

Population variation in *Fraxinus americana* L. (white ash) in a common garden at the edge of the species range

by

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Abstract

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High levels of intraspecific variation in morphology, physiology, phenology, and growth are commonly observed in tree species with large ranges that cover different climatic, edaphic, and biotic regions. It is important to understand how ecologically important traits vary in natural populations because it furthers our understanding of the roles of natural selection, gene flow, and genetic drift in shaping levels of adaptation and in affecting the distribution and abundance of species. My research explored genetic variation in physiologically important traits in *Fraxinus americana* L. (white ash) by examining 44 populations of 30-year-old trees growing in a common garden at the western edge of the species range.

The main objectives of my thesis research were to (1) determine if there are differences in morphology, phenology, gas exchange, and stomatal regulation among *F. americana* populations, (2) determine if intraspecific variation in these traits affects growth and survival in the species, and (3) correlate intraspecific variation in *F. americana* with a corresponding climate variable in order to define the causal mechanism driving genetic divergence in the species.

Stem circumference and survival varied significantly among the 44 populations of *F. americana* in the common garden. There was a 26-day range for the date of leaf emergence among populations in the common garden, and northern

populations have higher temperature requirements for bud break than southern populations. LMA in *F. americana* correlated with the mean annual precipitation at the location of population origin, indicating that populations from drier areas had thicker leaves. Foliar carbon isotope ratios ($\delta^{13}\text{C}$) indicated that there are differences in stomatal regulation of c_i/c_a among the 44 populations in the common garden. Populations originating from the western edge of the range have higher foliar $\delta^{13}\text{C}$, and thus higher water use efficiency, than populations originating from further east.

Intraspecific differences in morphology, phenology, and stomatal regulation were closely related to the climate of origination in *F. americana*. Populations with the highest stem circumference and survival at the edge of the species range originated from climates of similar annual temperature and precipitation to that of the common garden. Identifying the influence of temperature and precipitation on the growth and survival of *F. americana* from different regions is an important step towards understanding how this species will respond to future climate change scenarios and for determining the level of genetic variation for potential adaptive responses within the species.

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Chapter 1

Introduction

In the colonization of the terrestrial biosphere, plants evolved many adaptive traits, including a more rigid growth form and a vascular system that could deliver water and nutrients to aboveground tissues. In the millions of years since these developments, plants have evolved many other adaptations that have allowed them to colonize the large diversity of environments found on Earth. For example, *Welwitschia mirabilis*, a gymnosperm native to the Namib Desert in Africa, can tolerate the exceedingly low leaf water potential of -6.0 MPa (Herppich et al. 1997), and *Betula papyrifera* (paper birch) can survive temperatures below -80°C (Marchand 1991). Such interspecific differences among plants illustrate how plant species have succeeded in occupying diverse ecosystems from the tropics to the poles.

Although studies of interspecific variation in plant groups are necessary to understand the evolution of the diversity of plant species now in existence, studies of intraspecific differences are important for understanding the potential for adaptive evolution within plant species at a microevolutionary scale. It is these differences that will dictate species responses to changes in environmental conditions. For example, recent climate changes, including temperature shifts and changes in precipitation patterns that are a result of the anthropogenic release of carbon dioxide and other greenhouse gases into the atmosphere, will certainly affect the current ranges of many plant species. The rate of this climate change is potentially faster than any the Earth

has experienced since the end of the Younger Dryas, a period approximately 10,000 years ago, when northern hemisphere temperatures are thought to have risen 7°C in 50 years (Puhe and Ulrich 2001; Weart 2003).

The goal of my thesis research is to quantify the intraspecific variation of a deciduous tree species by comparing the responses of trees from different populations when all are grown in a common garden that is located in Kansas, at the western edge of the species range. The study species *Fraxinus americana* L. (white ash), a tree native to the temperate deciduous forests of North America, was examined for population variation in morphology, phenology, gas exchange physiology, and stomatal regulation. Additionally, I determined if intraspecific variation in these traits affected growth and survival in *F. americana* and attempted to correlate population variation with corresponding climate variables in order to elucidate the causal mechanism driving genetic divergence in the species. My main hypothesis was that *F. americana* trees would exhibit variation in all of the examined traits as a result of local adaptation to different climate conditions across the large range. This research can provide insights into how the species may respond to climate changes in its native range.

Plant Adaptation to Local Climate

Geber and Dawson (1993) performed a survey of literature published since 1986 and found over 100 studies of ecotypic or geographic differentiation for a wide variety of traits in plant populations. Differentiation for traits, including phenology,

morphology, growth form, temperature tolerance, drought tolerance, gas exchange, nutrient and edaphic relations, and tolerance to salts, metals, and/or airborne pollutants, were often related to environmental differences among population habitats. Here I describe documented cases of intraspecific variation in tree species that have been identified by other research groups.

Both gymnosperm and angiosperm trees are likely to exhibit genotypic variation for ecologically important traits, because these trees often have large ranges that cover different climatic, edaphic, and biotic regions (Puhe and Ulrich 2001). Much research has focused on the interaction of plant growth with the surrounding environment, and this research has revealed that the physiological differences among populations of tree species in phenology, morphology, and gas exchange are mainly driven either by temperature differences or by differences in water availability in the populations' local climate (Kozlowski et al. 1991).

Intraspecific differences in temperature tolerance among populations of plant species exist in two forms, heat tolerance and cold tolerance. Heat stress reduces the stability of membranes in plant tissues, causing a leakage of ions from cells and a reduction of photosynthesis (Taiz and Zeiger 2002). Tolerance to heat stress can be increased in plants through higher protein thermostability and through the production of heat shock proteins, which protect proteins from misfolding and inactivating under high temperatures (Kozlowski et al. 1991). Differences such as increased root to shoot ratios or reduction of the tissue osmotic potential have been found among provenances of plant species as adaptations to heat stress and high soil temperatures

(Hällgren et al. 1991). Colombo and colleagues (1992) found evidence that heat tolerance is heritable in *Picea mariana* (black spruce) families. One family consistently possessed above average heat tolerance, and two clones expressed differences in heat shock protein synthesis.

Populations of tree species, including *Cercis canadensis* (eastern redbud), *Cornus sericea* (red-osier dogwood), *Betula pubescens* (pubescent birch), and *Picea asperata* (Dragon spruce), also show ecotypic differences in cold tolerance (Donselman and Flint 1982; Karlson et al. 2003; Li et al. 2004; Welling et al. 2004). Cold tolerance is important for preventing chilling or freezing injury in plants at low temperatures. Chilling injury can decrease photosynthesis and respiration and can cause rapid wilting and necrosis of leaves in plants, while freezing injury can cause death in unacclimated or unhardened plants (Hällgren et al. 1991). Death of plants exposed to freezing temperatures is caused by the formation of intracellular ice crystals, and so many plants have several mechanisms to prevent this type of ice formation in tissues (Kozłowski et al. 1991). One mechanism of cold tolerance, supercooling, prevents the formation of intracellular ice crystals in temperatures below the freezing point through the formation of ice in the intercellular spaces, which releases heat energy as a result of the latent heat of fusion of water (Taiz and Zeiger 2002). Deep supercooling has been found in over 240 species of woody plants (Kozłowski et al. 1991) and is more common in lower latitudes where air temperatures do not fall below -40°C , the temperature at which ice nucleation spontaneously occurs (Taiz and Zeiger 2002). Resistance to freezing temperatures can

also be accomplished through cold hardening, the mechanism for survival in trees from higher latitudes. Cold hardening generally occurs in two stages; the first stage is induced in autumn by short days and chilling temperatures, causing the removal of water from cells which allows trees to survive temperatures below 0°C. Direct exposure to freezing temperatures then fully hardens plants to the point where temperatures of -50 to -100°C can be tolerated (Taiz and Zeiger 2002). Different amounts of cold hardiness can be attained in populations of the same tree species. For example, northern populations of *F. americana* were found to survive temperatures as low as -42.6°C, whereas southern populations were killed at -33.7°C (Alexander et al. 1984).

Tree species also have different phenological responses among populations to avoid cold damage, including timing for initiation of cold hardening in autumn and subsequent bud burst in the spring. For example, *Betula pendula* (European white birch) has large genetic variation for the duration of bud burst among eight clones (Rousi and Pusenius 2005). In addition, southern ecotypes of *Populus trichocarpa* (black cottonwood) show a significant delay in bud set under a range of photoperiods when compared to a northern ecotype (Howe et al. 1995). Four populations of *Quercus ithaburensis* (prickly-cupped oak) near the Mediterranean Sea show distinct phenological patterns of leaf abscission and leaf flushing – populations from the mildest climate had the shortest period of leaflessness and the highest proportion of evergreen trees while populations further from the sea had longer periods of

leaflessness and lower proportions of evergreen trees (Ne'eman 1993), illustrating the wide range of phenological patterns that can be found in one tree species.

Changes in water availability across a species range drive intraspecific differences in drought tolerance in trees (Schmidting and Froelich 1993; Roupsard et al. 1998; Adams and Kolb 2004). Water is one of the most important resources for tree growth; up to 90% of the variation in the annual width of tree rings can be explained by variations in rainfall (Kozlowski et al. 1991). Tree populations in drier habitats can have higher ratios of root mass to foliage area to enhance the ability of individual trees to obtain soil water (Li et al. 2000). Other drought adaptations in tree populations include osmotic adjustment, a net increase in the cellular solute content that is independent of volume changes from the loss of water, and the modulation of cell wall elasticity. Osmotic adjustment permits turgor-dependent processes, such as stomatal opening, photosynthesis, and root growth, to occur at lower leaf water potentials (Taiz and Zeiger 2002). Genotypes of *Betula pendula* (silver birch) from areas of lower rainfall display larger osmotic adjustment than higher-rainfall genotypes when grown in a common garden (Aspelmeier and Leuschner 2004). An increase in cell wall elasticity, measured as a decrease in the bulk elastic modulus (ϵ), is an adaptation to drought, because it lowers the leaf water content at which cell turgor pressure is lost (Hinckley et al. 1991). Several tree species, such as *Betula populifolia* (gray birch), *Quercus crispula*, and *Quercus serrata*, have been found to decrease ϵ (increase tissue elasticity) to effectively maintain cell turgor during water stress (Morse et al. 1993; Saito and Terashima 2004). The woody plant *Gutierrezia*

sarothrae (broom snakeweed) shows intraspecific variation in ϵ among populations, and populations with lower ϵ were able to maintain leaf turgor at lower relative water content than other populations in the native range (Wan et al. 1998). However, no intraspecific differences in cell wall elasticity have been demonstrated within tree species to date (Clifford et al. 1998).

Intraspecific differences in morphology and growth form have been documented in tree species as an adaptation to drought (Donselman and Flint 1982; Abrams 1994; Schuler 1994; Palmroth et al. 1999; Harris and Beever 2000; Bacelar et al. 2004). Some tree species contain populations with different widths of xylem vessels, and drought-tolerant populations often have narrower vessels that are less vulnerable to cavitation and the formation of embolisms that prevent water movement in the xylem (Hinckley et al. 1991). For example, mesic *Pseudotsuga menziesii* (Douglas fir) populations are more susceptible to stem and root cavitation when compared to xeric populations (Kavanagh et al. 1999). Other trees, such as *F. americana* and *Pinus sylvestris* (Scots pine), have different growth forms across the species range (Schuler 1994; Palmroth et al. 1999). In a common garden experiment in West Virginia, Schuler (1994) examined differences in growth form among *F. americana* populations and found that populations from West Virginia contained the highest percentage of crop trees. Palmroth and colleagues (1999) found that growth form in *P. sylvestris* is genetically controlled – the smallest, most branched trees examined in western Europe originated from the more southern, drier climates. Growth forms that are caused by climatic differences in the species range can evolve

into permanent features of the genotype and thus are another source for intraspecific variation in response to drought.

Many tree species, including *C. canadensis*, *Quercus rubra* (red oak), *Olea europaea* (olive), and *Cordyline australis* (cabbage tree), have different leaf morphologies among populations (Donselman and Flint 1982; Abrams 1994; Harris and Beever 2000; Bacelar et al. 2004). In drought-tolerant tree species, leaves are often thicker, have stomata that are highly responsive to vapor pressure deficit (VPD), and have a relatively thick, waxy cuticle that reduces cuticular transpiration (Hällgren et al. 1991). For example, *C. canadensis* has thicker and smaller leaves with increased pubescence and an efficient stomatal geometry that are correlated with evapotranspirational trends over the natural species range, reaching extremes in the xeric western populations (Donselman and Flint 1982).

In order to optimize growth and survival in a given location, plants must tightly regulate stomatal aperture in order to control carbon dioxide uptake and water loss via transpiration. It has been found that stomatal conductance and photosynthetic rates of xeric tree populations are generally lower than in those populations with greater access to water (Abrams 1994; Aspelmeier and Leuschner 2004). There are two main water-use strategies commonly used by trees in different climates – the prodigal water-use strategy, most often found in wetter environments, and the conservative water-use strategy, found in dry environments. Intraspecific differences in water-use strategies can be easily analyzed through the comparison of the carbon isotope ratios of foliar or woody tissue among tree populations. The stable carbon

isotope composition of leaves is a time-integrated index of the ratio of leaf internal to ambient CO₂ concentration (c_i/c_a) that can be used to infer photosynthetic water use efficiency, WUE (Farquhar et al. 1989; Ehleringer 1991).

Li (1999) examined the carbon isotope ratios of leaves from three native Australian populations of *Eucalyptus microtheca* (Coolibah). He determined that individuals of a southeastern population from a wetter habitat had a lower WUE and might employ the prodigal water-use strategy, whereas those in the northwestern and central populations with higher WUE may employ the conservative water-use strategy. Measurements of foliar carbon isotope ratios were also used to identify differences between *Pinus ponderosa* (Ponderosa pine) populations at low and high elevations in ecotone forests of northern Arizona (Adams and Kolb 2004). Low-elevation populations had a greater growth response to drought than high-elevation populations, although the increase in WUE due to drought was similar between populations (Adams and Kolb 2004). Intraspecific differences in drought tolerance are not only attributable to moisture differences among habitats, but may be caused by variations in exposure to sunlight among tree populations. Abrams (1994) found that *Prunus serotina* (black cherry) shade trees were more drought tolerant than sun-adapted trees and were able to maintain a higher rate of light-saturated photosynthesis with increasing drought.

