At roughly ten years old, both oak species are well before reported reproductive ages. *Q. macrocarpa* was the only species to develop acorns, though they were predated before maturation and collection (Appendix Fig. A6). In 2020, one northern tree developed acorns, and in 2021, three northern and one southern tree attempted reproduction.

Table 4. Test statistics from analysis of the full data set. Two populations (northern and southern) of two oak species (*Quercus macrocarpa* and *Quercus rubra*) were grown in a randomized block design (two blocks). At ten years of age, fitness, growth, phenological, and physiological measurements were taken for two years (2020-2021).

	Species			Year			Block			Species x Year			Year x Block			Species x Block			Population (Species)		
	df	*F/χ2*	*p*	*df*	*F/χ2*	*p*	*df*	*F/χ2*	*p*	*df*	*F/χ2*	*p*	*df*	*F/χ2*	*p*	*df*	*F/χ2*	*p*	*df*	*F/χ2*	*p*
Fitness																					
Survival	1	3.20	0.07	1	0.10	0.76	1	6.84	**0.01**	1	0.04	0.83	1	0.17	0.68	1	1.23	0.27	2	1.40	0.50
Morphology																					
Spring Height	1, 268	6.28	**0.01**	1, 268	2.66	0.10	1, 268	0.03	0.87	1, 268	0.38	0.54	1, 268	0.05	0.82	1, 268	0.02	0.88	1, 268	1.61	0.20
Fall Height	1, 270	5.58	**0.02**	1, 270	3.59	0.06	1, 270	0.62	0.43	1, 270	1.19	0.28	1, 270	0.28	0.60	1, 270	0.43	0.51	1, 270	1.58	0.21
Spring Stem Diameter	1, 268	0.39	0.53	1, 268	20.11	**<0.0001**	1, 268	0.31	0.58	1, 268	0.12	0.73	1, 268	1.35	0.25	1, 268	1.64	0.20	1, 268	4.27	**0.01**
Fall Stem Diameter	1, 270	0.05	0.82	1, 270	3.07	0.08	1, 270	2.26	0.13	1, 270	0.82	0.37	1, 270	0.94	0.33	1, 270	0.79	0.38	1, 270	3.38	**0.04**
Number of Leaves	1, 270	7.00	**0.01**	1, 270	41.53	**<0.0001**	1, 270	17.84	**<0.0001**	1, 270	2.33	0.13	1, 270	0.63	0.43	1, 270	1.09	0.30	1, 270	5.71	**0.004**
SLA	1, 268	23.02	**<0.0001**	1, 268	0.96	0.33	1, 268	6.68	**0.01**	1, 268	8.29	**0.004**	1, 268	29.54	**<0.0001**	1, 268	5.31	**0.02**	1, 268	8.61	**0.0002**
Presence of Galls	1	76.13	**<0.0001**	1	8.71	**0.003**	1	8.01	**0.005**	1	3.08	0.08	1	0.08	0.78	1	2.96	0.09	2	5.92	**0.05**
Phenology																					
Bud Burst	1, 269	14.12	**0.0002**	1, 269	918.90	**<0.0001**	1, 269	0.17	0.68	1, 269	4.95	**0.03**	1, 269	1.28	0.26	1, 269	2.02	0.16	1, 269	3.84	**0.02**
Full Canopy	1, 249	4.60	**0.03**	1, 249	604.10	**<0.0001**	1, 249	282.50	**<0.0001**	1, 249	1.32	0.25	1, 249	229.62	**<0.0001**	1, 249	1.03	0.31	1, 249	3.08	**0.05**
33% Senescence	1, 252	2.54	0.11	1, 252	4.74	**0.03**	1, 252	29.27	**<0.0001**	1, 252	0.61	0.43	1, 252	9.70	**0.002**	1, 252	0.50	0.48	1, 252	43.61	**<0.0001**
66% Senescence	1, 257	3.21	0.07	1, 257	0.24	0.63	1, 257	52.81	**<0.0001**	1, 257	0.00	0.96	1, 257	56.75	**<0.0001**	1, 257	1.41	0.24	1, 257	38.08	**<0.0001**
100% Senescence	1, 260	21.58	**<0.0001**	1, 260	204.60	**<0.0001**	1, 260	6.06	**0.01**	1, 260	15.40	**0.0001**	1, 260	4.62	**0.03**	1, 260	0.57	0.45	1, 260	48.08	**<0.0001**
Canopy Retention	1, 259	9.63	**0.002**	1, 259	540.50	**<0.0001**	1, 259	6.85	**0.01**	1, 259	10.37	**0.001**	1, 259	3.10	0.08	1, 259	1.67	0.20	1, 259	36.98	**<0.0001**
Physiology																					
Early Transpiration	1, 267	1.53	0.22	1, 267	78.98	**<0.0001**	1, 267	5.35	**0.02**	1, 267	2.55	0.11	1, 267	15.61	**<0.0001**	1, 267	22.87	**<0.0001**	1, 267	3.65	**0.03**
Mid Transpiration	1, 269	0.00	0.97	1, 269	126.30	**<0.0001**	1, 269	17.11	**<0.0001**	1, 269	6.20	**0.01**	1, 269	12.37	**0.001**	1, 269	5.73	**0.02**	1, 269	0.34	0.71
Late Transpiration	1, 268	0.06	0.81	1, 268	56.39	**<0.0001**	1, 268	39.43	**<0.0001**	1, 268	3.74	**0.05**	1, 268	15.92	**<0.0001**	1, 268	6.59	**0.01**	1, 268	1.24	0.29

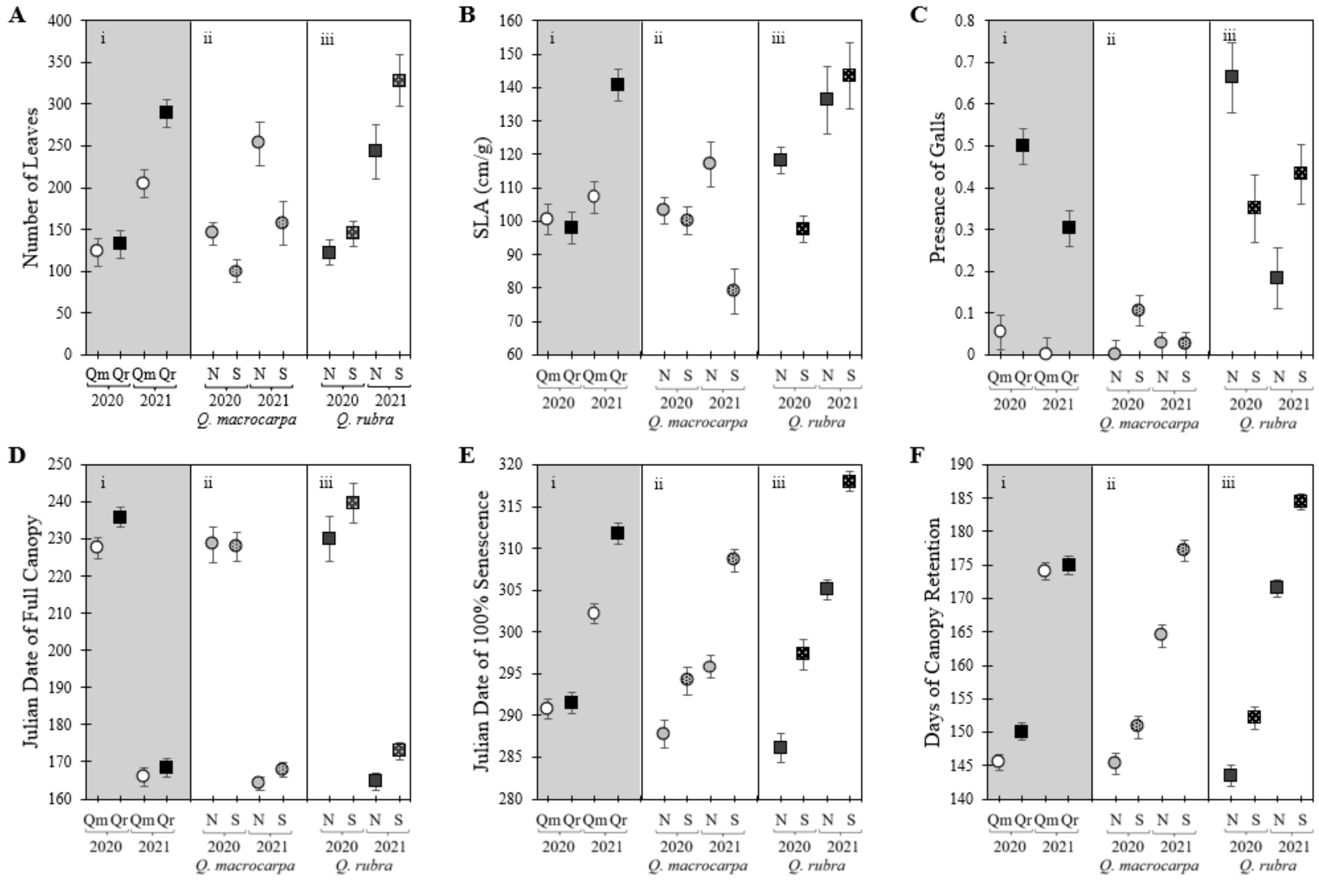

Figure 6. Least squares means and one standard error for plant traits and phenology. Two populations (N, northern; S, southern) are shown for each of two plant species (*Quercus macrocarpa*, Qm, circles and *Quercus rubra*, Qr, squares) measured in a common garden for two years (2020-2021). **A)** Leaf number**, B)** Specific Leaf area (SLA), **C)** Proportion of individuals with galls, **D)** Julian date of full canopy attainment, **E)** Julian date of 100% leaf senescence, and **F)** Total days of canopy retention. Panels **i)** Species differences in two years. Panels **ii)** Population differences for *Q. macrocarpa* (northern solid, southern stippled). Panels **iii)** Population differences for *Q. rubra* (northern solid, southern stippled).

Table 5A. Test statistics from analysis of data subsets: species (*Quercus macrocarpa*) and year (2020-2021). Plants were grown in a randomized block design (two blocks). At ten years of age, fitness, growth, phenological, and physiological measurements were recorded.

	2020									**2021**								
	Population			**Block**			**Population x Block**			**Population**			**Block**			**Population x Block**		
A. *Quercus macrocarpa*	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***
Fitness																		
Survival	1	0.00	1.00	1	0.00	1.00	1	3.87	**0.05**	1	0.00	1.00	1	0.00	1.00	1	3.87	**0.05**
Morphology																		
Spring Height	1	1.05	0.31	1	0.01	0.91	1	2.32	0.13	1	3.30	0.07	1	0.19	0.66	1	1.50	0.23
Fall Height	1	0.90	0.35	1	0.16	0.69	1	1.71	0.20	1	2.83	0.10	1	0.06	0.81	1	1.93	0.17
Spring Stem Diameter	1	0.50	0.48	1	0.67	0.42	1	0.49	0.49	1	0.06	0.81	1	0.00	0.98	1	0.06	0.81
Fall Stem Diameter	1	0.09	0.76	1	1.61	0.21	1	0.41	0.52	1	0.24	0.63	1	0.30	0.59	1	0.04	0.85
Leaf Number	1	5.44	**0.02**	1	1.05	0.31	1	0.11	0.74	1	5.93	**0.02**	1	2.60	0.11	1	0.36	0.55
Specific Leaf Area	1	0.68	0.41	1	8.35	**0.01**	1	2.49	0.12	1	15.86	**0.0002**	1	4.11	**0.05**	1	1.21	0.28
Presence of Galls	1	0.00	1.00	1	0.00	1.00	1	0.00	1.00	1	0.00	1.00	1	0.00	1.00	1	2.88	0.09
Phenology																		
Bud Burst	1	13.89	**0.001**	1	0.94	0.34	1	0.43	0.52	1	0.00	0.98	1	1.12	0.29	1	0.15	0.70
Full Canopy	1	0.11	0.74	1	9.70	**0.003**	1	0.09	0.77	1	1.78	0.19	1	0.02	0.88	1	0.68	0.41
33% Senescence	1	13.80	**0.001**	1	0.96	0.33	1	0.45	0.51	1	26.63	**<0.0001**	1	0.26	0.61	1	0.01	0.92
66% Senescence	1	24.94	**<0.0001**	1	1.81	0.18	1	2.81	0.10	1	22.44	**<0.0001**	1	0.85	0.36	1	0.44	0.51
100% Senescence	1	7.50	**0.01**	1	8.18	0.01	1	0.59	0.44	1	43.67	**<0.0001**	1	0.03	0.86	1	1.28	0.26
Canopy Retention	1	5.34	**0.02**	1	10.97	**0.002**	1	0.27	0.61	1	29.77	**<0.0001**	1	0.23	0.63	1	0.58	0.45

Table 5B. Test statistics from analysis of data subsets: species (*Quercus rubra*) and year (2020-2021). Plants were grown in a randomized block design (two blocks). At ten-years of age, fitness, growth, phenological, and physiological measurements were recorded.