Intraspecific variation in water relations occurs not only among populations separated geographically, but can also occur sympatrically between genders in dioecious tree species. For example, the tree ring cellulose of females in *Acer*

negundo (box elder) has a lower carbon isotope ratio than males, indicating less conservative water use, and thus female *A. negundo* trees are capable of more growth than male trees during wet years in Utah (Ward et al. 2002). This is an exception to the rule for many dioecious species, where male trees often exceed females in total plant size, plant height, growth rates, and frequencies in populations (Dickson 1991; Obeso 2002). Furthermore, differential responses of males and females have also been observed in *Populus tremuloides* (quaking aspen) in response to elevated CO₂ (Wang and Curtis 2001). Thus, intraspecific differences between genders can have important effects on more than just tree water relations, by also influencing the survival, distribution, and population structure of dioecious tree species.

Intraspecific differences in tree species are commonly observed, and a thorough understanding of the physiological tolerances of individual populations is important because this information can be used to determine the potential for adaptive evolution in a species. By understanding intraspecific differences in a tree species, it is also possible to ascertain which genotypes of a tree species may be most successful in a given region under both current and predicted future climates.

Predicted Climate Change

Rising [CO₂] in the atmosphere from the anthropogenic burning of fossil fuels is predicted to increase the global mean temperature by at least 1.4–5.8°C over the next century (McCarthy et al. 2001), and a more recent computer climate simulation found global temperatures could increase by up to 11°C (Stainforth et al. 2005). On a

global scale, the twentieth century has been the warmest since 1400, and this warmer atmosphere can lead to increased evapotranspiration from ecosystems and increased global precipitation (Puhe and Ulrich 2001), but may reduce local soil water availability for plants. Furthermore, on a regional scale, precipitation may either increase or decrease in a given area.

Within the native range of *F. americana*, namely the eastern half of the United States, the predicted temperature and precipitation changes differ across regions (NAST 2000). In the northeast, winter minimum temperatures are projected to increase by 2–5°C, while models predict a range of potential changes in precipitation, from 25% increases to small regional decreases, by 2100 (NAST 2000). There is an increasing likelihood for severe drought in the central regions of the United States (McCarthy et al. 2001), making this area vulnerable to vegetation changes as the drier climate facilitates the spread of grasslands to the east, displacing the deciduous forests (Lauenroth et al. 2004). In the southeast, climate model projections offer two opposing, yet plausible scenarios for climate changes over the next century. One model, the general circulation model (GCM) produced by the Canadian Climate Centre (United Kingdom), predicts a high degree of warming with little changes in rainfall, which translates into large decreases in soil moisture (NAST 2000). The Hadley Centre (United Kingdom) GCM, on the other hand, shows less warming and a 20% increase in precipitation (NAST 2000).

Another aspect of future climate change that will have a global effect is the projected increase in the variability of year-to-year precipitation in natural

ecosystems. The El Niño Southern Oscillation (ENSO) in the Pacific Ocean has been increasing in frequency since approximately 1976–1977 and has been recognized for some time as one of the causes for this global climatic variability (Puhe and Ulrich 2001). In addition, the North Atlantic Oscillation (NAO) has strengthened in recent decades to a historic maximum in the early 1990s and is one cause for increased climate variability in Europe (Beniston and Innes 1998). The current rapid rate of climate change may not be unprecedented in the history of the Earth, but human influences are altering a climate that has been stable for at least 10,000 years and these changes will affect the current distribution of plant species (Puhe and Ulrich 2001) and may result in rapid selection responses within populations.

Plant Adaptation to Climate Change

The survival of plant species in the future will require individuals in populations to tolerate changes in precipitation patterns and the maximum and minimum temperatures of their current ranges. In addition to tolerating climatic changes as they occur, tree species must also maintain a competitive advantage in order to persist in a given area. If, for example, the frequency of drought increases, as is expected in parts of the central United States, tree populations in these areas will begin to decline. Although some tree populations may go locally extinct because of climate change, there is the possibility that in the future nearby drought-tolerant populations of the same species may replace the lost population. Climate change fosters a dynamic period of local colonizations and extinctions in natural ecosystems.

Thus, before predictions can be made about the response of any tree species to future climate change, it is necessary to determine the different climatic and resource requirements within individual populations throughout the species range and to quantify genetic variation for responses to climate change factors.

In the past, plant species have responded to climate change by migration or adaptation. Migration during the Quaternary glacial and interglacial periods allowed tree species to survive large changes in the global climate by retreating to southern refuges and then recolonizing northern sites after the ice sheets receded (Tzedakis et al. 2002). The current fragmentation of habitats by humans, however, is likely to impede plant migration in the future. In addition, unlike migration during the early Holocene, species range expansion will depend on seedling establishment in occupied habitats (Etterson and Shaw 2001).

Thus, in order for species to survive future climate changes, populations will be more heavily dependent on adaptive evolution (relative to migration) than in the past. One major criterion for evolutionary responses within plant species is the existence of genetic variation among populations (Etterson and Shaw 2001), and therefore the potential for adaptive evolution in a plant species is dependent on the current genetic composition of the populations across its range. The few studies that have attempted to estimate the potential rates of evolutionary response in plant species have found that these rates may be too slow relative to the predicted rate of climate change (Etterson and Shaw 2001 for *Chamaecrista fasciculata*, sleeping plant; Potvin and Tousignant 1996 for *Brassica juncea*, leaf mustard). This potential

limitation to evolution suggests the necessity of additional gene flow between populations, fostered by management practices, in order to ensure the survival of certain plant species (Puhe and Ulrich 2001).

The Common Garden Approach

A common garden is an ideal experimental system with which to examine the differences in the morphology, physiology, growth, and survival of populations originating from sites across a species range. Since all populations are growing under the same conditions, any differences in the physiological responses and survival of populations are attributable to genetically based character divergence. Phenotypic plasticity, which in and of itself is a selectable character, is also important in long-lived species such as trees, because it allows an individual to adjust to gradual environmental changes and may be key for allowing the species to persist during rapid climate change.

Common garden experiments can be used to understand which populations are best adapted to a specific climate, and thus have the highest chance of survival at that location, as well as which populations are not well adapted and have a low chance of survival. The influence of climate on the growth of trees is particularly discernible where the trees grow in marginal environments (Puhe and Ulrich 2001), and so such studies may be particularly insightful at the edge of a species range. Moreover, it has been suggested (Hoffman and Blows 1993) that the effects of global change will first be manifested along the edges of the range for many plant species.

Forest growth in the interiors of continents is limited by the supply of water. As a result of decreasing precipitation and longer summer droughts, forests are replaced by grasslands (Puhe and Ulrich 2001), with the exception of riparian zones where trees are plentiful even in arid environments. In the continental interiors of the

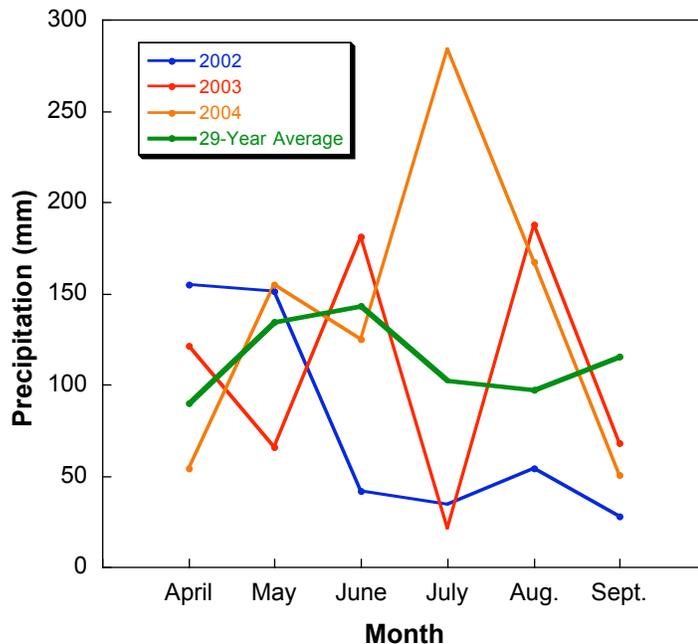


Figure 1-1. A comparison of monthly precipitation in Kansas during the growing seasons of 2002-2004 with the average precipitation (calculated from the last 29 years) reveals variable year-to-year precipitation patterns at this location.

United States, the temperate deciduous forests are replaced by prairie ecosystems. One area of transition between forest–grassland ecosystems is along the eastern border of Kansas. Here the amount of precipitation during the growing season in Kansas is highly variable from year to year (Figure 1-1). For example, in 2002 it remained much drier than average throughout the length of the growing season (total growing season precipitation = 464 mm), while 2003 and 2004 were both wetter than average, but with a very different amount of precipitation in July (total growing season precipitation = 645 and 835 mm, respectively). Because there is high interannual variation in the amount of precipitation, a common garden in Kansas can be used to assess drought tolerance among tree populations. A decrease in the amount

of precipitation has been predicted for the central regions of the United States, and possibly in the southeastern United States, so a common garden study in Kansas will also provide important insight into the potential for survival of tree populations originating from a variety of regions throughout the range.

Research Species: *Fraxinus americana*

The natural range for *F. americana* is large (see Figure 1-3), extending from Texas to Minnesota in the west and Nova Scotia to Florida in the east (Schlesinger 1990). Models for predicting potential suitable habitat for tree species under future climate change scenarios indicate that *F. americana* will decrease its range as the optimum latitude for its habitat shifts north (Iverson and Prasad 1998). Because *F. americana* is known to be genetically variable throughout its range, partly owing to the presence of polyploidy (Wright 1944), it is an ideal species for a common garden study. The differences in ploidy level vary across the species range, with diploids (46 chromosomes) occurring throughout the species range. Tetraploids (92 chromosomes), however, are restricted to regions south of latitude 35°N, and hexaploids (138 chromosomes) are found between latitudes 35° and 40°N (Black and Beckmann 1983; Schlesinger 1990). These genetic differences in geographic regions of the species range may contribute to different local adaptations among *F. americana* populations.

Because *F. americana* is a pioneer species in colonizing abandoned fields, it is important for forest regeneration across its range. Primarily characteristic of early and

intermediate stages of succession, the seedlings can establish in full sun, but are also shade tolerant (Schlesinger 1990). Mature individuals, however, are shade intolerant, but can persist for years in moderately dense shade and later reach the overstory by responding quickly to openings in the canopy (Nesom 2000). Additionally, the winged seeds provide food for many North American bird species (Schlesinger 1990). The wood of *F. americana* is valued for its strength, hardness, heavy weight, and elasticity and has been used in wooden baseball bats, furniture, and tool handles (Nesom 2000). It is a commonly planted tree in urban areas as well, because its large canopy provides shade in parks, campuses, and along streets (Nesom 2000).

Several pests and diseases pose a serious threat to the continued presence of *F. americana* in its natural habitat (Nesom 2000; Muirhead et al. 2006). Ash decline, a disease complex affecting *F. americana* from the Great Plains to the Atlantic coast at 39–45°N, is especially prevalent in the northeastern United States. Mycoplasma-like organisms are associated with dying trees, but ash decline is also thought to result from atmospheric pollution and frequent droughts (Nesom 2000). *Agrilus planipennis* (emerald ash borer), a wood-boring beetle native to southeast Asia, poses an enormous threat to *Fraxinus* species of North America and was first reported in the Great Lakes region during the summer of 2002 (Muirhead et al. 2006). Untreated trees in infested areas of Ontario, Michigan, and Ohio suffer high mortality, and *A. planipennis* range expansion in the Great Lakes region has continued despite deployment of an array of management strategies (Muirhead et al. 2006).

In addition to the ecological and economic characteristics that make *F. americana* an important component of temperate deciduous forests, there are several growth traits that are important to consider when performing research on the species. Shoot growth in *F. americana* is determinate, meaning that all leaves are preformed in the resting bud and the next year's leaves are formed as soon as the shoots stop expanding (Strobl and Bell 2000). This species, a ring-porous angiosperm, simultaneously reactivates the cambium of its branches and trunk in early spring. Ring-porous tree species form their first xylem vessels at or just prior to bud break in the spring, which gives the advantage of a water supply early in the growing season, although these species often have a shorter growing period than many diffuse-porous tree species (Aloni 1991). In addition, *F. americana* is a dioecious plant, although physiological or growth differences between genders have not been tested in this species to date.

Overview of Thesis Research

The overall goal of my thesis research was to analyze the intraspecific differences in morphology, physiology, growth, and survival of *F. americana* in a common garden at the edge of the species range in order to understand how populations and genders respond to a variable climate. I have chosen to concentrate mainly on population variation throughout the geographic range, rather than on variation due to ploidy, because population variation is generally attributable to the environmental conditions of the habitat of origination. Thus, the responses of *F.*

americana populations and
genotypes in Kansas can
provide insights into how the
species may respond to
climate changes across its
native range.

For my research, I
have utilized a *F. americana*
common garden (Figure 1-
2) located at 39.0°N,



Figure 1-2. Over 1100 trees were planted in Kansas in 1976 by the North Central Forest Experiment Station to create the *Fraxinus americana* common garden (pictured here in the spring of 2005).

95.2°W in the Nelson Environmental Study Area (University of Kansas) in Jefferson county, northeast of Lawrence, Kansas. The *F. americana* trees were grown from open-pollinated seeds that were collected from up to ten parent trees at each of the 31 locations that are represented in the common garden (Figure 1-3). The North Central Forest Experiment Station (USDA Forest Service) planted the seeds in a nursery in Illinois and later transplanted the one-year-old seedlings to the common garden in 1976 as part of a rangewide provenance test of *F. americana* (Clausen 1984a; Clausen 1984b; Rink and Kung 1991). The trees were 30 years old at the time of this study. The common garden is set up in a block design where each of the 44 populations was originally represented by 25 trees – five blocks with five replicate trees from each population (see Figure 5-1, Appendix). A nearby climate station operated by the University of Kansas Field Station and Ecological Reserves records

daily precipitation, daily minimum and maximum temperatures, and hourly relative humidity.

The second chapter of my thesis examines how the 44 populations of *F. americana* respond to a variable climate at the edge of their species range. I measured the stem circumference and survival of the 44 populations after 29 years at the common garden. To interpret differential growth and survival patterns among *F.*

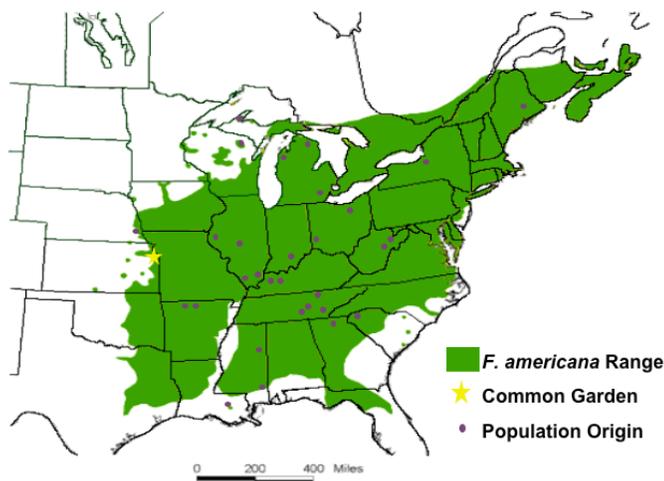


Figure 1-3. The native range of *Fraxinus americana* (white ash) spans much of the eastern United States and extends into southern Canada. The purple dots indicate the locations of origination of the *F. americana* populations that are present in the common garden, which is located at Nelson Environmental Study Area, near Lawrence, Kansas (indicated with a yellow star). (USDA Forest Service)

closest to the common garden would have the highest growth and survival, because they are more closely adapted to the variable amount of precipitation on the western edge of the range.

americana populations, I also analyzed phenology, leaf gas exchange physiology, and stomatal regulation in *F. americana* populations. These responses were compared to climate differences across the native range of *F. americana* to determine possible causal mechanisms for intraspecific variation. I hypothesized that the populations originating

The main objective of the third chapter of my thesis was to determine if any differences in morphology or water relations exist among populations and genders in *F. americana*. To examine the population effect, leaf mass per unit area, stomatal conductance, daily changes in leaf water potential, and foliar carbon isotope ratios were compared among eleven populations in the common garden. The eleven populations form an east–west gradient (at 39°N, the optimal latitude for growth and survival in the common garden) across the *F. americana* range where there is a large range in mean annual precipitation. My hypothesis was that the populations from the drier, western edge of the species range would have a higher WUE than the other *F. americana* populations. I also compared daily changes in leaf water potential, foliar carbon isotope ratios, and growth of male–female pairs of trees in order to determine if gender contributed to intraspecific variation in the species. I hypothesized that female *F. americana* trees would have a lower water status than male trees, as well as lower overall growth, as a result of a greater investment in reproductive structures.

Chapter 2

Potential causes for growth and survival differences among populations of *Fraxinus americana* L. (white ash) in a common garden at the edge of the species range

Abstract

Population variation in *Fraxinus americana* (white ash) in a common garden of 30-year-old trees at the edge of the species range in northeastern Kansas, USA was examined in order to (1) determine if intraspecific variation in various physiological traits affects growth and survival in *F. americana* and (2) correlate any intraspecific variation in *F. americana* to either the Kansas climate or to local climatic factors throughout the species range. No intraspecific differences were found for light-saturated photosynthesis (A_{sat}) or stomatal conductance (g_s) in the common garden. Phenology and stomatal regulation, however, were found to vary significantly among the 44 *F. americana* populations in the common garden, and the differences are correlated with climatic conditions at the locations of population origination. The growing degree day ($^{\circ}\text{D}$) for leaf emergence of *F. americana* populations in the common garden was positively correlated with the latitude of population origination ($R^2 = 0.47$). The *F. americana* populations with higher foliar carbon isotope ratios, and thus higher water use efficiency, had higher stem circumference and survival in the common garden. These intraspecific differences in physiology caused a wide range of growth and survival among populations of *F. americana* in the common garden. Overall, the differential growth and survival of *F. americana* populations at the edge of the species range is well-correlated with several temperature parameters at

the sites of population origination ($R^2 = 0.53\text{--}0.61$ and $R^2 = 0.67\text{--}0.71$, respectively). A thorough understanding of the physiological tolerances of individual populations can be used to determine the potential for adaptive evolution in the species, as well as which genotypes may be most successful in a given region under predicted future climates. Possible impacts of climate change on the growth and survival of *F. americana* populations in areas of the native range that are expected to become drier in the future are discussed.

Introduction

High levels of intraspecific variation in physiology, growth, and survival are commonly observed in tree species with large ranges that cover different climatic, edaphic, and biotic regions (Donselman and Flint 1982; Geber and Dawson 1993; Abrams 1994; Aspelmeier and Leushner 2004). Tree physiology often changes along climatic gradients as a result of adaptations to changes in temperature, water availability, or other abiotic and biotic factors throughout the species range. It is important to understand how ecologically important traits vary in natural populations because it furthers our understanding of the roles of natural selection, gene flow, and genetic drift in shaping levels of adaptation and in affecting the distribution and abundance of species (Jonas and Geber 1999).

Fraxinus americana L. (white ash) is an early successional species in the temperate deciduous forests of the United States. This hardwood tree is important for forest regeneration across its range, as it is often one of the first trees to colonize

abandoned fields (Schlesinger 1990). This species is an ideal candidate for studies aimed at examining intraspecific differences in the physiology of trees, because it has been shown to be genetically variable throughout its range (Wright 1944). In addition, growth and survival varied among populations of *F. americana* and were previously found to be correlated with the latitude of origination when 45 populations were grown in a common garden in West Virginia (Schuler 1994).

A thorough quantification of the intraspecific variation in the physiology of *F. americana* allows a better understanding of the potential for adaptive evolution in the species. Furthermore, it is possible, with this information, to determine which genotypes may be most successful in a given region under predicted future climates. A common garden study in northeastern Kansas, where interannual precipitation during the growing season is highly variable and droughts are frequent, provides important insights into the likelihood for survival of tree populations in an increasingly variable climate (NAST 2000; Lauenroth et al. 2004). Models for predicting potential suitable habitat for tree species under future climate change scenarios indicate that *F. americana* will decrease its range as the optimal latitude for its habitat shifts north (Iverson and Prasad 1998). In addition, several areas that are covered within the white ash range, specifically the midwestern and the southeastern United States, are predicted to become drier in the near future (NAST 2000; McCarthy et al. 2001), potentially decreasing the range of this species.

I quantified intraspecific differences in the physiology, growth, and survival of 44 *F. americana* populations in a common garden in northeastern Kansas at the

edge of the *F. americana* range, where the influence of climate on the growth of trees is easily discernible (Puhe and Ulrich 2001). Phenology and foliar carbon isotope ratios were analyzed to compare physiological adaptations to climate among the 44 *F. americana* populations. Additionally, leaf gas exchange was assessed in ten *F. americana* populations whose location of origination formed a latitudinal gradient centered around a longitude of 89°W. These ten populations were chosen to test the hypothesis that temperature and/or photoperiod are adaptive pressures that can cause differentiation in physiological traits among tree populations. Such genetic variation in physiological and developmental parameters among populations of plant species can improve the accuracy of models that predict species responses to climate change (Panek and Waring 1997).

My specific objectives were to (1) determine if there are differences in phenology, gas exchange, and stomatal regulation among *F. americana* populations, (2) determine if intraspecific variation in these traits affects growth and survival in *F. americana*, and (3) correlate intraspecific variation in *F. americana* to either the Kansas climate or to local climatic factors throughout the species range. I hypothesized that the populations originating closest to the common garden would have the highest growth and survival, because they are more closely adapted to temperature patterns and the variable amount of precipitation of the western edge of the species range.

Materials and Methods

Research species

F. americana is a ring-porous angiosperm native to the temperate deciduous forests of the eastern United States, with a natural range extending from Texas to Minnesota in the west and Nova Scotia to Florida in the east (Schlesinger 1990). Shoot growth in *F. americana* is determinate, and trees in the common garden only have one flush of leaves each growing season (R. Marchin, personal observation). There is a high degree of variation in climatic variables throughout the *F. americana* range (Table 2-

Table 2-1. Climatic data from the common garden (located in Jefferson county, KS, USA) and the locations represented in the common garden

| Location | Latitude (°N) | Longitude (°W) | Elevation (m) | Annual Precipitation (mm) | Annual Temperature (°C) | Growing Season VPD (kPa) |
|----------------------|---------------|----------------|---------------|---------------------------|-------------------------|--------------------------|
| Jefferson, KS | 39.0 | 95.2 | 299 | 879 | 11.9 | 0.633 |
| Ontonagon, MI | 46.6 | 89.5 | 408 | 820 | 4.3 | 0.400 |
| Forest, WI | 45.7 | 89.0 | 511 | 777 | 5.6 | 0.428 |
| Presque Isle, MI | 45.3 | 83.6 | 198 | 749 | 6.1 | 0.509 |
| Penobscot, ME | 44.8 | 69.0 | 85 | 1083 | 6.2 | 0.519 |
| Benzie, MI | 44.7 | 86.0 | 236 | 793 | 6.4 | 0.530 |
| Onondaga, NY | 42.7 | 76.1 | 381 | 940 | 8.3 | 0.579 |
| Washtenaw, MI | 42.2 | 83.7 | 259 | 799 | 9.0 | 0.637 |
| Wayne, OH | 40.7 | 82.0 | 265 | 969 | 9.7 | 0.592 |
| Otoe, NE | 40.6 | 95.7 | 259 | 765 | 10.8 | 0.639 |
| Adams, IL | 39.8 | 90.7 | 213 | 943 | 10.9 | 0.633 |
| Preble, OH | 39.6 | 84.7 | 305 | 1035 | 11.7 | 0.699 |
| Tucker, WV | 39.1 | 79.5 | 762 | 1261 | 9.3 | 0.463 |
| Effingham, IL | 39.1 | 88.4 | 183 | 1025 | 11.9 | 0.631 |
| Effingham, IL | 39.0 | 88.4 | 177 | 1025 | 11.9 | 0.631 |
| Randolph, WV | 38.9 | 79.7 | 975 | 1261 | 9.3 | 0.463 |
| Jackson, IN | 38.9 | 86.0 | 191 | 1142 | 12.3 | 0.670 |
| Jackson, IL | 37.7 | 89.4 | 158 | 1097 | 13.3 | 0.760 |
| Gallatin, IL | 37.6 | 88.3 | 152 | 1125 | 13.3 | 0.680 |
| Muhlenberg, KY | 37.3 | 87.2 | 128 | 1220 | 13.9 | 0.547 |
| Hopkins, KY | 37.3 | 87.6 | 139 | 1220 | 13.9 | 0.547 |
| Overton, TN | 36.5 | 85.4 | 357 | 1408 | 13.6 | 0.627 |
| Marion, AR | 36.4 | 92.8 | 274 | 1155 | 14.5 | 0.672 |
| Boone, AR | 36.4 | 93.0 | 274 | 1164 | 14.2 | 0.652 |
| Bledsoe, TN | 35.5 | 85.2 | 396 | 1408 | 13.6 | 0.680 |
| McMinn, TN | 35.3 | 84.5 | 251 | 1263 | 13.9 | 0.690 |
| Franklin, TN | 35.2 | 85.9 | 357 | 1408 | 13.6 | 0.680 |
| Pickens, SC | 35.0 | 83.0 | 229 | 1295 | 15.6 | 0.779 |
| Union, GA | 34.8 | 83.9 | 914 | 1378 | 15.3 | 0.738 |
| Oktibbeha, MS | 33.4 | 88.8 | 116 | 1383 | 17.2 | 0.639 |
| East Baton Rouge, LA | 31.5 | 91.0 | 9 | 1559 | 19.3 | 0.686 |
| George, MS | 30.8 | 88.8 | 76 | 1602 | 19.4 | 0.732 |

1), with mean annual temperature ranging from 4.3–19.4°C and mean annual precipitation ranging from 749–1602 mm.

Common garden and site characteristics

The common garden of *F. americana* trees was planted in 1976 and is located at the edge of the species range at 39.0°N, 95.2°W in the Nelson Environmental Study Area (University of Kansas) in

Jefferson county, Kansas. At the common garden, the average annual precipitation is 879 mm and the average

annual temperature is 11.9°C (Table 2-1). The 30-year-old *F. americana* trees in the common garden were grown from open-pollinated seeds that were collected from native parent trees at each of the 44 locations that are represented in the common garden (Figure 2-1). The common garden is set up in a block design (see Figure 5-1, Appendix) with each of the 44 populations represented by 25 trees (5 blocks with 5 replicate trees from each population). Six *F. americana* trees of approximately the same stem circumference growing at NESAs in similar soil and water conditions (see

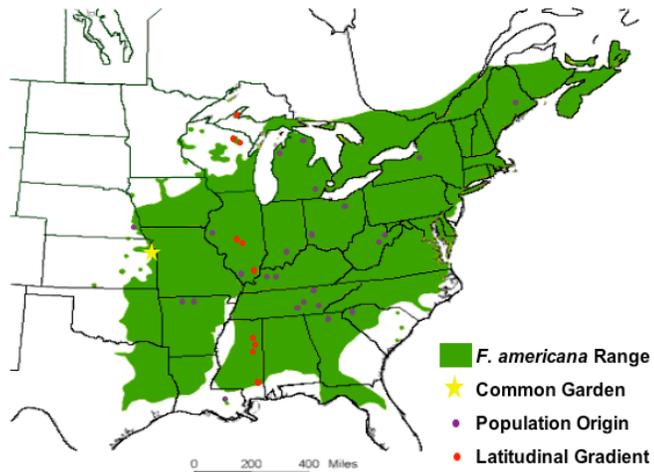


Figure 2-1. The common garden (indicated with a yellow star) is located at Nelson Environmental Study Area in northeastern Kansas. The purple dots (•) indicate locations of origination of the *Fraxinus americana* populations present in the common garden, while the red dots (•) indicate the populations chosen to represent a latitudinal gradient (along a longitude of 89°W). (USDA Forest Service)

Figure 5-4 and Table 5-1, Appendix) were chosen to compare populations in the common garden to a native Kansas population.

A nearby climate station operated by the University of Kansas Field Station and Ecological Reserves recorded daily precipitation, hourly minimum and maximum temperatures, and hourly relative humidity. Climate data for the locations of origination of *F. americana* populations in the common garden (Table 2-1) were obtained from climate records of the nearest meteorological station to each site (National Oceanic and Atmospheric Administration 2002; National Oceanic and Atmospheric Administration National Climate Data Center 2004). All meteorological stations were less than 150 km from the sites of origination of each *F. americana* population in the common garden. In order to improve accuracy, when possible, climate data from the two nearest meteorological stations were averaged if the nearest meteorological station was over 100 km from the site of origination of a *F. americana* population in the common garden.