	2020									**2021**								
	Population			**Block**			**Population x Block**			**Population**			**Block**			**Population x Block**		
B. *Quercus rubra*	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***	***df***	***F/χ2***	***p***
Fitness																		
Survival	1	1.33	0.25	1	8.73	**0.003**	1	1.22	0.27	1	2.49	0.11	1	6.94	**0.01**	1	2.28	0.13
Morphology																		
Spring Height	1	0.18	0.67	1	0.00	0.95	1	0.99	0.32	1	0.03	0.87	1	0.00	0.97	1	2.44	0.12
Fall Height	1	0.09	0.77	1	0.05	0.82	1	2.33	0.13	1	0.00	0.97	1	1.85	0.18	1	2.37	0.13
Spring Stem Diameter	1	2.34	0.13	1	0.02	0.88	1	0.27	0.61	1	6.46	**0.01**	1	2.48	0.12	1	0.45	0.51
Fall Stem Diameter	1	2.55	0.12	1	1.94	0.17	1	0.35	0.56	1	3.82	0.06	1	1.13	0.29	1	2.24	0.14
Leaf Number	1	1.12	0.30	1	6.11	**0.02**	1	0.27	0.60	1	3.53	0.07	1	10.59	**0.002**	1	2.29	0.14
SLA	1	13.45	**0.001**	1	1.57	0.21	1	0.94	0.34	1	0.26	0.61	1	14.89	**0.0003**	1	1.56	0.22
Presence of Galls	1	6.97	**0.01**	1	2.17	0.14	1	1.26	0.26	1	5.40	**0.02**	1	1.25	0.26	1	0.48	0.49
Phenology																		
Bud Burst	1	11.71	**0.001**	1	0.00	0.98	1	0.15	0.70	1	0.00	0.97	1	0.54	0.46	1	1.01	0.32
Full Canopy	1	9.38	**0.003**	1	0.00	1.00	1	1.97	0.17	1	6.37	**0.01**	1	7.36	**0.01**	1	0.43	0.51
33% Senescence	1	11.76	**0.001**	1	0.00	0.98	1	0.16	0.69	1	19.07	**<0.0001**	1	2.67	0.11	1	0.50	0.48
66% Senescence	1	36.02	**<0.0001**	1	3.71	0.06	1	0.99	0.32	1	27.00	**<0.0001**	1	0.67	0.42	1	4.19	**0.05**
100% Senescence	1	19.16	**<0.0001**	1	1.68	0.20	1	3.31	0.07	1	54.57	**<0.0001**	1	0.03	0.86	1	2.70	0.11
Canopy Retention	1	12.94	**0.001**	1	0.67	0.42	1	2.73	0.10	1	50.90	**<0.0001**	1	0.15	0.70	1	3.20	0.08

Transpiration

Overall, plants had higher rates of transpiration in 2021 than 2020, but transpiration did not differ between species on average (Table 4). Initial analyses showed that species transpired differently from year to year and at each block within years.

In each year of the study, transpiration peaked in early August and declined by late August, although not to the levels of early June (Fig. 9). At a species level, there was high variation between individuals, with a few trees transpiring at high rates compared to others during each measurement round. For instance, transpiration rates of *Q. macrocarpa* ranged between 22 mm hr^{-1} and 858 mm hr^{-1} in 2020 to 44 mm hr^{-1} and 2819 mm hr^{-1} in 2021. Meanwhile, *Q. macrocarpa* transpiration rates ranged from 26 mm hr^{-1} and 1896 mm hr^{-1} 2020 to 56 mm hr^{-1} 2369 mm hr^{-1} in 2021. In 2020, *Q. rubra* transpired 1.1x more than *Q. macrocarpa* on average (205 mm hr^{-1} and 186 mm hr^{-1}, respectively). In 2021, *Q. macrocarpa* transpired 1.2 times more than *Q. rubra* on average (402 mm $hr^{-1}$and 339 mm hr^{-1}, respectively).

Within species, northern and southern populations exhibited different transpiration patterns (Fig. 9). Although not always statistically significant (Table 6A), southern *Q. macrocarpa* tended to transpire at higher rates than northern *Q. macrocarpa*, and when the data was normalized (logged), that relationship persisted at every measurement (Fig. 9A). Southern *Q. macrocarpa* transpired significantly higher than the northern population in late August in both 2020 and 2022 (Table 6A). In the repeated measures analysis, there were only marginally significant differences between populations (Table 7A, $P < 0.08$). In contrast, the opposite was true for *Q. rubra*—the northern population tended to transpire at higher rates than the southern population, a pattern that held true for every measurement round when the data was normalized (Fig. 9B). Northern *Q. rubra* transpired at significantly higher rates than southern *Q. rubra* in July of 2020 and marginally so in late August of 2020 (Table 6B, $P < 0.06$). The repeated measures analysis was congruent with this result and showed that *Q. rubra* populations had significantly different transpiration rates across all three rounds of measurement (Table 7B).

The timing of measurements, both daily and hourly, frequently accounted for a significant amount of the variation in transpiration rates in both the subset analyses (Tables 6A, 6B) and the repeated measurements analysis (Tables 7A, 7B). However, the direction of these effects was not consistent. Transpiration measurements typically took seven days to complete and there were significant trends across some of the collection dates (i.e., Day of Year, Tables 6A, 6B, 7A, 7B). When analyzed separately by year and round, the day of the year had a significant impact on both *Q. macrocarpa* and *Q. rubra* transpiration rates in July 2021 and August 2020 (Tables 6A, 6B, 7A, 7B). In the repeated measures analysis, day of the year significantly affected transpiration of *Q. macrocarpa* in 2021 (Table 7A) and *Q. rubra* in both 2020 and 2021 (Table 7B). In the subset analysis, time of day significantly affected transpiration measurements for *Q. macrocarpa* in early August (Table 6A) and for *Q. rubra* in July 2021 and late August 2020 (Table 6B). In the repeated measures analysis (Table 7B), day of year was significant but time of day was not. The direction of effect of these covariates was not consistent.

Ambient conditions at the time of measurement also affected transpiration rates in some instances. Vapor pressure deficit (VPD) luminous flux (LUX), and soil moisture were all positively related to transpiration rate. In the subset analysis, VPD measured at the start of each measurement period significantly covaried with transpiration rates for three of the *Q. macrocarpa* measurements (July 2021, early August 2020, late August 2020, Table 6A) and one of the *Q. rubra* measurements (late August 2020, Table 6B). LUX at the start of each measurement also had significant effects on transpiration rate for *Q. macrocarpa* once (July 2021, Table 6A) and for *Q. rubra* twice (July 2020, late August 21, Table 6B). Soil moisture also significantly affected *Q. macrocarpa* once (late August 2021, Table 6A) and for *Q. rubra* twice (July 2020, late August

2021, Table 6B). In the repeated measurements analysis, the direction of the effect of VPD, LUX and soil moisture was also consistently positive. Considering all three measurement rounds together, VPD was significant for *Q. macrocarpa* in 2020 and for *Q. rubra* in both years. LUX was significant for *Q. macrocarpa* in 2021, and *Q. rubra* in both years. Soil moisture was significant for *Q. macrocarpa* in both years and not significant for *Q. rubra* in either year.

Aspects of the plant's phenotype including specific leaf area (SLA) and the number of leaves also affected transpiration rates. In the subset analyses, SLA did not influence transpiration rates for *Q. macrocarpa* in any year or measurement round (Table 6A), but did for *Q. rubra* in July 2021, Early August 2020, and late August 2021 (Table 6B). In each of these cases, plants with lower SLA (thicker leaves) had lower transpiration rates. The results were similar in the repeated measurements analysis (Tables 7A, 7B). SLA was never significant for *Q. macrocarpa* but was significant for *Q. rubra* in 2020 and marginally so in 2021 (Tables 7A, 7B, $P < 0.07$). In both the subset analysis and the repeated measures analysis, leaf number had consistently positive and significant effects on *Q. macrocarpa* transpiration rates (Tables 6A, 7A). The effect of leaf number was also positive and significant for *Q. rubra* in the subset analyses in July 2020 and 2021 and in early August and late August in 2021 (Table 6B) but was highly significant in both years in the repeated measures analysis (Table 7B).

Transpiration of *Q. macrocarpa* differed significantly between the blocks in the subset analyses in late August in 2020 (Table 6A) but was not significant in the repeated measures analysis (Table 7A). Block differences in transpiration were also detected for *Q. rubra* in July 2020 and 2021 in the subset analysis (Table 6B) and for both years in the repeated measures analysis (Table 7B). In each case, plants in Block 2 had significantly higher transpiration rates.

Table 5A. Test statistics for a generalized linear mixed model analysis of *Quercus macrocarpa* transpiration data. Data were taken in two years (2020, 2021). Species, years, and seasonal time periods (rounds) were analyzed separately.

A. *Q. macrocarpa*	**Round 1: July**				**Round 2: Early August**				**Round 3: Late August**			
	2020		**2021**		**2020**		**2021**		**2020**		**2021**	
Variables	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Population	1.85	0.17	1.56	0.21	0.01	0.94	1.56	0.21	7.00	**0.008**	4.89	**0.0269**
Day of Year	0.03	0.86	4.76	**0.029**	3.18	0.07	0.08	0.78	25.38	**<0.0001**	0.77	0.39
Time of Day	1.64	0.20	**<0.01**	0.96	4.41	**0.036**	1.82	0.18	1.26	0.26	**<0.01**	0.97
VPD start	1.29	0.26	6.23	**0.012**	4.61	**0.032**	0.45	0.50	4.25	**0.039**	0.52	0.47
LUX start	0.75	0.39	3.89	**0.049**	0.04	0.83	0.12	0.73	2.80	0.09	1.99	0.16
Mean Soil Moisture	0.34	0.56	2.14	0.14	0.35	0.55	0.13	0.72	0.18	0.67	5.02	**0.025**
SLA	0.71	0.40	0.04	0.83	0.13	0.72	0.03	0.86	1.22	0.27	1.09	0.30
Number of Leaves	9.69	**0.002**	6.42	**0.011**	6.16	**0.013**	6.34	**0.012**	6.51	**0.011**	12.94	**0.0003**
Block	1.75	0.19	1.11	0.29	1.42	0.23	0.71	0.40	8.73	**0.003**	**<0.01**	0.98

Table 6B. Test statistics for a generalized linear mixed model analysis of *Quercus rubra* transpiration data. Data were taken in two years (2020, 2021). Species, years, and seasonal time periods (rounds) were analyzed separately.

B. *Q. rubra*	**Round 1: July**				**Round 2: Early August**				**Round 3: Late August**			
	2020		**2021**		**2020**		**2021**		**2020**		**2021**	
Variables	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Population	0.28	0.60	6.97	**0.008**	1.16	0.28	1.11	0.29	0.13	0.72	3.58	0.06
Day of Year	0.63	0.43	10.75	**0.001**	0.07	0.79	8.55	**0.003**	11.32	**0.001**	2.43	0.12
Time of Day	1.41	0.24	4.44	**0.035**	3.53	0.06	2.2	0.14	12.56	**0.000**	2.07	0.15
VPD start	2.31	0.13	0.53	0.47	1.06	0.30	2.55	0.11	11.97	**0.001**	0.31	0.58
LUX start	8.82	**0.003**	< 0.01	1.00	0.61	0.44	0.6	0.44	0.29	0.59	4.91	**0.027**
Mean Soil Moisture	4.97	**0.026**	0.7	0.40	2.09	0.15	0.2	0.65	1.08	0.30	5.21	**0.023**
SLA	3.76	0.05	4.44	**0.035**	6.39	**0.012**	0.17	0.68	0.15	0.70	9.47	**0.002**
Number of Leaves	9.47	**0.002**	9.89	**0.002**	0.76	0.38	4.86	**0.027**	3.68	0.06	20.83	**< 0.0001**
Block	4.28	**0.039**	19.61	**< 0.0001**	0.00	0.96	2.11	0.15	0.10	0.75	0.16	0.69

Table 7A. Test statistics for a mixed model with an unstructured repeated measures matrix analysis of *Quercus macrocarpa* transpiration data. Data were taken in two years (2020, 2021). Species, years, and seasonal time periods (rounds) were analyzed jointly.

A. *Q. macrocarpa*

	2020			2021		
Variables	*df*	*F*	*p*	*df*	*F*	*p*
Population	1, 81.4	2.41	0.12	1, 64.6	3.13	0.08
Day of Year	1, 82.7	0.46	0.50	1, 93.4	31.25	**<0.0001**
Time of Day	1, 162.8	1.05	0.31	1, 129.6	1.81	0.18
VPD start	1, 156.2	34.39	**<0.0001**	1, 136.8	1.56	0.21
LUX start	1, 169.9	2.99	0.09	1, 127	14.22	**0.0002**
Mean Soil Moisture	1, 135.8	10.06	**0.002**	1, 171.8	6.33	**0.013**
SLA	1, 56.9	0.10	0.76	1, 64.5	0.99	0.32
Number of Leaves	1, 70.2	26.90	**<0.0001**	1, 68.1	15.28	**0.0002**
Block	1, 111.7	3.57	0.06	1, 69.3	< 0.01	1.00

Table 8B. Test statistics for a mixed model with an unstructured repeated measures matrix analysis of *Quercus rubra* transpiration data. Data were taken in two years (2020, 2021). Species, years, and seasonal time periods (rounds) were analyzed jointly.

B. *Q. rubra*

	2020			2021		
Variables	*df*	*F*	*p*	*df*	*F*	*p*
Population	1, 73.3	0.09	0.76	1, 59.4	5.15	**0.03**
Day of Year	1, 72.8	15.03	**0.0002**	1, 108.3	30.32	**<0.0001**
Time of Day	1, 159.1	8.24	**0.005**	1, 149.6	1.93	0.17
VPD start	1, 127.5	36.99	**<0.0001**	1, 161.4	6.13	**0.014**
LUX start	1, 135.3	6.21	**0.014**	1, 116.5	8.68	**0.004**
Mean Soil Moisture	1, 129.6	3.19	0.08	1, 131.4	0.24	0.63
SLA	1, 60	5.36	**0.024**	1, 57.8	3.41	0.07
Number of Leaves	1, 60.1	26.90	**<0.0001**	1, 66.4	15.75	**0.0002**
Block	1, 73.1	5.22	**0.025**	1, 65	12.98	**0.0006**

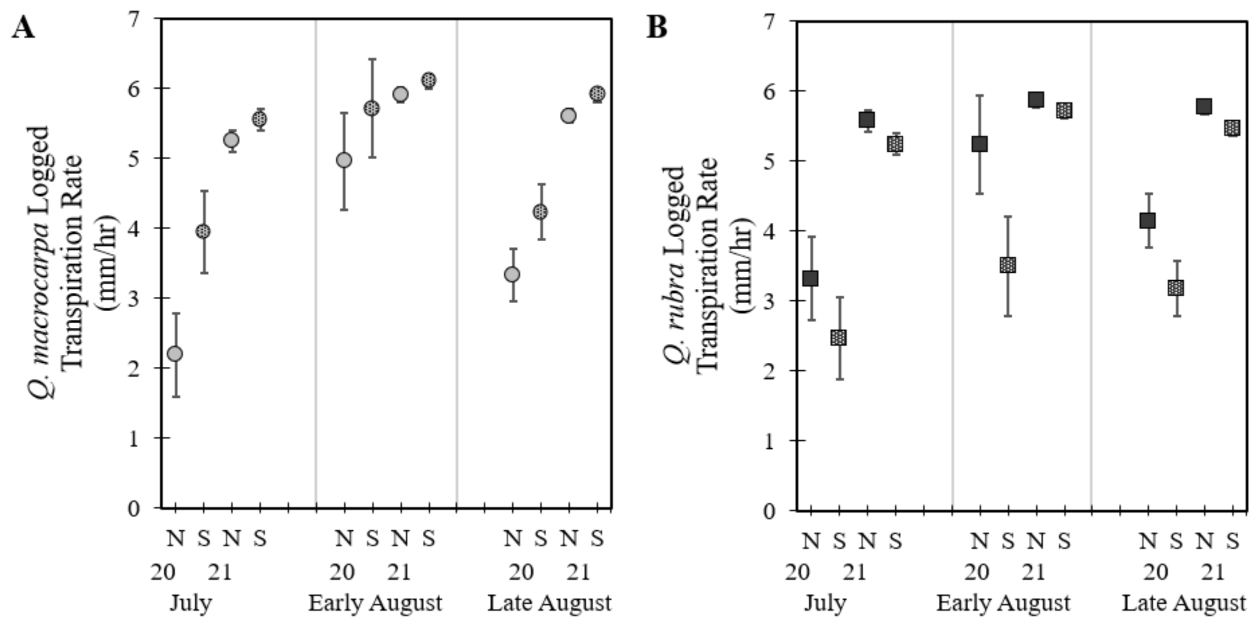

Figure 7. Logged transpiration rates. **A)** *Q. macrocarpa* and **B)** *Q. rubra*. Rates are partitioned by population, measurement round, and year. Gray circles: *Q. macrocarpa*, Black squares: *Q. rubra*; solid fill, northern populations; dotted fill, southern populations.

Transpiration Model Results

Measured transpiration rates were scaled to the growing season using the canopy retention of each population per species (Eqn. 8, canopy retention for *Q. macrocarpa*: northern mean 145.2 days and southern mean 150.3 days in 2020, northern mean 164.4 days and southern mean 177.1 days in 2021; canopy retention for *Q. rubra*: northern mean 143.6 days and southern mean 151.5 days in 2020, northern mean 171.7 days and southern mean 184.1 days in 2021). *Q. rubra* had the greatest mean overall predicted transpiration (Fig. 11). Using the maximum predicted transpiration rates led to the largest departures for each round and overall, but it was inconsistent which species and population had the largest departure over the measurement rounds. A southern *Q. macrocarpa* tree had the greatest predicted discharge departure in the first round of measurements, and the second largest departure was in the final round, by a southern *Q. rubra* tree. Minimum predicted discharge departures were by contrast largely homogenous, showing consistency across populations and species.

The average transpiration rate was chosen to represent each tree type in model figures and analysis. Over the course of measured rounds, *Q. rubra* showed a noticeable increase over time in predicted transpiration, while *Q. macrocarpa* transpired most in July but was relatively consistent. For both species, northern populations generally transpired less than southern populations, except in the late summer, when northern *Q. rubra* had the greatest scaled predicted transpiration (Table 8, Fig. 10). When seasonal averages were considered, northern populations transpired least for each species, with southern *Q. macrocarpa* transpiring least of all and southern *Q. rubra* transpiring most (Table 8, Fig. 10).

From the predicted transpiration, predicted discharge in the Stewart River was calculated (Eqn. 7). Larger values of predicted transpiration led to smaller values of predicted discharge, or streamflow. To compare these modelled values to reality, predicted discharge was subtracted from the measured discharge in the Stewart River in 2020. If a predicted discharge value was similar to the measured discharge, the resulting discharge departure value (positive or negative) was closer to zero (Table 9, Fig. 11). At most measured points, the discharge departure values neared zero, barring two extreme instances. In July, *Q. rubra* had large, negative departures, the values of which increased steadily until it had even larger positive departures in late August (Table 9, Fig. 11). *Q. macrocarpa* did not exhibit such marked variance.

Table 9. Predicted transpiration rates by round and on average in mm hr^{-1}. Values were isolated for the minimum, maximum, and average transpiration rates and scaled to the full growing season (Eqn. 9).

	Round One 7/20/20 - 7/29/20			**Round Two 8/5/20 - 8/12/20**			**Round Three 8/19/20 - 8/25/20**			**Average**		
Tree Type	***Ave.***	***Min.***	***Max.***	***Ave.***	***Min.***	***Max.***	***Ave.***	***Min.***	***Max.***	***Ave.***	***Min.***	***Max.***
QM 104	293.19	32.89	986.68	241.54	32.21	883.58	281.43	39.33	1129.42	272.40	61.56	579.36
QM 105	299.16	42.72	1287.66	251.43	34.82	676.64	288.08	64.05	805.39	279.56	57.79	766.37
QR 104	191.94	50.51	608.19	249.04	41.65	965.34	419.19	37.15	273.02	289.11	80.83	1062.40
QR 105	256.82	54.62	256.82	288.53	66.22	710.55	410.27	54.91	1189.32	318.54	95.57	786.66

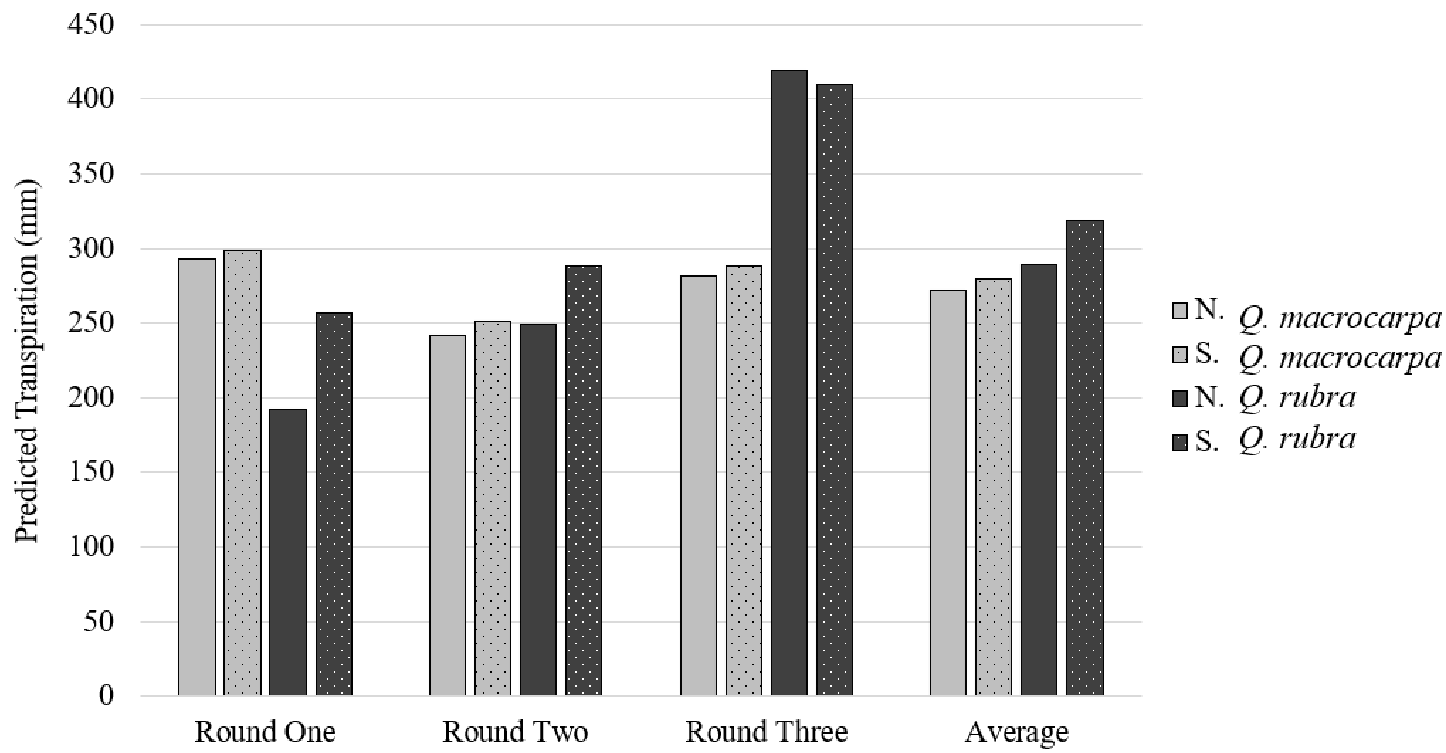

Figure 8. Predicted growing season transpiration by round and on average. *Q. macrocarpa* is depicted in gray tones on the left, and *Q. rubra* is in black on the right. The northern populations have solid bars; the southern populations have speckled bars. Values were scaled from field measurements to encompass the growing season based on a ten-hour day and the canopy retention of each tree type.

Table 10. Predicted discharge departures by round and on average in mm hr^{-1}. Values are calculated by subtracting Predicted Transpiration from the annual precipitation to get predicted discharge and then subtracting predicted discharge from the annual discharge (Eqn. 8).