For each origination site, mean growing season vapor pressure deficit (VPD), mean annual precipitation, mean growing season number of days of precipitation, mean annual temperature, mean growing season temperature, mean January temperature, minimum temperature, maximum temperature, and mean annual frost-free days, were compared with the physiological responses of *F. americana* populations in the common garden. For climatic variables representing growing season values, monthly climatic data from April to September were combined

because this is the growing season for *F. americana* trees in Kansas. The monthly VPD values were calculated as

$$\text{VPD} = (e_s - e_a) \quad (1)$$

where

$$e_a = \left[\frac{h}{100} \right] e_s \quad (2)$$

and e_a is the actual water vapor pressure present in the atmosphere, h is the mean monthly relative humidity, and e_s is the water vapor pressure in a saturated atmosphere, assuming leaf temperature is equal to the mean monthly air temperature (Rundel and Jarrell 1989), for each location of origination in the common garden. To determine the growing degree days ($^{\circ}\text{D}$) for each *F. americana* population in the common garden, the daily degree days after the arbitrary starting point of March 1 were calculated as

$$^{\circ}\text{D} = T_m - x \quad (3)$$

where

$$T_m = \frac{T_{\max} + T_{\min}}{2} \quad (4)$$

and T_{\max} is the maximum daily temperature, T_{\min} is the minimum daily temperature, and x is the threshold temperature (7°C). The value for x was chosen by comparing threshold temperatures found to predict phenological responses in other temperate deciduous tree species (Snyder et al. 1999; Borchert et al. 2005). Whenever the T_m is less than the threshold ($T_m < x$), the daily degree days are set equal to zero ($^{\circ}\text{D} = 0$). The daily degree days between March 1 and the mean date for leaf emergence in the

common garden are summed for the development period to determine the temperature sum for leaf emergence in each population (Ring et al. 1983).

Growth and Survival Analyses

Growth of each population of *F. americana* was measured as average stem circumference (cm) at a height of 0.61 m in the surviving individuals in the common garden. This height was chosen to minimize any skewing of stem circumference results by the large number of trees in the common garden that were branched below the DBH. Survival (%) of *F. americana* populations in the common garden was expressed as the number of live individuals in the spring of 2004 remaining from the original 25 trees per population that were planted in 1976.

Phenology

The average date of leaf emergence in the spring of 2005 was recorded for each *F. americana* population. Trees in the common garden were examined every 24–48 h for changes in phenology. The date of leaf emergence for each tree was recorded when approximately 15% of the leaf buds on a tree were fully open and every leaflet in these compound leaves was fully expanded.

Leaf gas exchange

Light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) were measured in the common garden for each of ten *F. americana* populations (n = 3-5 trees) with a

Table 2-2. Climatic data from the locations along the latitudinal gradient

| Location | Latitude (°N) | Longitude (°W) | Elevation (m) | Annual Precipitation (mm) | Annual Temperature (°C) | Growing Season VPD (kPa) |
|-----------------|--------------------------|---------------------------|--------------------------|--|--|---|
| Ontonagon, MI | 46.6 | 89.5 | 408 | 820 | 4.3 | 0.400 |
| Forest, WI | 45.7 | 89.0 | 511 | 777 | 5.6 | 0.428 |
| Effingham, IL | 39.1 | 88.4 | 183 | 1025 | 11.9 | 0.631 |
| Effingham, IL | 39.0 | 88.4 | 177 | 1025 | 11.9 | 0.631 |
| Gallatin, IL | 37.6 | 88.3 | 152 | 1125 | 13.3 | 0.680 |
| Oktibbeha, MS | 33.4 | 88.8 | 116 | 1383 | 17.2 | 0.639 |
| George, MS | 30.8 | 88.8 | 76 | 1602 | 19.4 | 0.732 |

location of origination forming a latitudinal gradient centered around a longitude of 89°W (Figure 2-1, Table 2-2). Measurements were made on one sun leaflet on the south side of each tree with an open-flow infrared gas analyzer equipped with a red-blue light source (LI-6400, LI-Cor, Lincoln, NE) between 1000 and 1400 h, a time interval previously determined to be the daily peak time for photosynthesis (see Figure 5-2, Appendix), on sunny, clear days (1-3 August 2005). Maximum daily temperatures during the sampling period ranged from 34.0–35.1°C. Sampling times for each population were randomized to account for diurnal differences in environmental conditions between and within consecutive days of this experiment.

Inside the gas exchange chamber, the CO₂ concentration was maintained at 380 ppm, a saturating light level of 1500 μmol m⁻² s⁻¹ was used (see Figure 5-3, Appendix), and the block temperature was kept at 30°C. The relative humidity inside the gas exchange chamber was not controlled and ranged from 44.2–59.5% during the measurement period. All sampled leaflets had a surface area greater than 6 cm² and filled the entire area of the cuvette. After the gas exchange of each leaflet in the cuvette had stabilized, four measurements were recorded over a period of two minutes and were later averaged to determine A_{sat} and g_s for each tree.

After gas exchange measurements were completed, each leaflet was harvested and assayed for total N on a leaf-area basis (N_a). Leaflet tissue for N analysis was dried at 70°C and ground with liquid nitrogen to a powder. The N content of leaf tissue (4–5 mg) was measured with a CN autoanalyzer (CE Instruments, Milan, Italy). Leaf mass per unit area (LMA; g m^{-2}) was calculated as the mean ratio of leaf dry mass to photosynthetic surface area on circular leaf discs ($n = 5$) with an area of 0.32 cm^2 . The N_a for each leaflet was then calculated as the product of the leaf N content and the LMA.

Carbon isotope ratios of leaf tissue

The average carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$) of leaf tissue (‰) was determined for all *F. americana* populations ($n = 1-5$ trees, although in only three populations was $n < 3$ trees) from leaflets collected in the summer of 2003. Leaflets were only collected from trees in one block (Block 2) of the common garden, where differences in soil water content were not observed (see Figure 5-4 and Table 5-1, Appendix). Three sun leaflets from each tree were collected on the south side of the tree canopy at a height of approximately 7.6 m on 3 October 2003 and brought back to the laboratory to be oven-dried (70°C, at least 48 hours). The trees from the native Kansas population of *F. americana* were sampled following the same procedure on 16 October 2003. Because height in the canopy can affect leaf carbon isotope ratios (Helle and Schleser 2004), we used this consistent method of collection in order to minimize possible isotopic differences due to light environment.

The three leaflets from each tree were combined and ground with liquid nitrogen to a fine powder. Carbon isotope ratios were then determined for foliar tissue (1.8–2.2 mg) of each replicate using an elemental analyzer (Carla Erba, Model 1108, Milano, Italy) coupled to a Finnigan MAT delta S isotope ratio mass spectrometer (San Jose, CA) at the Stable Isotope Ratio Facility for Environmental Research (University of Utah, Salt Lake City, UT). Carbon isotope ratios ($\delta^{13}\text{C}$) were calculated using the delta notation:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1)1000 \quad (5)$$

where R is the ratio of the heavy isotope (^{13}C) to the lighter isotope (^{12}C). The standard was PDB (belemnite carbonate standard from the PeeDee Formation, SC), and the precision of $\delta^{13}\text{C}$ measurements was $\pm 0.15\%$.

Statistical Analyses

Responses of stem circumference, survival, date of leaf emergence, g_s , and $\delta^{13}\text{C}$ were analyzed with one-way analyses of variance (ANOVA, $\alpha = 0.05$) with population as the main effect. All data were tested for normality with the Shapiro and Wilk's test ($p \leq 0.05$). Logarithmic transformations were used to fit responses to a normal distribution when possible. Data with a non-normal distribution were evaluated with the non-parametric Kruskal-Wallis test. Each response was tested separately for all 44 populations in the common garden and then again for the ten populations in the latitudinal gradient.

The $A_{\text{sat}}-N_a$ relationship for each population in the latitudinal gradient was calculated based on photosynthesis and area-based foliar N (N_a) from all sampled trees. Heterogeneity of slopes of this relationship was examined with an analysis of covariance ($\alpha = 0.05$) with N_a as the covariate, where a significant population $\times N_a$ interaction would indicate a significant difference in the slope of the $A_{\text{sat}}-N_a$ relationships. A significant difference in the main effect of population would indicate a significant difference in the y -intercept of the $A_{\text{sat}}-N_a$ relationships, reflecting a genetic difference in light-saturated photosynthesis (A_{sat}) across all foliar N concentrations.

When significant results were found in the above tests, linear regressions were calculated between the responses and several climatic parameters (mean growing season VPD, mean annual precipitation, mean growing season number of days of precipitation, mean annual temperature, mean growing season temperature, mean January temperature, minimum temperature, maximum temperature, mean annual frost-free days) to determine if there was a relationship between physiology, stem circumference, and survival and the climate at the population origination. These relationships were tested with separate regressions for all 44 populations in the common garden and for the ten populations in the latitudinal gradient. Second-order polynomial regressions were fit when appropriate. In addition, linear regressions were calculated between significant responses and stem circumference and survival to identify possible mechanisms impacting the demography of *F. americana*. All

statistical analyses were performed with the JMP 5.1 and MINITAB 14 statistical software packages.

Results

After 29 years in the northeastern Kansas climate, there was a significant difference among the 44 populations in the amount of stem circumference ($p < 0.0001$) and survival ($p < 0.0001$) in the common garden (Figure 2-2). The two populations with the highest mean levels of stem circumference (70–72 cm) and survival (100%) originated from latitudes close to the common garden (37.7°N, 36.5°N). The lowest mean stem circumference and survival in *F. americana* populations in the common garden were found in populations originating furthest from the common garden. One population from the southern edge of the range (East Baton Rouge, LA) had no surviving individuals in the common garden, while the northern-most population in the common garden (Ontonagon, MI) had a mean stem circumference of 17 cm and a mean survival of 44%.

In order to isolate the effect of latitudinal variation in climate on *F. americana*, ten of the 44 populations of *F. americana* in the common garden were chosen to form a latitudinal gradient (Figure 2-1, Table 2-2). The trends in stem circumference and survival among these ten populations were the same as those in all 44 populations, but the correlation coefficients were consistently higher ($p < 0.01$, Figure 2-2).

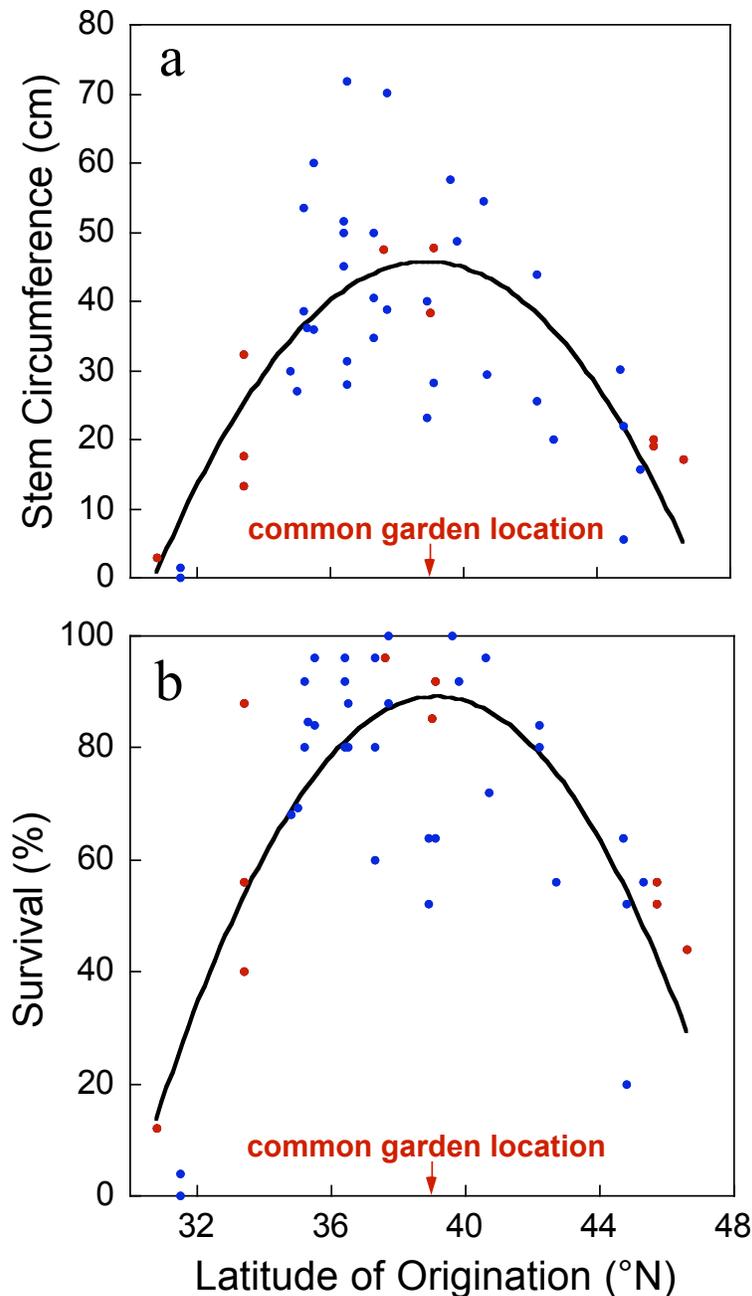


Figure 2-2. (a) Growth, as measured by average stem circumference, of the 44 *Fraxinus americana* populations ($n = 1-26$) in the common garden was highly variable among populations originating from different latitudes ($R^2 = 0.55$, $p < 0.0001$). (b) Survival among *F. americana* populations in the common garden was also variable among populations originating from different latitudes ($R^2 = 0.63$, $p < 0.0001$). Red dots (\bullet) indicate growth and survival among the ten populations in the latitudinal gradient ($R^2 = 0.85$ and $R^2 = 0.79$, respectively). Populations with the highest growth and survival originate from latitudes near the common garden (39°N).

Because temperature and precipitation vary widely across the *F. americana* range, I analyzed climatic variables to determine which factor best explained the trends in the growth and survival of *F. americana* at the edge of the species range. Variables were analyzed by comparing the coefficients of determination from second-order polynomial regression analyses. The relationships of all the climatic variables related to temperature, excluding maximum temperature (which had very low coefficients of determination), and stem circumference among all 44 populations in the common garden were similar, ranging from $R^2 = 0.53$ (for minimum temperature) to $R^2 = 0.61$ (for mean annual temperature). The relationships of all the climatic variables related to temperature, excluding maximum temperature (which had very low coefficients of determination), and survival among all 44 populations in the common garden were also similar, ranging from $R^2 = 0.67$ (for mean annual frost-free days) to $R^2 = 0.71$ (for mean annual temperature).