	Round One			**Round Two**			**Round Three**					
	7/20/20 - 7/29/20			**8/5/20 - 8/12/20**			**8/19/20 - 8/25/20**			**Average**		
Tree Type	***Ave.***	***Min.***	***Max.***	***Ave.***	***Min.***	***Max.***	***Ave.***	***Min.***	***Max.***	***Ave.***	***Min.***	***Max.***
QM 104	31.45	-296.59	905.44	-33.64	-297.46	775.50	16.63	-288.48	1085.33	5.25	-260.46	392.11
QM 105	28.92	-285.64	1241.48	-29.63	-295.34	491.96	15.33	-259.48	649.90	4.87	-267.15	602.03
QR 104	-92.79	-273.51	439.09	-19.83	-284.82	895.44	197.58	-290.57	10.82	31.37	-234.77	1019.46
QR 105	-25.11	-271.50	-25.11	13.53	-257.36	527.78	161.88	-271.14	1111.19	50.10	-221.59	620.53

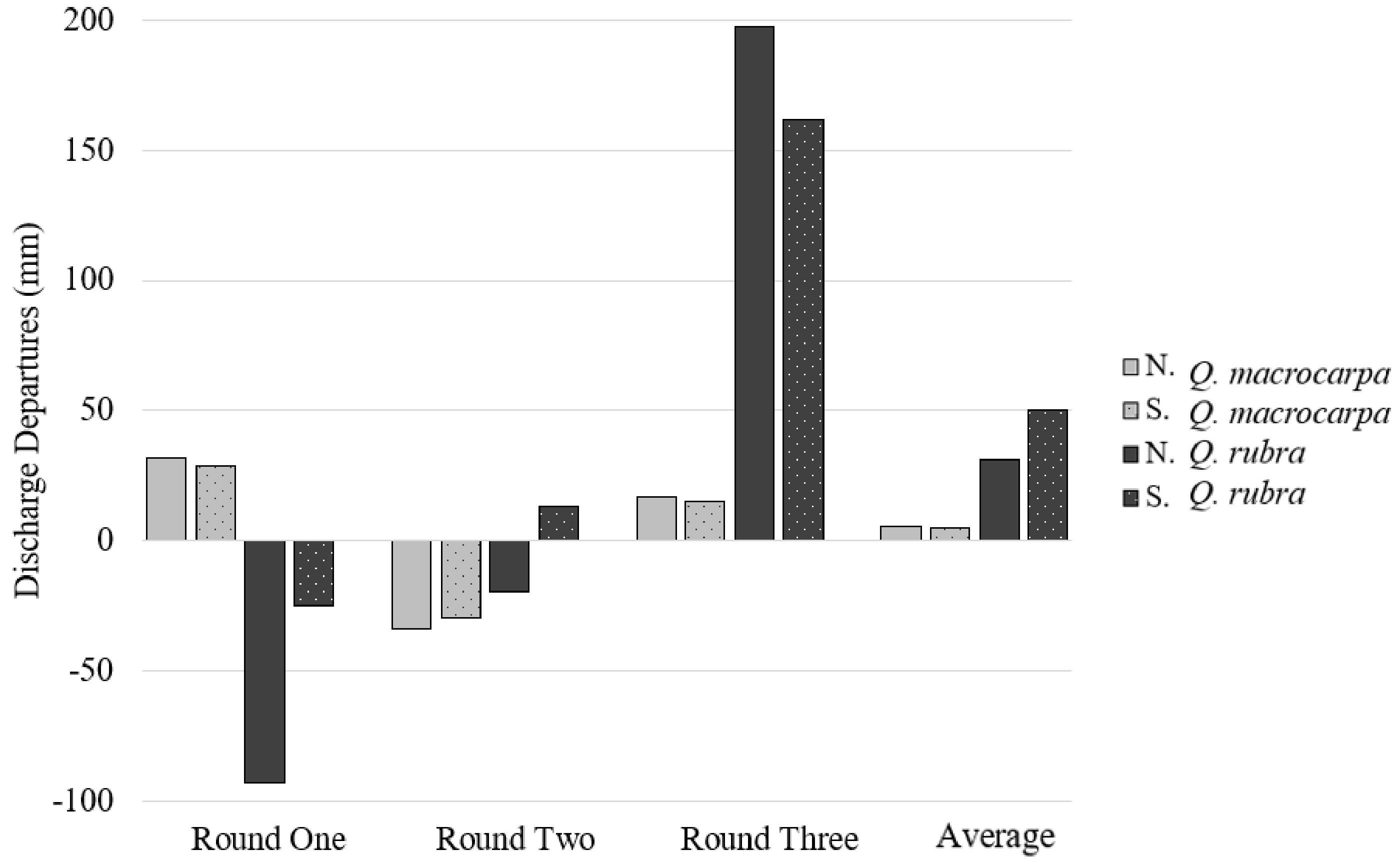

Figure 9. Discharge departures by round and on average. *Q. macrocarpa* is depicted in gray tones on the left, and *Q. rubra* is in black on the right. The northern populations have solid bars; the southern populations have speckled bars.

Discussion

This research contributes to the growing body of evidence suggesting that assisted population migration is an effective management strategy to mitigate the effects of climate change. In this experiment, a northern and southern population of two Minnesota species that are predicted to increase in local abundance with climate change, *Quercus macrocarpa* and *Quercus rubra*, were planted in a northern site. Climate shifts may be in favor of altered species compositions in northern forests, as has been modelled many times over (Tirmenstein 1991, Gucker 2011). Some models project that conditions in northern Minnesota will shift to favor an oak-hickory forest composition rather than the historical aspen-birch systems (Lal *et al.* 2011), and many models suggest that *Quercus macrocarpa* and *Quercus rubra* will have a neutral or beneficial responses to predicted climate shifts in our study region (Matthews *et al.* 2018).

In an adverse environment, these two species maintained their cohorts well, indicating that climate shifts have created hospitable long-term conditions for these species. Similarly to previous findings (Etterson *et al.* 2020), this study showed that there was genetic differentiation between the two populations for each species for traits that are important for climate adaptation. These differences were especially strong for fall phenology traits and notable for physiological traits linked to water use adaptation, such as specific leaf area (SLA).

This work is a departure from previous studies in that transpiration measurements were also taken and revealed population differences that have not previously been reported in the literature. Importantly, measured transpiration varied at a population level for both species. A model constructed using field precipitation, discharge, and transpiration measurements demonstrated that each population for each species would have a unique impact on the discharge of the Stewart River.

Southern Populations Demonstrated Higher Climate Suitability

Both *Q. rubra* and *Q. macrocarpa* persisted in high numbers even a decade after their 2013 planting, with no lower than 84% survival across populations (northern *Q. rubra* in 2021). Even though *Q. macrocarpa* significantly outlived *Q. rubra* as of 2021, these oaks have thrived at the very northernmost edge of their traditional ranges (Tirmenstein 1991, Gucker 2011). *Q. macrocarpa* is renowned for its cold and drought tolerance; *Q. rubra*, while hardy, is perhaps not as adapted to northern winters and the ongoing drought conditions during the study (Tirmenstein 1991, Gucker 2011). Only *Q. rubra's* population differed significantly in survivorship. Southern conspecifics survived more than the northern *Q. rubra* population, indicating that conditions present in the northern planting sites were actually better suited to the nonlocal population. Perhaps the same stressors that led to lower species-wide survival acted on population-level adaptations.

Q. macrocarpa is a savannah species well adapted to drought and is known to invest early in belowground biomass, whereas *Q. rubra*, more often found in denser forests, dedicates early growth to aboveground gains in height and trunk diameter (Tirmenstein 1991, Gucker 2011). *Q. rubra* also had thinner and more numerous leaves than *Q. macrocarpa*. Southern *Q. rubra* consistently had the most leaves, and the largest canopies of the four study populations. *Q. macrocarpa* also had significantly lower SLA than *Q. rubra,* aligning with broader literature (Reich *et al.* 1995). Southern *Q. macrocarpa* had the lowest SLA (thicker leaves) than all others. Interestingly, in previous work, seedling *Q. rubra* had lower SLA than seedling *Q. macrocarpa* (Etterson *et al.* 2020). Developing fewer, thicker leaves with smaller surface area is a common water conservation and light-intake limiting strategy in warmer, droughty, open environments (Sanchez-Gomez *et al.* 2013, Bacelar *et al.* 2006, Geng *et al.* 2006, Navas and Garnier 2002), which makes sense for populations of a savannah species and for populations that originated from warmer parts of the species range. *Q. rubra* more often competes for light and grows in environments with less water stress (Tirmenstein 1991, Gucker 2011). It makes sense that this species had higher SLA (thinner leaves) that are more prone to water loss than thicker leaves. As the climate grows warmer, the higher SLA of *Q. rubra* puts it at a competitive disadvantage compared to species that can conserve water more easily, such as *Q. macrocarpa.*

Northern populations burst their buds before southern in both species, indicating that northern populations are calibrated to take advantage of a later spring and shorter growing season (Kunkel *et al*. 2004). A European study compared oak and beech phenology across a latitudinal gradient from Norway to Portugal from 1969-2017 and found that spring phenology stages were becoming earlier as climate warmed; the authors further suggested that oak may be more phenologically adaptive to climate change than beech, since it can get an earlier start in the spring due to greater temperature sensitivity (Wenden *et al.* 2019). Northern populations also senesced earlier at the end of the growing season, which echoed the 2013 findings of these trees (Etterson *et al.* 2020). A forty-year American study of *Quercus alba* examined fitness and phenology in populations from across a latitudinal gradient. The authors found that southerly populations senesced later and grew faster than northerly populations, mirroring my findings, and further determined that northern populations were disadvantaged in growth and ultimately survival (Thomas *et al.* 2024). These and other studies emphasize my findings of advanced and extended phenology in southern oak populations when compared to northern conspecifics (Vitasse *et al.* 2009, Knott *et al.* 2022). Overall, for my trees, *Q. rubra* retained its canopy longer than *Q. macrocarpa*.

Interannual Weather Variability

2021 was a year of greater extremes than 2020. It had the lowest and highest measured temperatures at the field sites (-16.3 and 38.4 °C) and more overall rainfall (421.3 cm compared to 364.7 cm in 2020). Periods of little to no rainfall were also more common and longer lasting in 2021 than in 2020.

Over the two years of the study, weather fluctuations induced plastic responses for many traits. Early-season cold snaps in 2021 caused tissue damage to developing leaves, which had cascading effects on subsequent measurements of phenology. Damaged leaves did not reach full size, prompting the growth of replacement leaves and increasing canopy fullness in some cases. Despite freezing damage, full canopy attainment appeared more consistent between years than budburst timing, indicating that it may depict population phenology more accurately. Given budburst's prevalence in the literature as a phenological benchmark, full canopy's reliability is a possibility that bears further exploration.

Species and Population Climate Suitability

Survivorship was high for all trees planted in the study which suggests that these species are good candidates for assisted migration into northern Minnesota sites. Both species persisted in high numbers even a decade after their 2013 planting, with no lower than 84% survival across populations (northern *Q. rubra* in 2021). Only one tree died during 2020-2021 which was not sufficient to detect differences in mortality between species or their populations. This echoed earlier studies on these trees, which reported higher survivorship in *Q. macrocarpa* across all study sites in the larger experiment (see *Study Area*) six years after planting (Etterson *et al.* 2020). Even though *Q. macrocarpa* significantly outlived *Q. rubra* at my field sites as of 2021, these oaks have thrived at the very northernmost edge of their traditional ranges (Tirmenstein 1991, Gucker 2011). In fact, southern *Q. rubra* had significantly higher survivorship than northern, despite its translocation northwards.

Plant Traits

Though similar in survivorship, the two oak species had phenotypic traits that suggested that they have different life history strategies. *Q. macrocarpa*, a savannah species well adapted to drought, is known to invest early in belowground biomass, whereas *Q. rubra*, more often found in forests, dedicates early growth to aboveground gains in height and trunk diameter (Tirmenstein 1991, Gucker 2011). In a previous study, this was hypothesized to explain differences in height between *Q. macrocarpa* and *Q. rubra* (Etterson *et al.* 2020) that were also observed here. Height was strongly differentiated at the start and end of the growing season, with *Q. rubra* surpassing *Q. macrocarpa's* height by seven cm in spring. Though the height gap lessened by fall, *Q. rubra* remained significantly taller than *Q. macrocarpa*. Within species, *Q. rubra* had the only population differentiation in basal stem diameter. This may be due to *Q. rubra's* propensity for resprouting. Several trees were observed to have broken off and regrown since initial plantings, but they were far outnumbered by saplings demonstrating continuous growth and health.

Southern *Q. rubra* reached canopy attainment consistently before the northern population, while *Q. macrocarpa* populations were not strongly differentiated through all phases of canopy attainment. Still, northern populations burst their buds before southern in both species, indicating that northern populations are calibrated to take advantage of a later spring and shorter growing season (Kunkel *et al*. 2004). Lingering winter weather disrupted this population level pattern in 2021, yet species differentiation remained. Because bursting its buds later allowed *Q.*

rubra to somewhat avoid the spring freezes in 2021, *Q. rubra* may be more robust to freezing interference than *Q. macrocarpa*. Northern populations senesced earlier at the end of the growing season, which echoed the 2013 findings of these trees (Etterson *et al.* 2020). There was strong species differentiation in the timing of fall benchmarks, as well. *Q. macrocarpa* reached 33% senescence faster than *Q. rubra,* but *Q. rubra* arrived at 100% senescence first. Overall, *Q. rubra* retained its canopy longer than *Q. macrocarpa*. Population-level differences demonstrated much firmer patterning through senescence; in both species, northern populations senesced sooner.

It should be noted that in 2020, field observations ended on a monitoring cutoff date before all trees had fully senesced. In 2021, a more variable weather year, observations continued until all trees were fully at 100% senescence. Despite earlier budburst, northern populations had significantly shorter canopy retention overall than southern. Southern trees continued to photosynthesize in hospitable conditions for longer than their northern counterparts, without discernable costs to survival or other growth traits.

In terms of suitability, species and populations cannot solely be considered in terms of individual traits, since individuals in turn interact with a larger ecosystem. *Q. rubra* proved more susceptible to parasitoid wasps, for example, and *Q. macrocarpa* unexpectedly produced acorns. Traits such as disease and pest resistance can make the difference in tree survival and forest health, as can reproductive success and hybridization (Trumbore *et al.* 2015, Wingfield *et al.* 2015, Budde *et al.* 2016). Both galls and acorns represent a biomass investment for the affected individuals; resources are dedicated to replacement leaves and potential offspring rather than height, roots, or other vital functions.

Novel Transpiration Differentiation in Quercus Populations

Q. macrocarpa is a savannah species with lower water needs than *Q. rubra*, which is generally found in mesic forest stands and along floodplains (Tirmenstein 1991, Gucker 2011). Though species were expected to differ in transpiration, initial analyses showed complicated interplay between year, block, and species (Table 4), so ensuing statistical tests analyzed species separately.

Testing population-level differences in transpiration and other water-use traits is a relatively novel and largely underexplored topic. To date, the only other study known to have detected transpiration differentiation among tree populations was conducted by Cantürk *et al.* (2023) in Turkey. Three-year-old seedlings of *Castanea sativa* varied significantly across populations in several gas exchange parameters including transpiration. While novel, this study evaluated seedlings grown in pots over a brief time period. The controlled, common conditions were carefully designed to showcase any genetic differentiation in the targeted traits.

My study, conversely, measures trees that have spent over ten years in common garden blocks grown within an extant forest. All the weather variability, soil water content, community interactions, and other stressors of a natural setting have been present throughout my trees' lives, and even still, my study is one of the few to demonstrate population differentiation in tree transpiration rates. It is perhaps the very first to detect it in *Q. macrocarpa* and *Q. rubra*. In late August 2020 and 2021, *Q. macrocarpa* populations significantly differed in their transpiration rate and therefore water use. One instance alone would be remarkable enough, but this study detected a consistent pattern between years. Furthermore, in a year with more water stress, *Q. rubra* also demonstrated significant population differentiation in midsummer transpiration. Transpiration differed by population multiple times in multiple species. This suggests true population differences, a pattern that may become more evident over time or if additional sites from the original experiment are measured. These differences were also evident even under the variable conditions present in an uncontrolled environment, suggesting that variance in population transpiration may have common and persistent effects on local hydrology.

Modeling Differentiation Indicates Varied Watershed Compatibility for Populations

Modeling demonstrated the potential impacts of each tree type if it were scaled to be the homogenous forest cover of the Stewart River watershed. Greater predicted transpiration (Table 8, Fig. 10) led to lesser predicted discharge. Between the two species, *Q. macrocarpa* showed the most stable patterns of predicted transpiration over the measurement period (July, early August, late August) and less predicted transpiration on average (Fig. 10). *Q. rubra*, on the other hand, had a lesser predicted transpiration rate than *Q. macrocarpa* in July, yet steadily increased over the measurement period until it far exceeded *Q. macrocarpa* by late August and had greater transpiration on average for the measurement period (Fig. 10). Information such as this could be useful to know for watershed managers when considering what tree species to plant, especially in river systems where water availability varies over time due to factors such as spring snowmelt, hydropower production, or drinking water reservoir operation.

Within species, predicted transpiration for northern populations was always less than for southern populations. Northern populations therefore led to greater predicted discharge, or higher streamflow, than southern populations. Increases to streamflow, if drastic, could lead to stream channel instability, but given the drought and deluge patterns of precipitation under climate change, a higher baseflow in the Stewart River could provide a buffer to drought in its watershed (Cornelis *et al.* 2019).

It could also be important to know which choices in forest cover might maintain present conditions. Stability in fundamental resources such as water availability plays an important role in ecosystem resilience (van Meerbeek *et al.* 2021). In Figure 11, modelled discharge departures close to zero indicate that the scaled transpiration at that time resulted in streamflow nearest to the real, measured discharge of the Stewart River. *Q. macrocarpa* had the smallest divergences between predicted and real discharge in July, late August, and overall (Fig. 11). *Q. rubra* generated the smallest discharge departures in early August. However, its discharge departures in July were strongly negative, and its discharge departures in late August were even more strongly positive (Fig. 11). Northern *Q. rubra* showed the greatest fluctuations over time, resulting in the lowest modelled discharge departures in July and the highest discharge departures by late August. Also of note: at each time point, populations mostly varied in similar directions if not magnitudes, but in early August, *Q. rubra* had the only instance of opposite trends between the northern and southern populations. Between species, *Q. macrocarpa* clearly demonstrates the most stable impact on watershed discharge. Within species, southern populations uniformly resulted in discharge departures closer to zero and therefore closer to real, measured discharge, indicating that southern populations of both species might maintain more stable discharge as the predominant forest cover of the Stewart River watershed.

The fact that populations are demonstrably different in their impacts to the watershed reinforces that transpiration could be an important consideration in forest management decisions. Northern Minnesota receives higher snowfall than southern Minnesota, and proximity to Lake Superior notably increases the amount of rainfall present in the arrowhead region of Minnesota (Runkle *et al.* 2022). Populations historically present in the northern region of this study are more likely to have local adaptations that rely on greater water availability than populations from further away from Lake Superior. Climate shifts that lead to drought and deluge patterns of water availability will challenge these populations during extended dry periods.

Quercus species are known for higher levels of drought tolerance than many boreal species (Kunz *et al.* 2018, Baquedano and Castillo 2007, Picon *et al.* 1996). As exhibited by the adjusted strategies in leaf morphology for each species in this study, these trees responded significantly to variations in weather and water availability. *Quercus* species may therefore compensate enough morphologically to minimize transpiration variation from year to year; a different boreal species might exhibit stronger reactions to weather fluctuations.

Conclusions

In northern Minnesota and around the world, the longstanding conditions that led to the evolution of local phenological traits in these species have shifted. In their place are warmer temperatures, altered water availability patterns, and an extending growing season (Zeng *et al.* 2011, Kunkel *et al.* 2004). Southern populations present plant traits that are likely more suitable to present growing conditions in the north. Northern populations may be falling out of alignment with local climate, at the cost of lost growth opportunities and greater winter stresses at the margins of the growing season.

H1) Neither species demonstrated variation in fitness between populations. *Q. rubra*, perhaps due to its early-life investments in aboveground biomass, did have a few significant differences in growth between its populations. Most population-level differences in morphology were in traits closely linked to transpiration, such as number of leaves and specific leaf area.

H2) Southern populations began their seasons later than northern, but they kept their leaves longer into the fall. The result was a longer period of canopy retention, and thus a longer growing season for the southern trees. This season aligned with hospitable growing conditions at the study sites, indicating that the phenology of southern populations is better aligned with local climatic conditions in the north.

H3) Transpiration varied by year and block, but it did not vary as a simple function of species. The effect of species on transpiration was contingent on what year it was and which block the measurement took place in.

H4) There were indeed population level differences in transpiration for both species. *Q. macrocarpa* demonstrated population level differentiation twice at the end of the growing season, while *Q. rubra* exhibited differentiation at midsummer of a drought year. Which population tended to transpire most differed by species. For *Q. macrocarpa*, southern populations usually transpired more than northern populations. For *Q. rubra*, northern populations transpired more than southern.

Given the results of this study, there are avenues for future work in detecting population differentiation in other tree species. Projections continue to emerge on how climates may shift to suit altered species compositions in local forests. Many of the species predicted to thrive in northern Minnesota are poorly understood in terms of population differentiation for plant traits, potential hydrologic impacts, and climate suitability. The modeling methods used here could be modified to represent differing proportions of forest cover for these tree types in the Stewart River watershed, or they could be adapted to examine potential watershed impacts of other species and populations.

Paradigms in ecology and resource stewardship must shift towards proactive management strategies to promote ecosystem resilience. Distinctions in species and population level traits present an opportunity to counteract the desynchronization of climate shifts and local adaptations, especially in heavily forested regions such as northern Minnesota. Better understanding of local adaptations such as phenology and water use strategies creates opportunities for tree species and populations to be realigned with the growing conditions that suit them, bolstering forest health against the ongoing and worsening effects of climate change.

Bibliography

Adams, H.D., Luce, C.H., Breshears, D.D., Allen, C.D., Weiler, M., Hale, V.C., Smith, A.M.S., Huxman, T.E. 2012. Ecohydrological consequences of drought and infestation triggered tree die off: insights and hypotheses. *Ecohydrology* 5:145-159.

Aitken, S.N., Adams, W.T., Schermann, N., Fuchigami, L.H. 1996. Family variation for fall cold hardiness in two Washington populations of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco). *Forest Ecology and Management* 80(1-3): 187-195.

Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S. 2008. Adaptation, migration, or extirpation: Climate change outcomes for tree populations. *Evolutionary Applications* 1(1): 95-111.

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O. 2013. Potential for evolutionary responses to climate change - evidence from tree populations. *Global Change Biology* 19:1645-1661.

Amano, T., Freckleton, R.P., Queenborough, S.A., Doxford, S.W., Smithers, R.J., Sparks, T.H., Sutherland, W.J. 2014. Links between plant species' spatial and temporal responses to a warming climate. *Proceedings of the Royal Society B*.

Aubin, I., Garbe, C.M., Colombo, S., Drever, C.R., McKenney, D.W., Messier, C., Pedlar, J., Saner, M.A., Venier, L., Wellstead, A.M., Winder, R., Witten, E., Ste-Marie, C. 2011. Why we disagree about assisted migration: Ethical implications of a key debate regarding the future of Canada's forests. *The Forestry Chronicle* 87(9): 755-765.

Axler, R., C. Kleist, G. Host, C.Hagley, M.Lonsdale, J. Reed, J. Schomberg, N. Will, J. Henneck, G. Sjerven, E.Ruzycki, T.Carlson, B. Munson, and C. Richards. 2009. *LakeSuperiorStreams: Community Partnerships for Understanding Water Quality and Stormwater Impacts at the Head of the Great Lakes (http://lakesuperiorstreams.org).* University of Minnesota-Duluth, Duluth, MN 55812.

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Gonçalves, B., Ferreria, H.F., Correia, C.M. 2006. Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: Changes on structure and chemical composition of foliage and oxidative damage. *Plant Science* 170(3): 596-605.

Baker, D.G., Watson, B.F., Skaggs, R.H. 1985. The Minnesota long-term temperature record. *Climatic Change* 7: 225-236.

Baquedano, F.J., and Castillo, F. 2007. Drought tolerance in the Mediterranean species *Quercus coccifera*, *Quercus ilex, Pinus halepensis*, and *Juniperus phoenicea*. *Photosynthetica* 45(2): 229-238.

Beardmore, T. and Winder, R. 2011. Review of science-based assessments of species vulnerability: Contributions to decision-making for assisted migration. The Forestry Chronicle 87(6): 745-754.

Borkowski, D.S., Hoban, S.M., Chatwin, W., Romero-Severson, J. 2017. Rangewide population differentiation and population substructure in *Quercus rubra* L. *Tree Genetics and Genomes* 13.

Bowden, J.D. and Bauerle, W.L. 2008. Measuring and modeling the variation in species-specific transpiration in temperate deciduous hardwoods. *Tree physiology* 28(11): 1675-1683.

Bradbury, J.P., Dean, W.E, Anderson, R.Y. 1993. *Holocene climatic and limbologic history of the north-central United States as recorded in the varved sediments of Elk Lake, Minnesota: A synthesis.* Elk Lake, Minnesota: Evidence for Rapid Climate Change in the North-Central United States.

Bradshaw, A.D. 1984. The importance of evolutionary ideas in ecology—and vice versa. *Evolutionary Ideas in Ecology*: 1-25.

Budde, K.B., Nielsen, L.R., Ravn, H.P., Kjær. 2016. The natural evolutionary potential of tree populations to cope with newly introduced pests and pathogens—lessons learned from forest health catastrophes in recent decades. *Current Forestry Reports* 2: 18-29.

Buma, B. and Livneh, B. 2015. Potential effects of forest disturbances and management on water resources in a warmer climate. *Forest Science* 61(5): 895-903.

Cantürk, U., Çobanoğlu, H., Beyazyüz, F., Koç, İ. 2023. Investigation of leaf gas exchange parameters of several chestnut population seedlings at the end of the growing season. *Turkish Journal of Agriculture – Food Science and Technology* 11(10).

Clark, J.S. 1988. Effect of climate change on fire regimes in northwestern Minnesota. *Nature* 334: 233-235.