For the ten populations along the latitudinal gradient, where the longitudinal effects were removed, even stronger trends were found between the relationships of all the climatic variables related to temperature, excluding maximum temperature (which was not a significant predictor of the response), and stem circumference and survival of *F. americana* populations in the common garden. The range of coefficients of determination for stem circumference was $R^2 = 0.72$ (for mean annual frost-free days) to $R^2 = 0.86$ (for mean January temperature), while for survival the range was from $R^2 = 0.69$ (for mean growing season temperature) to $R^2 = 0.80$ (for mean January temperature). Additionally, unlike in the analysis of all 44 populations

in the common garden, there were also significant correlations of stem circumference with mean annual precipitation ($R^2 = 0.74$) and mean growing season VPD ($R^2 = 0.64$) in the populations originating from along the latitudinal gradient. Survival was significantly correlated with mean annual precipitation ($R^2 = 0.76$) in these populations as well.

Phenology

The date of leaf emergence in the spring of 2005 varied significantly ($p < 0.001$) among the 44 populations in

the common garden. A population from Oktibbeha, MS had the earliest mean date of leaf emergence in the common garden (11 April), and the latest mean date of leaf emergence was in a population from Washtenaw, MI (6 May), which makes the range in leaf emergence nearly a month for multiple populations of *F. americana* in Kansas. The average

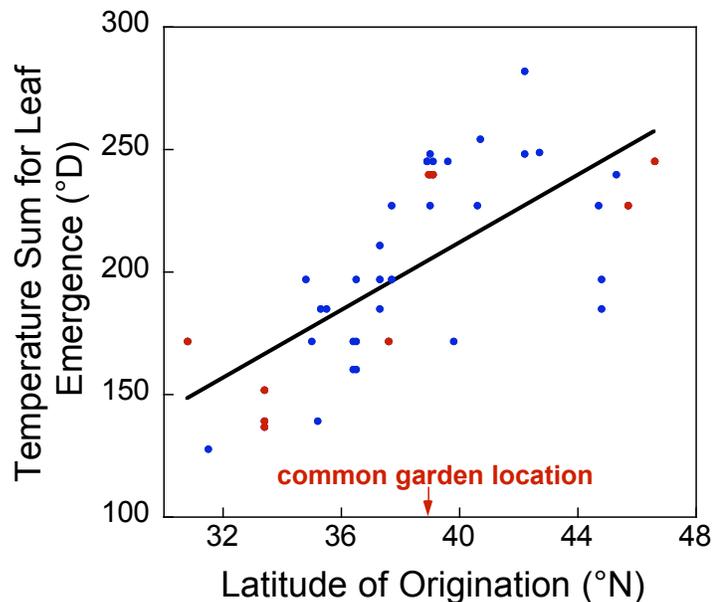


Figure 2-3. The average growing degree day ($^{\circ}\text{D}$) temperature sum ($n = 1-26$) required for leaf emergence in the spring was significantly correlated with latitude of origination for the 44 populations in the common garden ($R^2 = 0.47$, $p < 0.0001$). Red dots (\bullet) indicate the ten populations in the latitudinal gradient ($R^2 = 0.65$). Populations originating from locations further north require a greater temperature sum in the common garden before leaves will emerge in the spring.

growing degree day ($^{\circ}\text{D}$) temperature sum required for leaf emergence in the spring was significantly correlated with latitude of origination for the 44 populations in the common garden ($R^2 = 0.47$, $p < 0.0001$, Figure 2-3). Populations originating from locations further north required a greater temperature sum in the common garden before leaves emerged in the spring.

Along the latitudinal gradient, the date of leaf emergence also varied significantly ($p < 0.001$) among the ten *F. americana* populations. The correlation between latitude and average growing degree day ($^{\circ}\text{D}$) temperature sum in the common garden was significant ($R^2 = 0.65$, $p < 0.05$) among these ten populations (Figure 2-3). When compared to the northern populations, leaf emergence in populations from southern latitudes required lower temperature sums in the common garden.

Gas exchange

Leaf gas exchange parameters, A_{sat} and g_s , did not vary significantly ($p = 0.35$ and $p = 0.25$, respectively) among the ten populations in the latitudinal gradient (Table 2-3). Average A_{sat} was $9.35 \pm 1.49 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for the *F. americana* populations in the latitudinal gradient, and the average g_s was $0.119 \pm 0.033 \text{ mol m}^{-2} \text{ s}^{-1}$.

To further analyze A_{sat} in the populations in the latitudinal gradient and to account for possible variation within populations due to sun or shade adaptations of individual leaves, the $A_{\text{sat}}-N_a$ relationships were analyzed with an analysis of covariance where a significant result would indicate population differences in A_{sat} .

Table 2-3. Leaf gas exchange in *F. americana* populations (n = 3-5 trees) originating from locations along the latitudinal gradient

| Location | Latitude | A_{sat} (± SD) | g_s (± SD) | Slope of A_{sat}-N Relationship |
|-----------------|-----------------|-------------------------------|-----------------------------|--|
| Ontonagon, MI | 46.6 | 7.94 ± 4.26 | 0.127 ± 0.136 | 9.6 |
| Forest, WI | 45.7 | 6.46 ± 1.67 | 0.062 ± 0.018 | 2.6 |
| Forest, WI | 45.7 | 8.20 ± 2.09 | 0.080 ± 0.027 | -4.6 |
| Effingham, IL | 39.1 | 9.35 ± 3.30 | 0.111 ± 0.078 | 12.5 |
| Effingham, IL | 39.0 | 9.98 ± 2.11 | 0.118 ± 0.037 | 3.1 |
| Gallatin, IL | 37.6 | 11.27 ± 2.68 | 0.150 ± 0.076 | 3.2 |
| Oktibbeha, MS | 33.4 | 11.25 ± 1.83 | 0.159 ± 0.068 | 1.3 |
| Oktibbeha, MS | 33.4 | 9.18 ± 3.71 | 0.118 ± 0.082 | -2.7 |
| Oktibbeha, MS | 33.4 | 10.04 ± 2.52 | 0.104 ± 0.033 | 2.3 |
| George, MS | 30.8 | 9.85 ± 4.26 | 0.164 ± 0.101 | 10.2 |

However, even accounting for this added variation within populations failed to reveal a significant ($p = 0.52$) population difference in A_{sat} in *F. americana* (Table 2-3).

Variation in the slope of the A_{sat}-N_a relationship was high among populations, ranging from -4.6 to 12.5.

Leaf carbon isotope ratios

Carbon isotope ratios indicated that the ratio of intracellular to atmospheric CO₂ concentrations (c_i/c_a) was significantly different among the 44 *F. americana* populations in the common garden ($p < 0.01$). The mean carbon isotope ratios among *F. americana* populations in the common garden ranged from approximately -29.5 to -27‰. Although there was a significant population effect on the $\delta^{13}\text{C}$ of the 44 *F. americana* populations in the common garden, no significant correlations between $\delta^{13}\text{C}$ and a climatic variable could be found.

Among the ten populations originating along the latitudinal gradient, $\delta^{13}\text{C}$ were also significantly different in the common garden ($p < 0.01$). Although

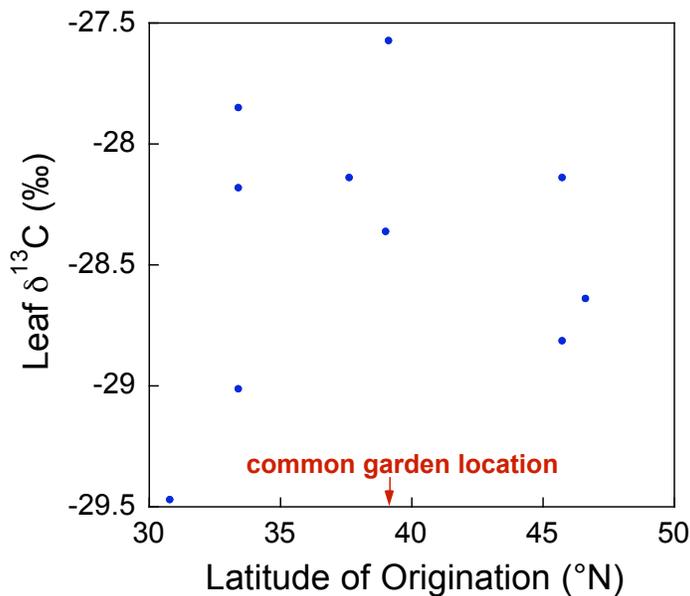


Figure 2-6. Average carbon isotope ratios of leaf tissue from populations ($n = 3-5$) along the latitudinal gradient are not significantly correlated with latitude of population origination (analyzed as a second-order polynomial regression: $R^2 = 0.49$, $p = 0.09$). There was a trend for higher carbon isotope ratios in the populations that originate from latitudes closest to the common garden.

origination latitude did not have a significant effect ($p = 0.09$) upon the mean carbon isotope ratios among these ten populations, there was a trend for populations that originated closer to the latitude of the common garden, 39°N , to have the highest mean carbon isotope ratios ($R^2 = 0.49$; Figure 2-6). The foliar carbon isotope ratios were significantly and

positively correlated with stem circumference ($R^2 = 0.41$) and survival ($R^2 = 0.72$) in these ten populations (Figure 2-7).

Discussion

This common garden study examined the physiology, growth, and survival of 44 populations of *F. americana* originating from locations throughout its native range. The trees in the study were 30 years old and were growing at the western edge of the range, where the influence of climate on trees should be more pronounced (Shortle et al. 2000) and where the effects of global change may first be evident (Hoffman and

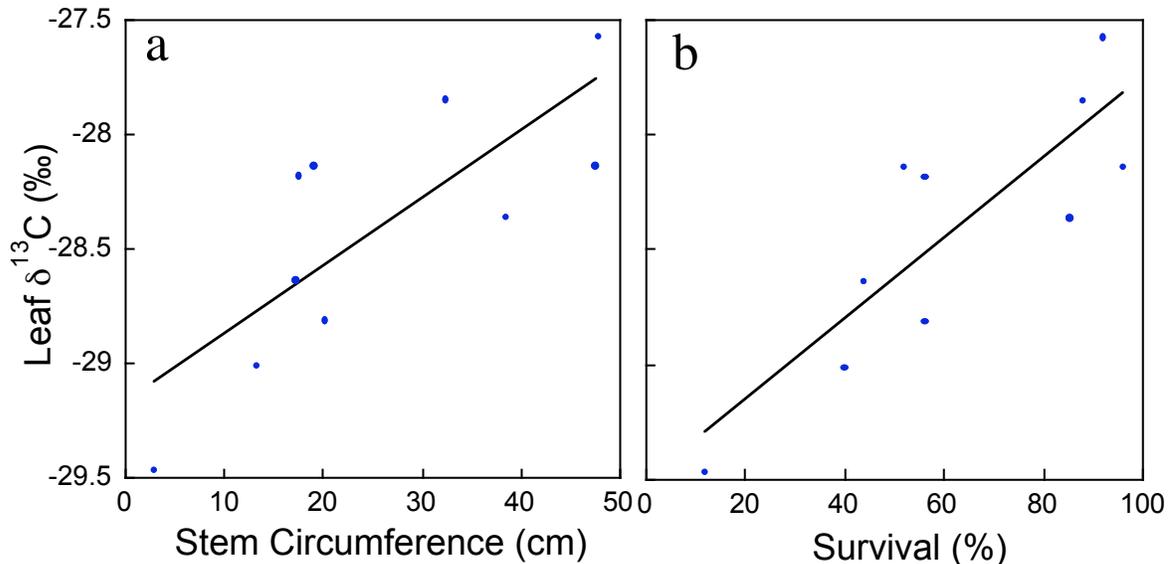


Figure 2-7. Average carbon isotope ratios of leaf tissue from the populations ($n = 3-5$) along the latitudinal gradient are highly correlated with (a) stem circumference and (b) survival in the common garden ($R^2 = 0.41$, $p < 0.05$; $R^2 = 0.72$, $p < 0.01$). Populations with the highest stem circumference and survival have higher carbon isotope ratios and are more conservative in their water use.

Blows 1993). Very few common garden studies include examination of older, mature trees, and even fewer studies can examine genetic differentiation in such a large number of populations of mature trees. The study revealed that genetic variation exists in the development and physiology of *F. americana* populations.

One objective of the study was to determine if there were differences in phenology among *F. americana* populations. Intraspecific variation in the cold tolerance of *F. americana* populations has previously been identified through the demonstration of different killing temperatures for stem tissue from various populations across the species range (Alexander et al. 1984). The study found that the northern-most populations had lower killing temperatures (-42.6°C , -41.4°C) when compared to the southern-most populations (-33.7°C , -30.8°C) in two different years (Alexander et al. 1984). The phenological measurement used in this study, date of

leaf emergence, also revealed the presence of population variation in *F. americana* (Figure 2-3). The range for mean date of leaf emergence among *F. americana* populations in the Kansas common garden was 26 days, which is a comparable range to reported time intervals of similar phenological measurements in other deciduous temperate tree species (Scotti-Saintagne et al. 2004; Borchert et al. 2005). In one study, the range for the date of bud burst among 278 individuals of a full-sib (F_1) *Quercus robur* (common oak) family was found to be 20 days (Scotti-Saintagne et al. 2004).

Warming in the spring, winter chilling, and photoperiod can all influence the phenology of tree species, although there is no consensus on the relative importance of these factors (Hunter and Lechowicz 1992). Genotypic differences among populations also contribute to variation in phenology in deciduous tree species (Rousi and Pusenius 2005). In this study with *F. americana*, northern populations have higher temperature requirements for bud break than southern populations (Figure 2-3). Studies examining differences in flowering time among tree populations along altitudinal gradients found that flowering occurs later in the spring in populations at higher altitudes (Roller 1978; Koch 1994 as cited in Larcher 2003). Because southern populations of *F. americana* have the earliest date of leaf emergence in the common garden, these populations are the most susceptible to damaging late-spring frosts, which may explain the lower survival of these populations in Kansas (Figure 2-2). It is possible that the observed differences in leaf emergence in the common garden are controlled by variations in the chilling requirement among *F. americana* populations,

as has been found in *Acer rubrum* (red maple) (Perry and Wang 1960). Further research, however, would be required to fully elucidate the controls on bud break in *F. americana*, as a combination of spring temperature, winter chilling, and photoperiod may influence this trait.