Clark, J.S. 1990. Fire and climate change during the last 750 yr in northwestern Minnesota. *Ecological Monographs* 60(2): 135-159.

Conroy, G.C., Shimizu-Kimura, Y., Lamont, R.W., Ogbourne, S.M. 2019. A multidisciplinary approach to inform assisted migration of the restricted rainforest tree, *Fontainea rostrata*. *PLoS ONE* 14(1).

Cornelis, W., Waweru, G., Araya, T. 2019. *Building Resilience Against Drought and Floods: The Soil-Water Management Perspective.* Sustainable Agriculture Reviews, vol 29. p 125-142.

Correll, D.L., Jordan, T.E., Weller, D.E. 1997. *Failure of agricultural riparian buffers to protect surface waters from groundwater nitrate contamination.* Groundwater/Surface Water Ecotones: Biological and Hydrological Interactions and Management Options. Cambridge University Press, New York. 162-165.

Craft, K.J. and Ashley, M.V. 2006. Population differentiation among three species of white oak in northeastern Illinois. *Canadian Journal of Forest Research* 36: 206-215.

Craft, K.J. and Ashley, M.V. 2007. Landscape genetic structure of bur oak (Quercus macrocarpa) savannas in Illinois. *Forest Ecology and Management* 239(1-3): 13-20.

Cwynar, L.C. and MacDonald, G.M. 1987. Geographical variation of lodgepole pine in relation to population history. *The American Naturalist* 129(3): 463-469.

Daubree, J.B. and Kremer, A. 1993. Genetic and phenological differentiation between introduced and natural populations of *Quercus rubra* L. *Annals of Forest Science* 50: 271-280.

Davis, M.B. and Shaw, R.G. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science* 292(5517): 673-679.

Dore, M.H.I. 2005. Climate change and changes in global precipitation patterns: What do we know? *Environment International* 31(8): 1167-1181.

Duveneck, M.J., Scheller, R.M., White, M.A., Handler, S.D., Ravenscroft, C. 2014. Climate change effects on northern Great Lake (USA) forests: a case for preserving diversity. *Ecosphere* 17:237-249.

Eichenlaub, V.L. 1987. Lakes, effects on climate. *Climatology*.

Ellison, D., Morris, C.E., Locatelli, B., Sheil, D., Cohen, J., Murdiyarso, D., Gutierrez, V., van Noordwijk, M., Creed, I.R., Pokorny, J., Gaveau, D., Spracklen, D.V., Tobella, A.B., Ilstedt, U., Teuling, A.J., Gebrehiwot, S.G., Sands, D.C., Muys, B., Verbist, B., Springgay, E.. Sugandi, Y., Sullivan, C.A. 2017. Trees, forests, and water: Cool insights for a hot world. *Global Environmental Change*, 4: 51-61.

Etterson, J.R., Cornett, M.W., White, M.A., Kavajecz, L.C. 2020. Assisted migration across fixed seed zones detects adaptation lags in two major North American tree species. *Ecological Applications*, 30(5).

Faluyi, M.O. and Irmak, S. 2023. Northeastern American forests: Natural disturbances, climate change impact, and the utilization of increasingly damaged forest trees for biofuel production. *Forests* 14.

Fernandez, C.W., Mielke, L., Stefanski, A., Bermudez, R., Hobbie, S.E., Montgomery, R.A, Reich, P.B., Kennedy, P.G. 2023. Climate change-induced stress disrupts ectomycorrhizal interaction networks at the boreal-temperate ecotone. *PNAS* 120(34).

García-Hernández, M.A., Toledo-Aceves, T., López-Barrera, F., Sosa, V.J., Paz, H. 2019. Effects of environmental filters on early establishment of cloud forest trees along elevation gradients: Implications for assisted migration. *Forest Ecology and Management* 432: 427-435.

Garman and Moring. 1991. Initial effects of deforestation on physical characteristics of a boreal river. *Hydrobiologia* 209:29-37.

Geiss, C.E., Umbanhower, C.E., Camill, P., Banerjee, S.K. 2003. Sediment magnetic properties reveal Holocene climate change along the Minnesota prairie-forest ecotone. *Journal of Paleolimnology* 30: 151-166.

Geng, Y.P., Pan, X.Y., Xu, C.Y., Zhang, W.J., Li, B., Chen, J.K. 2006. Phenotypic plasticity of invasive *Alternanthera philoxeroides* in relation to different water availability, compared to its native congener. *Acta Oecologica* 30(3): 380-385.

Gömöry, D, Krajmerová, D., Hrivnák, M., Longauer, R. 2020. Assisted migration vs. close-to-nature forestry: What are the prospects for tree populations under climate change? *Central European Forestry Journal* 66(2): 63-70.

Grady, K.C., Ferrier, S.M., Kolb, T.E., Hart, S.C., Allan, G.J., Whitham, T.G. 2011. Genetic variation in productivity of foundation riparian species at the edge of their distribution: implications for restoration and assisted migration in a warming climate. *Global Change Biology* 17(12): 3724-3735.

Grady, K.C., Kolb, T.E., Ikeda, D.H., Whitham, T.G. 2015. A bridge too far: Cold and pathogen constraints to assisted migration of riparian forests. *Restoration Ecology* 23(6): 811-820.

Gray, L.K., Gylander, T., Mbogga, M.S., Chen, P.Y., Hamann, A. 2011. Assisted migration to address climate change: Recommendations for aspen reforestation in western Canada. *Ecological Applications* 21(5): 1591-1603.

Gucker, C.L. 2011. *Quercus macrocarpa. In: Fire Effects Information System, [Online].* U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer).

Hanratty, M.P. and Stefan, H.G. 1998. Simulating climate change effects in a Minnesota agricultural watershed. *Journal of Environmental Quality* 27(6): 1524-1532.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist* 173(5): 579-588.

Hewitt, G.M. 1999. Post-glacial recolonization of European biota. *Biological Journal of the Linnean Society* 68(1-2): 87-112.

Hiesey, W.M. and Nobs, M.A. 1982. *Experimental Studies on the Nature of Species: VI. Interspecific Hybrid Derivatives between Facultatively Apomictic Species of Bluegrasses and their Responses to Contrasting Environments.* Carnegie Institution of Washington Publication 636. Washington, D.C.

Huff, A. and Thomas, A. 2014. *Lake Superior climate change impacts and adaptation*. Report prepared for the Lake Superior Lakewide Action and Management Plan - Superior Work Group. Retrieved from the University of Minnesota Digital Conservancy.

Johnson, S.L. and Stefan, H.G. 2006. Indicators of climate warming in Minnesota: lake ICE covers and snowmelt runoff. *Climatic Change* 75: 421-453.

Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R., Hector, A., Huss-Danell, K., Jumpponen, A., Minns, A., Mulder, C.P.H., Pereira, J.S., Prinz, A., Scherer-Lorenzen, M., Siamantziouras, A.S.D., Terry, A.C., Troumbis, A.Y., Lawton, J.H. 2001. Local adaptation enhances performance of common plant species. *Ecology Letters* 4(6): 536-544.

Kawecki, T.J. and Ebert, D. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7:1225-1241.

Khodwekar, S. and Gailing, O. 2017. Evidence for environment-dependent introgression of adaptive genes between two red oak species with different drought adaptations. *American Journal of Botany* 104(7): 1088-1098.

Kittelson, P.M., Pinahs, C., Dwyer, J., Ingersoll, A., Mans, E., Rieke, J., Rutman, B., Volenec, M. 2009. Age structure and genetic diversity of four *Quercus macrocarpa* (Michx.) populations in fragmented oak savanna along the central Minnesota River valley. *The American Midland Naturalist* 161(2): 301-312.

Knott, J.A., Liang, L., Dukes, J.S., Swihart, R.K., Fei, S. 2022. Phenological response to climate variation in a northern red oak plantation: Links to survival and productivity. *Ecology* 104(3).

Konings, A.G., Saatchi, S.S., Frankenberg, C., Keller, M., Leshyk, V., Anderegg, W.R.L., Humphrey, V., Matheny, A.M., Trugman, A., Sack, L., Agee, E., barnes, M.L., Binks, O., Cawse-Nicholson, K., Christoffersen, B.O., Entekhabi, D., Gentine, P., Holtzman, N.M., Katul, G.G., Liu, Y., Longo, M., Martinez-Vilalta, J., McDowell, N., Meir, P., Mencuccini, M., Mrad, A., Novick, K.A., Oliveira, R.S., Siquiera, P., Steele-Dunne, S.C., Thompson, D.R., Wang, Y., Wehr, R., Wood, J.D., Xu, X., Zuidema, P.A. 2021. Detecting forest response to droughts with global observations of vegetation water content. *Global Change Biology* 27(23): 6005-6024.

Koralewski, T.E., Wang, H.H., Grant, W.E., Byram, T.D. 2015. Plants on the move: Assisted migration of forest trees in the face of climate change. *Forest Ecology and Management* 344: 30-37.

Kranabetter, J.M, Stoehr, M., O'Neill, G.A. 2015. Ectomycorrhizal fungal maladaptation and growth reductions associated with assisted migration of Douglas-fir. New Phytology 206: 1135-1144.

Kunkel, K.E., Easterling, D.R., Hubbard, K., Redmond, K. 2004. Temporal variations in frost-free season in the United States: 1895–2000. *Geophysical Research Letters* 31.

Kunz, J., Löffler, G., Bauhus, J. 2018. Minor European broadleaved tree species are more drought-tolerant than *Fagus sylvatica* but not more tolerant than *Quercus petraea*. *Forest Ecology and Management* 414: 15-27.

Lal, P., Alavalapati, J., Mercer, D. 2011. *Socio-Economic Impacts of Climate Change on Rural Communities in the United States.* USDA Forest Service - General Technical Report PNW-GTR.

Ledger, M.E., Brown, L.E., Edwards, F., Milner, A.M., Woodward, G. 2013. Drought alters the structure and functioning of complex food webs. Nature Climate Change, 2013. 3:223-227.

Leech, M.L., Almuedo, P.L., O'Neill, G. 2011. Assisted migration: Adapting forest management to a changing climate. *Journal of Ecosystems and Management* 12(3): 18-34.

Li, C. 2000. Population differences in water-use efficiency of *Eucalyptus microtheca* seedlings under different watering regimes. *Physiologia Plantarum* 108(2): 134-139.

Li L., Yang, Z.L., Matheny, A.M., Zheng, H., Swenson, S.C., Lawrence, D.M., Barlage, M., Yan, B., McDowell, N.G., Leung, L.R. 2021. Representation of Plant Hydraulics in the Noah-MP Land Surface Model: Model Development and Multiscale Evaluation. *Journal of Advanced Modeling Earth Systems* 13(4).

Liess, S., Twine, T.E., Snyder, P.K., Hutchison, W.D., Konar-Steenberg, G., Keeler, B.L., Brauman, K.A. 2022. High-resolution climate projections over Minnesota for the 21st century. *Earth and Space Science* 9(3).

Lindback, E.C., Rauschendorfer, J.K., Burton, A.J., Kuelheim, C., Cavaleri, M.A. 2023. Common garden study reveals frost-tolerant northern seed sources are best suited to expand range of *Quercus rubra*. *Forest Ecology and Management* 539.

Little, E. 1971. *Maps of species ranges for Q. macrocarpa and Q. rubra*. United States Forest Service.

Magni, C.R., Ducousso, A., Caron, H., Petit, R.J., Kremer, A. 2005. Chloroplast DNA variation of *Quercus rubra* L. in North America and comparison with other *Fagaceae*. *Molecular Ecology* 14(2): 513-524.

Martin R. J. and Kincer, J. B. 1934. *Climatic summary of the United States, Section 46—Southeastern Minnesota*. USDA Weather Bureau, Superintendent of Documents, Washington, DC.

Martínez-Berdeja, A., Hamilton, J.A., Bontemps, A., Schmitt, J., Wright, J.W. 2019. Evidence for population differentiation among Jeffrey and Ponderosa pines in survival, growth and phenology. *Forest Ecology and Management* 434(28): 40-48.

Matheny, A.M., Bohrer, G., Vogel, C.S., Morin, T.H., He, L., de Moraes Frasson, R.P. Mirfenderesgi, G., Schäfer, K.V.R., Gough, C.M., Ivanov, V.Y., Curtis, P.S. 2014. Species-specific transpiration responses to intermediate disturbance in a northern hardwood forest. *JGR Biogeosciences* 119(12): 2292-2311.

Matheny, A.M., Bohrer, G., Garrity, S.R., Morin, T.H., Howard, C.J., Vogel, C.S. 2015. Observations of stem water storage in trees of opposing hydraulic strategies. *Ecosphere* 6(9): 1-13.

Matheny, A.M., Fiorella, R.P., Bohrer, G., Poulsen, C.J., Morin, T.H., Wunderlich, A., Vogel, C.S., Curtis, P.S. 2016. Contrasting strategies of hydraulic control in two codominant temperate tree species. *Ecohydrology* 10(3): 1815.