The timing of bud burst in trees is important because a delay in canopy development can lead to large reductions in growth, because peak solar radiation precedes the peak in mean daily temperature (Cannell 1989; Rousi and Puseenius 2005). Other common garden studies with deciduous trees have found that measurements of phenology, including the timing of bud burst, are weakly correlated with height growth (Rousi and Puseenius 2005), and thus populations with an earlier date of leaf emergence may have the advantage of additional carbon gain in early spring. Therefore, phenological traits partially explain why northern *F. americana* populations have lower stem circumference in the common garden than populations originating from the optimal latitude, 39°N (Figure 2-2). In forest ecosystems, early greening of canopies is critical for net ecosystem primary production. A difference of a few days in canopy development accounted for more than 20% of the interannual change in net photosynthetic production in a northeastern North American forest (Myneni et al. 1997).

The second trait examined for intraspecific variation in *F. americana* was the leaf gas exchange parameters, A_{sat} and g_s , which have been found to be a source of population variation in other tree species, such as *B. pendula* and *F. pennsylvanica* (Abrams 1994; Aspelmeier and Leuschner 2004). The amount and intensity of solar

radiation in different habitats is a source of population variation in A_{sat} (Fitter and Hay 2002), and this variation among genotypes is maintained by simultaneous changes in biochemical and stomatal characteristics during photosynthesis (Geber and Dawson 1997). The current study found no differences in either A_{sat} or g_s in the *F. americana* populations along the latitudinal gradient (Table 2-3). Since differences in A_{sat} among populations were analyzed by the comparison of the $A_{\text{sat}}-N_a$ relationship for each population, the lack of intraspecific variation in A_{sat} was not caused by variations in the foliar concentrations of nitrogen. In fact, as foliar nitrogen did not vary significantly among *F. americana* populations, it is likely that differences among populations in photosynthetic nitrogen use efficiency (PNUE), the rate of photosynthesis per unit leaf N, also do not exist. Although photosynthetic capacity is a mechanism that may explain variation in growth among populations of some tree species (Abrams 1994; Anella and Whitlow 2000; Aspelmeier and Leuschner 2004), it is likely that A_{sat} does not influence the differential growth of *F. americana* populations in the common garden. The lack of a significant difference in g_s , however, among the latitudinal gradient of *F. americana* populations may simply reflect the sampling method for obtaining these values. This measurement was obtained by averaging g_s over a period of two minutes, which is likely not long enough to capture any differentiation of g_s among trees at the population level. Other evidence collected in this study, namely the estimates of c_i/c_a obtained from foliar $\delta^{13}\text{C}$, suggests that there are population differences in g_s when considered over a longer time period.

Foliar $\delta^{13}\text{C}$ of plants provides an integrated measure of c_i/c_a throughout the period of leaf development, and $\delta^{13}\text{C}$ can thus be used to compare stomatal regulation and to estimate intrinsic water use efficiency (WUE) among plant populations (Farquhar et al. 1989; Ehleringer 1991). Past studies have found $\delta^{13}\text{C}$ of leaf tissue to be correlated to climatic variables such as VPD (Panek and Waring 1997) or seasonal rainfall (Li et al. 2000). Thus, $\delta^{13}\text{C}$ provide an opportunity to compare population responses to climatic variation within a site over time (Panek and Waring 1997). Foliar $\delta^{13}\text{C}$, and thus stomatal regulation, were found to vary significantly among the 44 *F. americana* populations in the common garden. Intraspecific differences in $\delta^{13}\text{C}$ have also been found in other tree species, including *Pseudotsuga menziesii*, *Eucalyptus microtheca*, *Faidherbia albida*, *B. pendula*, *Pinus ponderosa*, and *Pinus sylvestris* (Panek and Waring 1997; Roupsard et al. 1998; Li 1999; Palmroth et al. 1999; Adams and Kolb 2004; Aspelmeier and Leuschner 2004).

Foliar $\delta^{13}\text{C}$ from the latitudinal gradient of ten *F. americana* populations in the common garden were not significantly correlated with latitude of origination, although there was a trend for the highest carbon isotope ratios to be found in trees originating from latitudes closest to 39°N (Figure 2-6). In a similarly aged common garden of *P. sylvestris* genotypes, there was also no correlation between $\delta^{13}\text{C}$ and latitude (Palmroth et al. 1999). This non-significant trend revealed that the lowest c_i/c_a in the populations along the latitudinal gradient was found in trees originating from areas with a high mean VPD during the growing season (Table 2-2). Foliar $\delta^{13}\text{C}$ in other tree species have been shown to be highly correlated to the VPD in natural

ecosystems (Panek and Waring 1997), and thus this climatic variable may influence the observed differences in stomatal regulation in *F. americana*.

Variations in rainfall have been found to explain 80-90% of the variation in the annual width of tree rings in some species (Kozlowski et al. 1991). Because water availability can be such an important driver of plant growth, it is not surprising to find that the differences in $\delta^{13}\text{C}$ among *F. americana* populations are significantly correlated with stem circumference in the common garden (Figure 2-7). A higher WUE allowed the *F. americana* populations originating near 39°N to have the highest growth and survival in the common garden, where year-to-year precipitation is variable and there is high evaporative demand during the growing season (Table 2-1).

The multiple significant correlations between the climate of the locations of origination and the physiology, growth, and survival of *F. americana* populations indicates that individual populations are adapted to the local climate conditions of their natural habitats. The long time period that the trees have been growing in the relatively harsher climate at the edge of the species range provides strong evidence for local adaptation in this species; even if some degree of acclimation has occurred over the past 29 years, the trees still display significant differences in many physiological parameters. Other common garden studies that have examined similarly aged trees have also found intraspecific variations among populations and correlations with the climate of origin (Palmroth et al. 1999). If the physiological responses of trees do not adjust to a new climate, the growth and survival in many populations will be reduced as temperature and precipitation patterns shift in the

future. These findings have important implications for the future of forest ecosystems in the eastern United States.

Several predictions about the future of individual *F. americana* populations can be made as a result of this study. Some models predict a decrease in the amount of precipitation in the midwestern United States and large decreases in soil moisture may occur in the southeastern United States (NAST 2000; McCarthy et al. 2001), which will cause the environment in these areas to become more similar to the current conditions in Kansas. Thus,

the responses of several *F. americana* populations in the common garden, those from the midwestern and the southeastern United States, can be used to

Table 2-5. *Fraxinus americana* stem circumference and survival of individuals collected from locations in the midwestern and southeastern United States

| Location | Stem Circumference (cm) | Survival (%) |
|----------------------|-------------------------|--------------|
| East Baton Rouge, LA | 0.70 | 2 |
| Oktibbeha, MS | 21.02 | 62 |
| George, MS | 2.96 | 12 |
| Marion, AR | 50.72 | 94 |
| Boone, AR | 45.16 | 80 |
| Otoe, NE | 54.52 | 96 |

predict the possible physiological responses in their natural habitat and the likelihood for survival in the future climate. In the populations from these areas, mean stem circumference in the common garden varied from 0.7–54.5 cm and mean survival varied from 2–96%, indicating a wide range of responses among populations to the relatively harsher climate at the edge of the species range (Table 2-5). Although many important characteristics that affect tree growth at these sites cannot be accounted for in this study, such as geologic features and microclimate variation, the results

presented here indicate that some populations may become locally extinct if the climate becomes drier in these areas.

Chapter 3

Genetically based variation in the morphology and water relations of *Fraxinus americana* L. (white ash) populations is driven by precipitation differences in the native habitat

Abstract

Water is a major limiting resource for the growth of tree species along the ecotone between forests and grasslands in the midwestern United States. In order to accurately predict future changes in the abundance and distribution of tree species in the temperate deciduous forests of the eastern United States, intraspecific differences in the morphology and water relations of common forest tree species must be investigated. The current study investigates the effects of population and gender on the water relations of 30-year-old *Fraxinus americana* L. (white ash) trees, a dioecious hardwood species, in a common garden at the edge of the species range. One morphological parameter, leaf mass per unit area (LMA), was measured to identify population differences in the morphology of *F. americana* that may be caused by differences in water availability in its natural range. Three physiological parameters were also measured to provide information about the water relations of *F. americana* trees over a range of time scales, including the instantaneous measurement of stomatal conductance (g_s), the difference in midday and predawn water potential ($\Psi^{\text{MD-PD}}$), and the foliar carbon isotope ratio ($\delta^{13}\text{C}$), which integrates stomatal regulation over the entire period of leaf formation. No significant differences in g_s were found in this study, but this may reflect the instantaneous variability of this measure. Genetically based differences in water relations among populations of *F.*

americana were revealed by LMA, $\Psi^{\text{MD-PD}}$, and $\delta^{13}\text{C}$ measurements. Populations of *F. americana* from the wetter, eastern portion of the range have lower $\delta^{13}\text{C}$ and thus are less conservative in their water use. Furthermore, the highest LMA (thickness), a leaf trait associated with drought tolerance, was found in populations from the drier, western edge of the range. The $\Psi^{\text{MD-PD}}$ data also supports the conclusion that there are intraspecific differences in water relations among *F. americana* populations. The western populations, which are more conservative in their water use, had the highest stem circumference and survival at the edge of the species range. There were no significant differences in the stem circumference or water relations between genders in *F. americana*. Identifying the influence of morphology and water relations on the differential growth and survival of *F. americana* from different regions is an important step towards understanding how this species will respond to climate change scenarios and for determining the level of genetic variation within the species for potential adaptive responses to future changes in inter-annual water availability.

Introduction

Tree growth in the continental interior of the United States is limited by water supply (Puhe and Ulrich 2001). The ecotone between the temperate deciduous forests of the eastern United States and the midwestern prairies is located at a longitude of approximately 95°W , where low mean annual precipitation and a variable rainfall pattern during the growing season limit further forest growth to the west. Along the eastern coast of the United States, mean annual precipitation is higher than at this

forest–grassland ecotone, creating a climatic gradient that may allow for the formation of local adaptations among tree populations of forest species. Some climate predictions show that the midwestern United States, including the forest–grassland ecotone, will become drier in the near future and the likelihood for severe droughts will increase (McCarthy et al. 2001), potentially influencing the distribution and persistence of tree populations in this area. A recent analysis of three global circulation models revealed predictions that the grasslands of the central United States will spread east, displacing the deciduous forest here by 2100 (Lauenroth et al. 2004).

Of primary importance for predicting the future of tree species in the forest–grassland ecotone is an understanding of whether adaptation to differences in water availability has occurred throughout the species range. Common garden studies comparing mature trees from populations throughout the range are valuable tools for characterizing any intraspecific variation present in a species. Once differences in physiological responses among tree populations have been examined, this information can be used to more accurately predict the effects of increasingly variable rainfall on future species distributions.

Intraspecific differences in morphology and water relations are commonly observed in tree species with large ranges that cover different climatic, edaphic, and biotic regions (Li 1999; Ponton et al. 2002; Adams and Kolb 2004; Aspelmeier and Leuschner 2004; Fischer et al. 2004). Tree species in the temperate deciduous forests of the eastern United States include *Pinus strobus* (eastern white pine), *Quercus*

rubra (northern red oak), *Quercus alba* (white oak), *Acer saccharum* (sugar maple), *Acer rubrum* (red maple), *Fraxinus americana* (white ash), *Betula alleghaniensis* (yellow birch), *Fagus grandifolia* (American beech), *Prunus serotina* (black cherry), *Tsuga canadensis* (eastern hemlock), *Ulmus americana* (American elm), and *Liriodendron tulipifera* (yellow poplar) (Schlesinger 1990; Isebrands et al. 2000). A review of the literature reveals that several of these species have been found to exhibit genetically based population variation in physiological responses to water availability. Xerophytic populations of *Q. rubra* exhibit genetic traits in ecophysiology and morphology that improve drought tolerance compared with populations from a mesic site (Kubiske and Abrams 1992). Similarly, populations of *A. rubrum* originating from bogs have genetically based differences in leaf gas exchange that cause stomatal conductance (g_s) to remain lower during a drought than in populations from upland sources (Abrams and Kubiske 1990). Genetically based differences have been found among *P. serotina* families for g_s and net photosynthetic rates, which then impacted the growth of these families under different ozone treatments (Ferdinand et al. 2000; Kouterick et al. 2000). Additionally, differences in leaf morphology exist among populations of *A. rubrum* and *P. serotina* from contrasting environments, including traits such as thickness, specific mass, stomatal density, and guard cell length (Abrams et al. 1992; Ferdinand et al. 2000).

Intraspecific differences in water relations have also been found between genders of some dioecious tree species. In natural populations of dioecious tree species, males often exceed females in total plant size, plant height, growth rates, and

frequencies in populations (Dickson 1991; Obeso 2002). However, females of *Acer negundo* (box elder) are capable of more growth than males during wet years in Utah (Ward et al. 2002). The reproductive success of these dioecious species will be impacted by any differences in water relations between genders, which may alter the survival and distribution of each gender as precipitation patterns shift in the future.

As global temperatures continue to increase and precipitation patterns shift in the eastern United States, the abundance and interaction of the dominant tree species in the temperate deciduous forests of the eastern United States may also shift in potentially unpredictable ways. In order to more accurately predict changes in species composition in these forests, intraspecific differences in the water relations of important forest tree species must be carefully investigated. Knowledge of the different water use strategies and drought tolerances among populations and between genders throughout the tree species range is important for the maintenance of forest ecosystems.

This study utilizes a common garden approach to determine if genetically based intraspecific variation in morphology and water relations exists among eleven populations of *F. americana* originating along a precipitation gradient in the native range. Specifically, leaf mass per unit area (LMA), g_s , leaf water potential (Ψ), and foliar carbon isotope ratios ($\delta^{13}C$) of the eleven populations growing in a common garden in Kansas were compared to determine if intraspecific differences are correlated with precipitation patterns at the site of population origin. The morphological parameter, LMA (leaf thickness), is a trait often found to be associated

with drought tolerance among plant populations (Li et al 2000; Li et al 2004). The other three measurements provide information about the water relations of trees over different time scales, including g_s obtained instantaneously, Ψ^{MD-PD} that estimates daily changes in cell turgor pressure, and the foliar $\delta^{13}C$ that integrates stomatal regulation over the period of leaf formation (up to weeks). The second objective of this study is to determine if there is any effect of gender on the water relations of *F. americana*, a dioecious species. Two parameters, Ψ^{MD-PD} and foliar $\delta^{13}C$, were compared to examine the impact of gender on the water relations of this dioecious species. Any impacts of intraspecific variation in morphology and water relations on the growth and survival of *F. americana* at the edge of the species range are also examined.

Materials and Methods

Research Species

The research species *F. americana* is an early successional, dioecious species in the temperate deciduous forests of the eastern United States. This hardwood tree is important for forest regeneration across its range, as it is often one of the first trees to colonize abandoned fields (Schlesinger 1990). It is an ideal candidate for studies aimed at examining intraspecific differences of important physiological traits, because *F. americana* is genetically variable throughout its range (Wright 1944). For example, height and survival vary among populations of *F. americana* and were

correlated with the latitude of origination for each population in a common garden in West Virginia (Schuler 1994).

Common garden and site characteristics

The common garden of *F. americana* was planted in 1976 and is located at the edge of the species range at 39.0°N, 95.2°W in the Nelson Environmental Study Area (University of Kansas) in Jefferson county, Kansas. The average annual precipitation is 879 mm and the average annual temperature is 11.9°C at

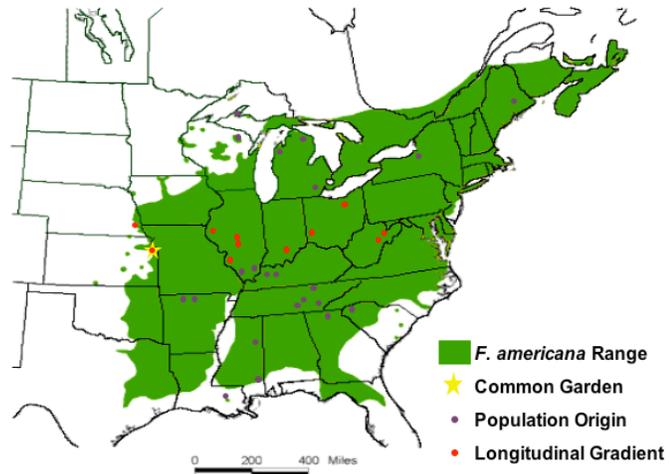


Figure 3-1. The common garden (indicated with a yellow star) is located at Nelson Environmental Study Area in northeastern Kansas. The purple dots (•) indicate locations of origination of the *Fraxinus americana* populations present in the common garden, while the red dots (•) indicate the eleven *F. americana* populations in the longitudinal gradient in this study. (USDA Forest Service)

the common garden (Table 3-1). The *F. americana* trees in the common garden were 30 years old at the time of study and were grown from open-pollinated seeds collected from native parent trees at each location represented in the experiment (Figure 3-1). Six *F. americana* trees of approximately the same stem circumference growing at NESAs in similar soil and water conditions (see Figure 5-4 and Table 5-1, Appendix) were chosen to compare populations in the common garden to a native Kansas population. The eleven populations chosen to analyze population differences

Table 3-1. Climatic data from the locations in the precipitation gradient

| Location | Latitude (°N) | Longitude (°W) | Elevation (m) | Annual Precipitation (mm) | Annual Temperature (°C) | Growing Season VPD (kPa) |
|-----------------|--------------------------|---------------------------|--------------------------|--|--|---|
| Jefferson, KS | 39.0 | 95.2 | 299 | 879 | 11.9 | 0.633 |
| Otoe, NE | 40.6 | 95.7 | 259 | 765 | 10.8 | 0.639 |
| Adams, IL | 39.8 | 90.7 | 213 | 943 | 10.9 | 0.633 |
| Jackson, IL | 37.7 | 89.4 | 158 | 1097 | 13.3 | 0.760 |
| Effingham, IL | 39.1 | 88.4 | 183 | 1025 | 11.9 | 0.631 |
| Effingham, IL | 39.0 | 88.4 | 177 | 1025 | 11.9 | 0.631 |
| Jackson, IN | 38.9 | 86.0 | 191 | 1142 | 12.3 | 0.670 |
| Preble, OH | 39.6 | 84.7 | 305 | 1035 | 11.7 | 0.699 |
| Wayne, OH | 40.7 | 82.0 | 265 | 969 | 9.7 | 0.592 |
| Randolph, WV | 38.9 | 79.7 | 975 | 1261 | 9.3 | 0.463 |
| Tucker, WV | 39.1 | 79.5 | 762 | 1261 | 9.3 | 0.463 |

in morphology and water relations form a precipitation gradient along a latitude of 39°N, the optimal latitude for growth and survival in the common garden (see Figure 2-2, Chapter 2).

The common garden is set up in a block design (see Figure 5-1, Appendix) where each of the 44 populations was originally represented by 25 trees (5 blocks with 5 replicate trees from each population). To analyze gender differences in *F. americana*, pairs of male and female trees were chosen from the 44 populations in the common garden. Each gender pair contained trees from the same population growing in the same block in order to minimize any population or microclimate effect on tree physiology and growth.

A nearby climate station operated by the University of Kansas Field Station and Ecological Reserves records daily precipitation, hourly temperature, and hourly relative humidity. Climate data for the locations of origination of *F. americana* populations in the common garden (Table 3-1) was obtained from climate records of the nearest meteorological station to each site (National Oceanic and Atmospheric

Administration 2002; National Oceanic and Atmospheric Administration National Climatic Data Center 2004). All meteorological stations were less than 150 km from the sites of origination of each *F. americana* population. In order to improve accuracy, when possible, climate data from the two nearest meteorological stations were averaged if the nearest meteorological station was over 100 km from the site of origination of a *F. americana* population in the common garden.

This climatic data, including mean growing season vapor pressure deficit (VPD), mean annual precipitation, and mean growing season number of days of precipitation, was used to analyze the morphology and physiology of *F. americana* populations in the common garden. For climatic variables representing growing season values, monthly climatic data from April to September was combined because this is the growing season for *F. americana* trees in Kansas. Monthly VPD values were calculated as

$$\text{VPD} = (e_s - e_a) \quad (1)$$

where

$$e_a = \left[\frac{h}{100} \right] e_s \quad (2)$$

and e_a is the actual water vapor pressure present in the atmosphere, h is the mean relative humidity, and e_s is the water vapor pressure in a saturated atmosphere, assuming leaf temperature is equal to the mean air temperature (Rundel and Jarrell 1989), for each location of origination in the common garden.

Growth and Survival Analyses

Growth of each population of *F. americana* was measured as average stem circumference (cm) at a height of 0.61 m in the surviving individuals in the common garden. This height was chosen to minimize any skewing of stem circumference results by the large number of trees in the common garden that were branched below the DBH. Survival (%) of *F. americana* populations in Kansas is expressed as the number of live individuals in the spring of 2004 remaining from the original 25 trees per population that were planted in 1976.

Morphology

Leaf mass per unit area (LMA; mg cm^{-2}) was measured in the *F. americana* populations ($n = 5$ trees) from the precipitation gradient by collecting one leaflet per tree on 8-11 July 2005. Only sun leaflets from the south side of the tree were collected for analysis. Leaflet tissue was dried at 70°C for at least 48 hours. LMA for each tree was calculated as the average ratio of leaf dry mass to photosynthetic surface area of five leaf discs (surface area = 0.32 cm^2) per leaflet.

Leaf gas exchange

Stomatal conductance (g_s) was measured in the *F. americana* populations ($n = 5$ trees) from the precipitation gradient on sunny, clear days in the common garden on 8-11 July 2005. Measurements were made on sun leaflets on the south side of the tree with an open-flow infrared gas analyzer equipped with a red-blue light source (LI-

6400, LI-Cor, Lincoln, NE) between 1000 and 1400 h, a time interval previously determined to be the daily peak time for photosynthesis (see Figure 5-2, Appendix). Maximum daily temperatures during the sampling period ranged from 30.6–31.3°C. Sampling times for each population were randomized to account for diurnal differences in environmental conditions between and within consecutive days of this experiment.

Inside the sampling chamber, the CO₂ concentration was maintained at 380 ppm, a saturating light level of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was used (see Figure 5-3, Appendix), and block temperature was kept at 30°C. The relative humidity inside the gas exchange chamber was not controlled and ranged from 43.2–62.8% during the measurement period. All sampled leaflets had a surface area greater than 6 cm² and filled the entire area of the cuvette. After the gas exchange of each leaflet in the cuvette had stabilized, four measurements were recorded over a period of two minutes and were later averaged to determine g_s for each tree.

Leaf water potentials

Predawn and midday water potentials (Ψ^{PD} and Ψ^{MD} , respectively) for *F. americana* populations (n = 4-10 trees) in the precipitation gradient were performed on 10 July 2005, 21 July 2005, and 8 September 2005. One sun leaf from each tree was collected from the south side of the canopy at a height of 3.4 m, sealed in a plastic bag with a wet paper towel, and placed in a dark cooler. Water potential measurements were obtained with a pressure chamber (P.M.S. Instruments, Corvallis, OR) in the

laboratory within three hours of leaf sampling, a time interval shown to have no effect on resulting water potential measurements for these trees (see Figure 5-5, Appendix). On 10 July 2005, leaves were sampled at 0430-0600 h and at 1100-1230 h. On 21 July 2005, leaves were sampled at 0400-0600 h and at 1100-1300 h. On 8 September 2005, leaves were sampled at 0500-0700 h and at 1230-1400 h. The Ψ^{PD} and Ψ^{MD} for the analysis of the effect of gender on the water relations of *F. americana* were also measured on 21 July 2005. Leaves were sampled as described above.

To remove the variation in water potentials due to differences in soil water content throughout the common garden, the difference between predawn and midday leaf water potentials (Ψ^{MD-PD}) was the measurement used in statistical analyses.

Carbon isotope ratios of leaf tissue

The average carbon isotope ratio ($^{13}C/^{12}C$) of leaf tissue (‰) was determined for *F. americana* populations (n = 3-6 trees) in the summer of 2004. Leaflets were only collected from trees in one block (Block 2) of the common garden, where differences in soil water content were not observed (see Figure 5-4 and Table 5-1, Appendix). Three sun leaflets from each tree were collected on 27 August 2004 from the south side of the canopy at a height of approximately 7.6 m and brought back to the laboratory to be oven-dried (70°C, at least 48 hours). Trees representing a native Kansas population of *F. americana* were sampled following the same procedure on 3 September 2004. Foliar $\delta^{13}C$ for the analysis of the effect of gender on the water relations of *F. americana* were determined in the summers of 2003 and 2004, as

described above. In 2003, the leaves were collected on 3 and 16 October. Because height in the canopy can affect leaf $\delta^{13}\text{C}$ (Helle and Schleser 2004), we used this consistent method of collection in order to minimize possible isotopic differences due to light environment.

The three leaflets from each tree were combined and ground with liquid nitrogen to a fine powder. Carbon isotope ratios were then determined for foliar tissue (1.8–2.2 mg) of each replicate using an elemental analyzer (Carla Erba, Model 1110, Milano, Italy) coupled to a ThermoFinnigan Delta Plus gas isotope mass spectrometer (Bremen, Germany) at the Stable Isotope Mass Spectrometry Laboratory (Kansas State University, Manhattan, KS). Carbon isotope ratios ($\delta^{13}\text{C}$) were calculated using the delta notation:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1)1000 \quad (6)$$

where R is the ratio of the heavy isotope (^{13}C) to the lighter isotope (^{12}C). The standard was PDB (belemnite carbonate standard from the PeeDee Formation, SC), and the precision of $\delta^{13}\text{C}$ measurements was $\pm 0.15\%$.

Statistical Analyses

Responses of stem circumference, survival, LMA, g_s , $\Psi^{\text{MD-PD}}$, and $\delta^{13}\text{C}$ were analyzed with one-way analyses of variance (ANOVA, $\alpha = 0.05$) with population as the main effect. All data were tested for normality with the Shapiro and Wilk's test ($p \leq 0.05$). Logarithmic and power transformations were used to fit responses to a normal distribution when possible. Data with a non-normal distribution were evaluated with

the non-parametric Kruskal-Wallis test. Additionally, the three sets of $\Psi^{\text{MD-PD}}$ data were analyzed with a two-way analysis of variance ($\alpha = 0.05$) with population and date as the main effects and population \times date as the interaction term.

The responses of stem circumference, $\Psi^{\text{MD-PD}}$, and foliar $\delta^{13}\text{C}$ were also analyzed with a paired comparisons analyses of variance ($\alpha = 0.05$) with gender and pair as the main effects. Twenty pairs were compared in the $\Psi^{\text{MD-PD}}$ analysis, 14 pairs were compared in the foliar $\delta^{13}\text{C}$ analysis in 2003 and 2004, and 44 pairs were compared in the stem circumference analysis.

When significant results were found in the above tests, linear regressions were calculated between the responses and several climatic parameters (mean growing season VPD, mean annual precipitation, mean growing season number of days of precipitation) to determine if there was a relationship between morphology and physiology and the climate of population origination. Linear regressions were also calculated between significant responses and stem circumference and survival to identify possible mechanisms impacting the demography of *F. americana*. All statistical analyses were performed with the JMP 5.1 and MINITAB 14 statistical software packages.

Results

Population Differences

LMA varied significantly ($p < 0.05$) among populations in the *F. americana* common garden, and western populations were found to have thicker leaves than eastern

populations. In *F. americana*, the LMA correlated ($R^2 = 0.58$, $p < 0.05$) with the mean annual precipitation at the location of population origin, also indicating that populations from drier areas have thicker leaves (Figure 3-2).