Matheny, A.M. 2021. Stressors reveal ecosystems' hidden characteristics. *JGR Biogeosciences* 126(8).

Matthews, S.N., Iverson, L.R., Peters, M.P., Prasad, A.M. 2018. *Assessing potential climate change pressures across the conterminous United States: mapping plant hardiness zones, heat zones, growing degree days, and cumulative drought severity throughout this century*. RMAP-NRS-9. U.S. Department of Agriculture, Forest Service, Northern Research Station. Newtown Square, PA. 31 p.

McLachlan, J.S., Clark, J.S., Manos, P.S. 2005. Molecular indicators of tree migration capacity under rapid climate change. *Ecology* 86(8): 2088-2098.

McLauchlan, K.K., Lascu, I., Myrbo, A., Leavitt, P.R. 2013. Variable ecosystem response to climate change during the Holocene in northern Minnesota, USA. *GSA Bulletin* 125(3-4): 445-452.

Melillo, J.M., Richmond, T.C., Yohe, G.W. 2014. *Climate change impacts in the United States: The third national climate assessment.* U.S. Global Change Research Program.

Míguez-Soto, B., Fernández-Cruz, J., Fernández-López, J. 2019. Mediterranean and Northern Iberian gene pools of wild *Castanea sativa* Mill. are two differentiated ecotypes originated under natural divergent selection. *PLoS ONE* 14(2).

MN DNR. 2024a. *Climate trends*. Minnesota Department of Natural Resources.

MN DNR. 2024b. *Northern Mesic Mixed Forest - FDn43*. Minnesota Department of Natural Resources.

Moser, W.K., Butler-Leopold, P., Hausman, C., Iverson, L., Ontl, T., Brandt, L., Matthews, S., Peters, M., Prasad, A. 2019. *The impact of climate change on forest systems in the northern United States: Projections and implications for forest management.* Chapter, Achieving sustainable management of boreal and temperate forests. Burleigh Dodds Science Publishing. 50 p.

Muller, J.J., Nagel, L.M., Palik, B.J. 2019. Forest adaptation strategies aimed at climate change: Assessing the performance of future climate-adapted tree species in a northern Minnesota pine ecosystem. *Forest Ecology and Management* 451.

Navas, M.L. and Garnier, E. 2002. Plasticity of whole plant and leaf traits in *Rubia peregrina* in response to light, nutrient and water availability. *Acta Oecologica* 23(6): 375-383.

Novotny, E.V. and Stefan, H.G. 2007. Stream flow in Minnesota: Indicator of climate change. *Journal of Hydrology* 334(3-4): 319-333.

Ohte, N., Tokuchi, N., Katsuyama, M., Hobara, S., Asano, Y., Koba, K. 2003. Episodic increases in nitrate concentrations in streamwater due to the partial dieback of a pine forest in Japan: runoff generation processes control seasonality. Hydrological Processes 17: 237-249.

Palik, B.J., Clark, P.W., D'Amato, A.W., Swanston, C., Nagel, L. 2022. Operationalizing forest-assisted migration in the context of climate change adaptation: Examples from the eastern USA. *Ecosphere* 13(10).

Pappas, C., Matheny, A.M., Baltzer, J.L., Barr, A.G., Black, T.A., Bohrer, G., Detto, M., Maillet, J. Roy, A., Sonnentag, O., Stephens, J. 2018. Boreal tree hydrodynamics: Asynchronous, diverging, yet complementary. *Tree Physiology* 38(7): 953–964.

Park, A. and Rodgers, J.L. 2023. Provenance trials in the service of forestry assisted migration: A review of North American field trials and experiments. *Forest Ecology and Management* 537.

Pedlar, J.H., McKenney, D.W., Aubin, I, Beardmore, T., Beaulieu, J., Iverson, L., O'Neill, G.A., Winder, R.S., Ste-Marie, C. 2012. Placing forestry in the assisted migration debate. *BioScience* 62(9): 835-842.

Person, M., Roy, P., Wright, H., Gutowski, Jr., W., Ito, E., Winter, T., Rosenberry, D., Cohen, D. 2007. Hydrologic response of the Crow Wing Watershed, Minnesota, to mid-Holocene climate change. *GSA Bulletin* 119(3-4): 363-376.

Pettenkofer, T., Burkhardt, K., Ammer, C., Vor, T., Finkeldey, R., Müller, M., Krutovsky, K., Vornam, B., Leinemann, L., Gailing, O. 2019. Genetic diversity and differentiation of introduced red oak (*Quercus rubra*) in Germany in comparison with reference native North American populations. *European Journal of Forest Research* 138: 275-285.

Picon, C., Guehl, J.M., Ferhi, A. 1996. Leaf gas exchange and carbon isotope composition responses to drought in a drought-avoiding (*Pinus pinaster*) and a drought-tolerant (*Quercus petraea*) species under present and elevated atmospheric CO2 concentrations. *Plant, Cell, and Environment* 19(2): 182-190.

Poyatos, R., Granda, V., Molowny-Horas, R., Mencuccini, M., Steppe, K., Martínez-Vilalta, J. 2016. SAPFLUXNET: Towards a global database of sap flow measurements. *Tree Physiology* 36: 1449-1455.

Rantz, S.E. 1982. *Measurement and Computation of Streamflow: Volumes I and II.* USGS Water Supply Paper 2175. 631 p.

Reich, P.B., Walters, M.B., Kloeppel, B.D., Ellsworth, D.S. 1995. Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. *Oecologia* 104: 24-30.

Reich, P.B., Bermudez, R., Montgomery, R.A., Rich, R.L., Rice, K.E., Hobbie, S.E., Stefanski, A. 2022. Even modest climate change may lead to major transitions in boreal forests. Nature 608: 540-545.

Rich, R.L, Stefanski, A., Montgomery, R.A., Hobbie, S.E., Kimball, B.A., Reich, P.B. 2015. Design and performance of combined infrared canopy and belowground warming in the B4WarmED (Boreal Forest Warming at an Ecotone in Danger) experiment. Global Change Biology 21(6): 2334-2348.

Runkle, J., Kunkel, K.E., Frankson, R., Easterling, D.R., Champion, S.M. 2022. *Minnesota State Climate Summary 2022.* NOAA Technical Report NESDIS 150-MN. NOAA/NESDIS. Silver Spring, MD. 4 p.

Sabater, A.M., Valiente, J.A., Bellot, J., Vilagrosa, A. 2023. Testing transpiration rates of juvenile Aleppo pine trees using the heat ratio method under laboratory conditions. *Ecohydrology* 16(8).

Sáenz-Romero, C., Lindig-Cisneros, R.A., Joyce, D.G., Beaulieu, J., St. Clair, J. B., Jaquish, B.C. 2007. Assisted migration of forest populations for adapting trees to climate change. *Revista Chapingo: Serie Ciencias Forestales y del Ambiente* 22(3): 303-323.

Sáenz-Romero, C., O'Neill, G., Aitken, S.N., Lindig-Cisneros, R. 2021. Assisted migration field tests in Canada and Mexico: Lessons, limitations, and challenges. *Forests* 12(9).

Sánchez-Gómez, D., Robson, T.M., Gascó, A., Gil- Pelegrín, E., Aranda, I. 2013. Differences in the leaf functional traits of six beech (*Fagus sylvatica* L.) populations are reflected in their response to water limitation. *Environmental and Experimental Botany* 87: 110-119.

Savolainen, O., Pyhäjärvi, T., Knürr, T. 2007. Gene Flow and Local Adaptation in Trees. *Annual Review of Ecology, Evolution, and Systematics* 38:595-619.

Schnabel, A. and Hamrick, J.L. 1990. Comparative analysis of population genetic structure in *Quercus macrocarpa* and *Q. gambelii* (*Fagaceae*). *Systematic Botany* 15(2): 240-251.

Schreiber, S.G., Ding, C., Hamann, A., Hacke, U.G., Thomas, B.R., Brouard, J.S. 2013. Frost hardiness vs. growth performance in trembling aspen: An experimental test of assisted migration. Journal of *Applied Ecology* 50(4): 939-949.

Scott, R.W. and Huff, F.A. 1996. Impacts of the Great Lakes on regional climate conditions. *Journal of Great Lakes Research* 22(4): 845-863.

Sendall, K.M., Reich, P.B., Zhao, C., Hou, J., Wei, X., Stefanski, A., Rice, K., Rich, R.L., Montgomery, R.A. 2015. Acclimation of photosynthetic temperature optima of temperate and boreal tree species in response to experimental forest warming. *Global Change Biology* 21(3): 1342-1357.

Shaw, R.G. and Etterson, J.R. 2012. Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. *New Phytologist* 195(4): 752-765.

Smith, D.M. and Allen, S.J. 1996. Measurement of sap flow in plant stems. *Journal of Experimental Botany* 47(12): 1833-1844.

Smith, D.S., Schweitzer, J.A., Turk, P., Bailey, J.K., Hart, S.C., Shuster, S.M., Whitham, T.G. 2012. Soil-mediated local adaptation alters seedling survival and performance. *Plant and Soil* 352: 243-251.

Solomon S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. 2007. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC Fourth Assessment Report (AR4). Cambridge University Press. Cambridge, United Kingdom and New York, NY, USA. 996 p.

Sork, V.L., Stowe, K.A., Hochwender, C. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. *The American Naturalist* 142(6).

Stannard, D.I. 1988. *Use of a hemispherical chamber for measurement of evapotranspiration.* Open-file report 88-452. U.S. Geological Survey. 22 p.

Ste-Marie, C., Nelson, E.A., Dabros, A., Bonneau, M.E. 2011. Assisted migration: Introduction to a multifaceted concept. *Forestry Chronicle* 8:724-730.

Stone, M. 2012. *Protocols for Collection of Streamflow, Water-Quality, Streambed-Sediment, Periphyton, Macroinvertebrate, Fish, and Habitat Data to Describe Stream Quality for the Hydrobiological Monitoring Program, Equus Beds Aquifer Storage and Recovery Program, City of Wichita, Kansas.* Open-File Report 2012–1055, U.S. Department of the Interior U.S. Geological Survey.

Swanston, C., Janowiak, M., Iverson, L., Parker, L., Mladenoff, D., Brandt, L., Butler, P., St. Pierre, M., Prasad, A., Matthews, S., Peters, M., Higgins, D., Dorland, A. 2011. *Ecosystem vulnerability assessment and synthesis: a report from the Climate Change Response Framework Project in northern Wisconsin*. General Technical Report, USDA Forest Service, Northern Research Station. Newtown Square, PA. 142 p.

Sykes, M.T. 2009. *Climate change impacts: Vegetation*. Encyclopedia of Life Sciences. John Wiley and Sons, Ltd. Chichester.

Teshome, D.T., Zharare, G.E., Naidoo, S. 2020. The threat of the combined effect of biotic and abiotic stress factors in forestry under a changing climate. *Frontiers in Plant Science* 11.

Thomas, A.M., Coggeshall, M.V., O'Connor, P.A., Nelson, C.D. 2024. Climate adaptation in white oak (*Quercus alba* L.): A forty-year study of growth and phenology. *Forests* 15(3).

Thomsen, J.E., Bohrer, G. Matheny A.M., Ivanov, V.Y., He, L., Renninger, H.J., Schäfer, K.V.R. 2013. Contrasting Hydraulic Strategies during Dry Soil Conditions in *Quercus rubra* and *Acer rubrum* in a Sandy Site in Michigan. *Forests* 4(4):1106-1120.

Thornthwaite, C.W. 1948. An approach toward a rational classification of climate. *Geographical Review* 38(1): 55-94.

Tirmenstein, D.A. 1991. *Quercus rubra. In: Fire Effects Information System, [Online].* U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer).

Trumbore, S., Brando, P., Hartmann, H. 2015. Forest health and global change. *Science* 349(6250): 814-818.

Turnipseed, D.P. and Sauer, V.B. 2010. *Discharge Measurements at Gaging Stations*. USGS Techniques and Methods Book 3, Chapter A8, 87 p.

Umbanhowar, Jr., C.E. 2004. Interaction of fire, climate, and vegetation change at a large landscape scale in the Big Woods of Minnesota, USA. *The Holocene* 14(5): 661-676.

van der Ent, R.J., Savenije, H.H.G., Schaefli, B., Steele-Dunne, S.C. 2010. Origin and fate of atmospheric moisture over continents. *Water Resources Research* 46(9).

van Meerbeek, K., Jucker, T., Svenning, J.C. 2021. Unifying the concepts of stability and resilience in ecology. *Journal of Ecology* 109: 3114-3132.

van Tienderen, P.H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* 45(6):1317-1331.

Vitasse, Y., Delzon, S., Dufrêne, E., Pontailler, J.Y., Louvet, J.M., Kremer, A., Michalet, R. 2009. Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? *Agricultural and Forest Meteorology* 149(5): 735-744.

Vitt P., Havens, K., Kramer, A.T., Sollenberger, D, Yates, E. 2010. Assisted migration of plants: Changes in latitudes, changes in attitudes. *Biological Conservation* 143(1): 18-27.

Way, D.A. and Montgomery, R.A. 2015. Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell, and Environment* 38(9): 1725-1736.

Wenden, B., Mariadassou, M., Chmielewski, F.M., Vitasse, Y. 2019. Shifts in the temperature-sensitive periods for spring phenology in European beech and pedunculate oak clones across latitudes and over recent decades. *Global Change Biology* 26(3): 1808-1819.

Williams, M.I. and Dumroese, R.K. 2013. Preparing for climate change: Forestry and assisted migration. *Journal of Forestry* 111(4): 287-297.

Wingfield, M.J., Brockerhoff, E.G., Wingfield, B.D., Slippers, B. 2015. Planted forest health: The need for a global strategy. *Science* 349(6250): 832-836.

Zeng, H., Jia, G., Epstein, H. 2011. Recent changes in phenology over the northern high latitudes detected from multi-satellite data. *Environmental Research Letters* 6.

Zeng, Q., Marquis, B., Delagrange, S., Zhou, P., Yuan, S., Sylvain, J.D., Raymond, P., Rossi, S. 2022. Late frost as a driver of ecotypic differentiation in sugar maple: Implications for assisted migration. *Agricultural and Forest Meteorology* 323.

Zhang, J. and Marshall, J.D. 1994. Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Canadian Journal of Forest Research* 24(1): 92-99.

Zhu, Y., Cheng, Z., Feng, K., Chen, Z., Cao, C., Huang, J., Ye, H. and Gao, Y. 2022. Influencing factors for transpiration rate: A numerical simulation of an individual leaf system. *Thermal Science and Engineering Progress* 27.

Appendix

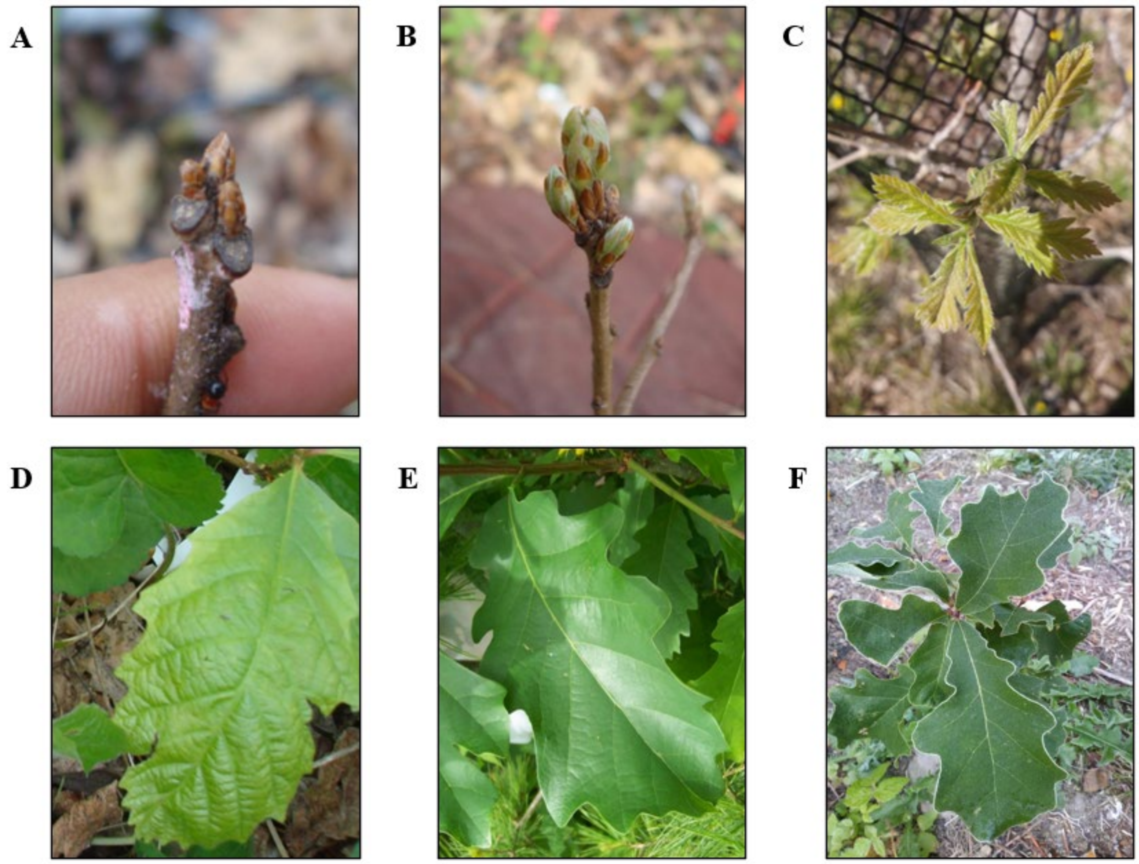

Figure A1. Phenological stages of canopy progression, as demonstrated by *Quercus macrocarpa*. **A)** all buds tightly closed, **B)** first bud scales separating, **C)** first immature leaf or leaves visible, **D)** example of a nearly mature leaf, **E)** first mature leaf or leaves present, **F)** all leaves mature leaves, or full canopy attainment (picture shows full canopy of a study tree at the end of the growing season, with frost rime edging the leaves). Photos A, B, D, and E taken from phenological protocols of the B4warmed project, ©Artur Stefanski 2020.

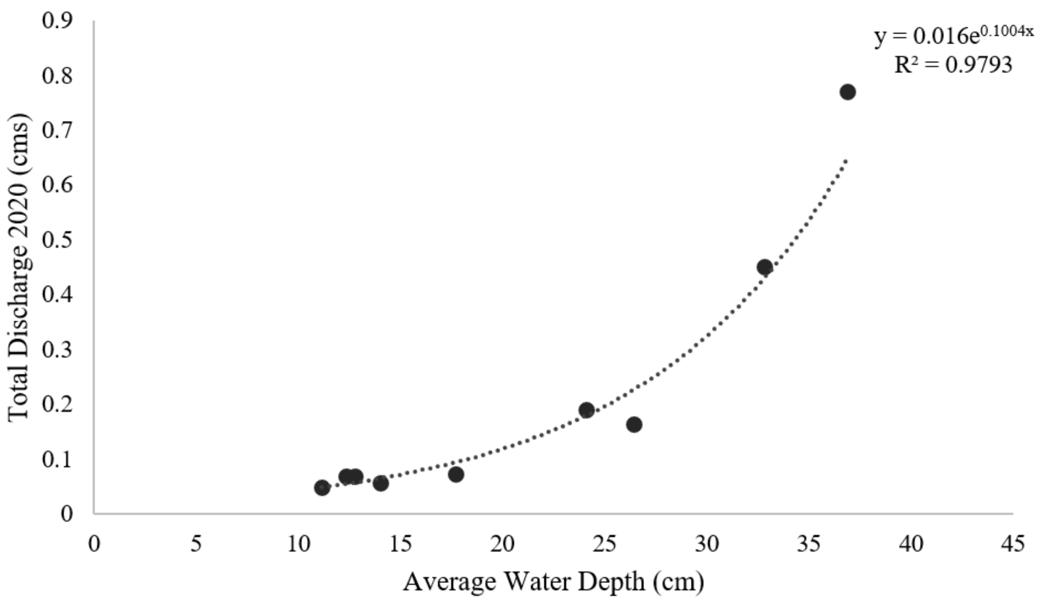

Figure A2: 2020 stream curve for the Stewart River. The total discharge measured via stream cross sections plotted against the average water depth at the time of that cross section. Nine measurements were taken throughout the year: 6/28/2020, 6/30/2020, 7/23/2020, 7/29/2020, 8/6/2020, 8/11/2020, 8/23/2020, 9/20/2020, 10/22/2020.

Figure A3. Weather station installed at Block 1. For sensors included, see ***Environmental Measurements***. The station was installed on the first day and taken down on the last day of field measurements each year.

Figure A4. Setup for transpiration measurements with the portable rapid evapotranspiration chamber.

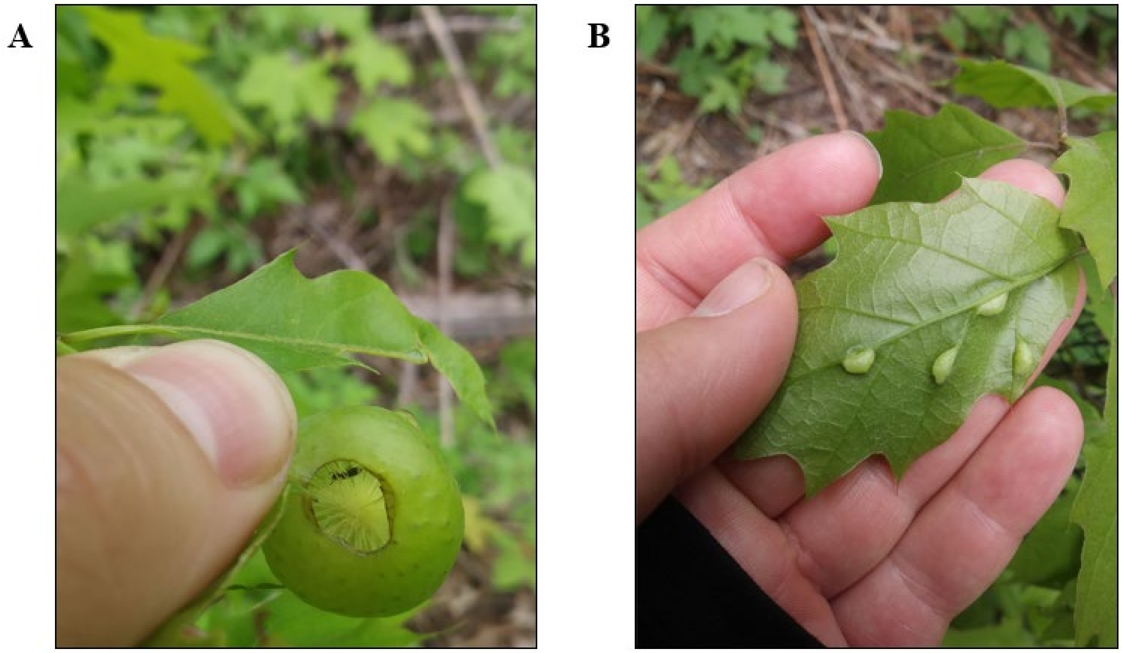

Figure A5. Types of galls formed in leaf tissue. **A)** Oak apple galls formed by wasps of the family *Cynipidae.* B) Pocket vein galls formed by larval flies of the family *Cecidomyiidae.*

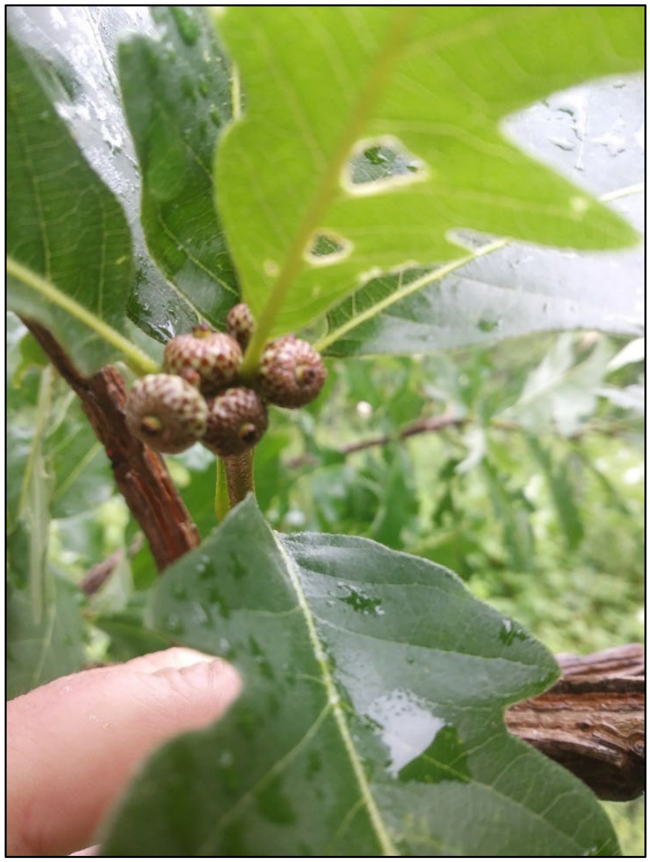

Figure A6. Acorns on *Q. macrocarpa* study tree. There were four overall instances of acorn growth, one in 2020, three in 2023 (see *Differences in Reproduction*). No acorns grew to maturity before predation.