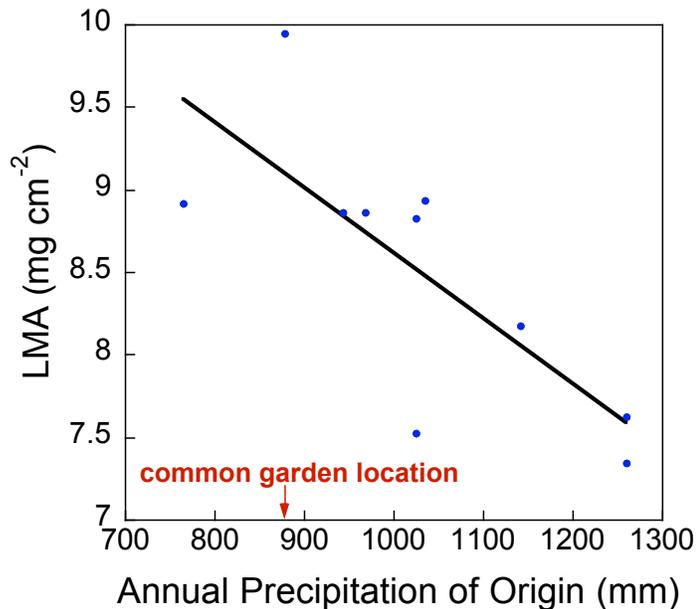


Figure 3-2. Average leaf mass per unit area ($n = 5$ trees) varies significantly ($p < 0.05$) among *F. americana* populations in the common garden. Populations originating from drier locations have thicker leaves ($R^2 = 0.58$).

Furthermore, LMA positively correlated ($R^2 = 0.50$, $p < 0.05$) with stem

circumference in these *F. americana* populations in the common garden. The greatest stem circumference among the populations analyzed in this study was found in those populations with high LMA, which originated from drier climates.

The effect of population on the water relations of *F. americana* in a common garden at the edge of the range was tested with three different parameters: g_s , Ψ^{MD-PD} , and foliar $\delta^{13}C$. There were no significant differences ($p = 0.80$) in g_s among the eleven *F. americana* populations in the common garden (Figure 3-3). Mean g_s values for *F. americana* populations were $0.341 \pm 0.029 \text{ mol m}^{-2} \text{ s}^{-1}$.

Ψ^{MD-PD} varied significantly ($p < 0.05$) among *F. americana* populations throughout the growing season (Table 3-2). Although many populations maintained

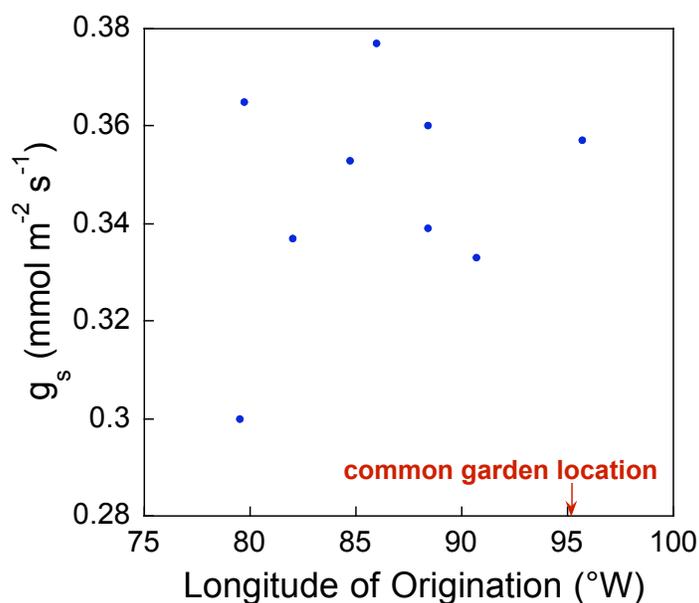


Figure 3-3. Average stomatal conductance did not vary significantly among the eleven *F. americana* populations ($n = 5$ trees) along the longitudinal gradient ($p = 0.80$).

potential when compared to the other nine populations in the common garden during the last half of the growing season, while populations from Preble, OH and Jackson, IN were on the opposite end of the range of $\Psi^{\text{MD-PD}}$ for the same sampling times. Values of $\Psi^{\text{MD-PD}}$ in the common garden throughout the growing season did not significantly correlate with the mean annual precipitation at the site of origin (Figure 3-4).

The differences in water relations revealed by foliar $\delta^{13}\text{C}$ of the eleven populations in the common garden were highly significant ($p = 0.0001$) and

similar $\Psi^{\text{MD-PD}}$ at each time sampled in the summer of 2005, there were several populations in the common garden with significantly different $\Psi^{\text{MD-PD}}$ from the remaining populations (Figure 3-4). Trees originating from Tucker, WV and Wayne, OH had larger daily changes in water

Table 3-2. $\Psi^{\text{MD-PD}}$ of *F. americana* populations in the common garden throughout the growing season

The $\Psi^{\text{MD-PD}}$ were sampled on July 10, July 21, and September 8 of 2005 ($n = 4-10$ trees) and were analyzed with a two-way ANOVA.

| Factor | p-value |
|-------------------|---------|
| Population | 0.03 * |
| Date | 0.10 |
| Population * Date | 0.69 |

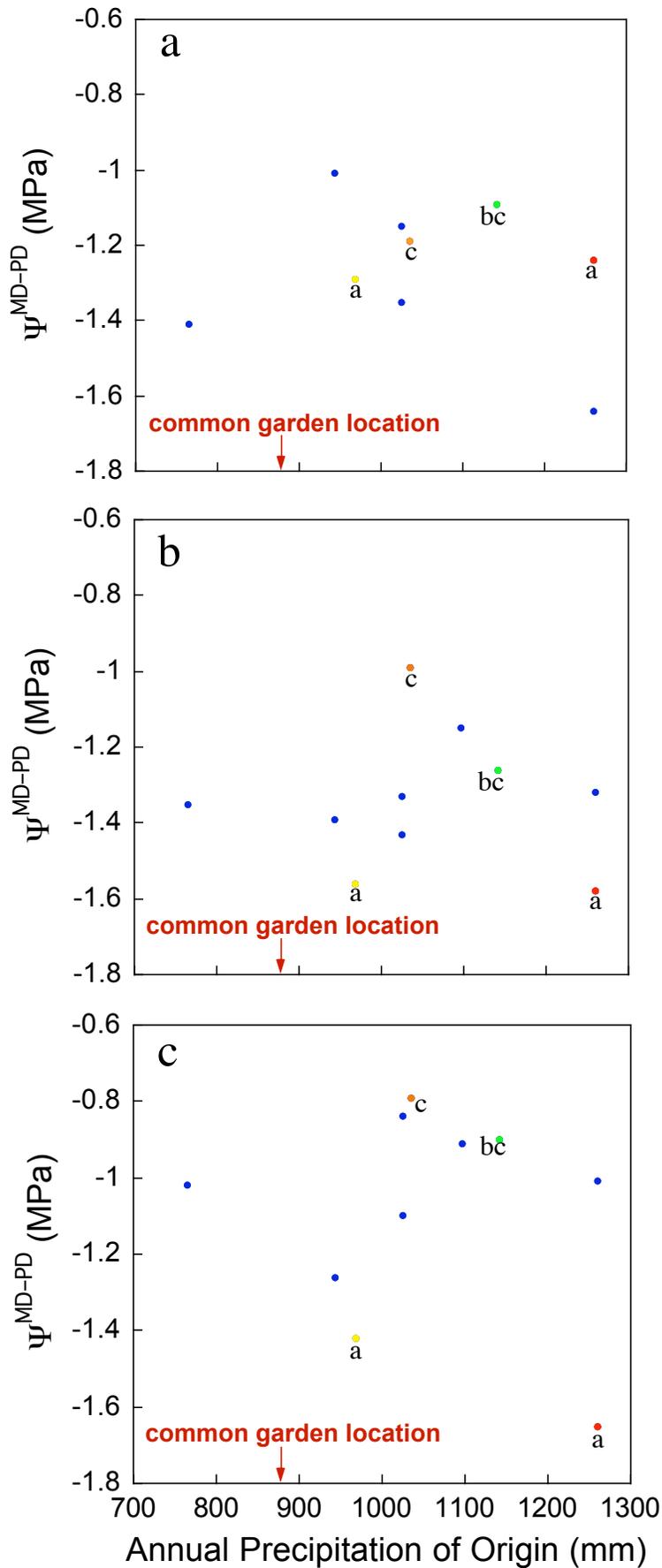


Figure 3-4. The average difference between midday and predawn water potentials ($n = 4-10$ trees) in the common garden is not significantly correlated with mean annual precipitation at the site of population origin for any time sampled: (a) July 10, (b) July 21, or (c) September 8. Colored populations originated from: (red, •) Tucker, WV; (orange, •) Preble, OH; (yellow, •) Wayne, OH; (green, •) Jackson, IN. Populations with different letters had significantly different Ψ^{MD-PD} when all three sampling dates analyzed with a two-way ANOVA. Populations originating from areas with low VPD tend to have more negative Ψ^{MD-PD} throughout the growing season in Kansas.

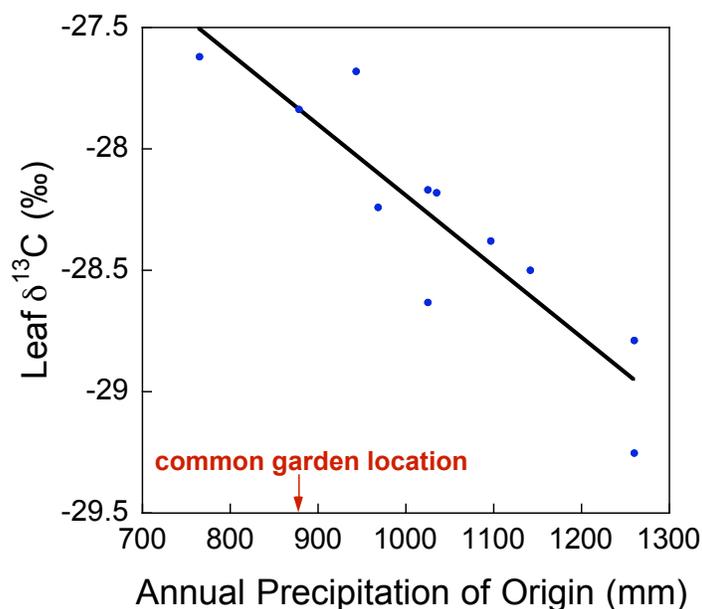


Figure 3-5. Average foliar carbon isotope ratios ($n = 3-6$) in the summer of 2004 were significantly correlated with mean annual precipitation of the location of population origin ($R^2 = 0.81$, $p = 0.0001$). Populations originating from wetter areas are less conservative in their water use in the common garden.

correlated ($R^2 = 0.81$) with the mean annual precipitation at the site of origin (Figure 3-5). Populations from wetter sites of origin had lower foliar $\delta^{13}\text{C}$ than populations from drier areas. These foliar $\delta^{13}\text{C}$ were also significantly correlated ($R^2 = 0.45$, $p < 0.05$) with the probability of survival in

the common garden in Kansas (Figure 3-6). Populations with the highest survival in the common garden at the edge of the species range are those with higher foliar $\delta^{13}\text{C}$ and, hence, more conservative water use.

Gender Differences

The effect of gender on the water relations of *F. americana* in a common garden at the edge of the range was tested with three different parameters: $\Psi^{\text{MD-PD}}$, foliar $\delta^{13}\text{C}$, and stem circumference. No significant differences in $\Psi^{\text{MD-PD}}$ or foliar $\delta^{13}\text{C}$ were found in male and female pairs of *F. americana* trees ($p = 0.18$, $p = 0.91$ respectively). The mean $\Psi^{\text{MD-PD}}$ for males was -1.30 ± 0.61 MPa, while the mean

$\Psi^{\text{MD-PD}}$ for females was -1.50 ± 0.43 MPa. In 2003, the mean $\delta^{13}\text{C}$ found for males was -28.11 ± 0.68 ‰, while the mean $\delta^{13}\text{C}$ found for females was -28.19 ± 0.69 ‰.

In 2004, the mean $\delta^{13}\text{C}$ for males was -28.42 ± 0.51 ‰, while the mean $\delta^{13}\text{C}$ for females was -28.39 ± 0.74 ‰.

There was also no significant ($p = 0.31$) difference in the

stem circumference of male versus female trees in the common garden. The mean stem circumference for males was 54.2 ± 20.1 cm, while the mean stem circumference for females was 52.1 ± 18.3 cm.

Discussion

Genetically based differences in morphology and water relations among populations of *F. americana* were revealed by the measurements of LMA, $\Psi^{\text{MD-PD}}$, and foliar $\delta^{13}\text{C}$ (Table 3-2, Figures 3-2, 3-4, 3-5). Similar differences have been found in other tree species of the temperate deciduous forests of the eastern United States (Abrams et al. 1992 for *P. serotina*; Bauerle et al. 2003 for *A. rubrum*). This is the first time,

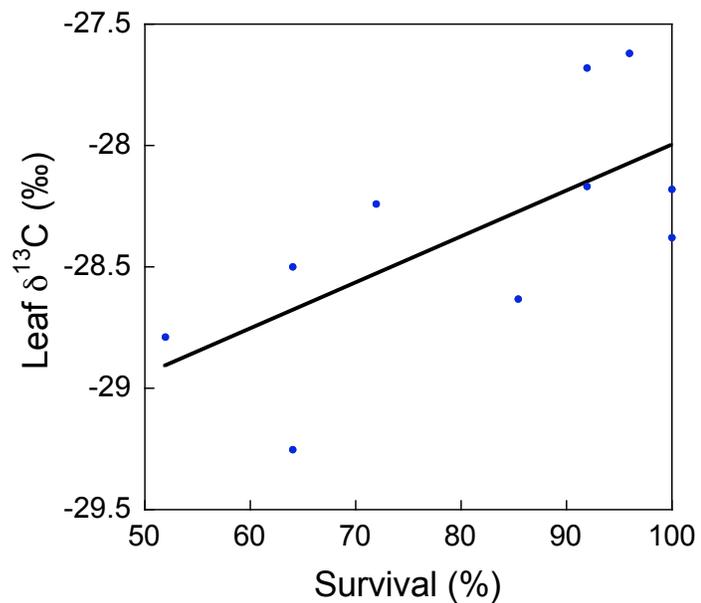


Figure 3-6. Average foliar carbon isotope ratios ($n=3-6$) in the summer of 2004 were correlated to the survival of each population in the common garden at the edge of the species range ($R^2 = 0.45$, $p < 0.05$). Populations more conservative in water use in the common garden had the highest survival.

however, these physiological adaptations to water availability have been identified for *F. americana*, and these findings may impact future predictions concerning the species response to climate change in its native range.

Leaves with high LMA (thicker leaves) contain more mesophyll tissue and thus have a higher water use efficiency (WUE), and these traits are often associated with drought tolerance in tree populations (Li et al. 2000; Li et al. 2004). The morphological differences found in this study indicate that differentiation in LMA has occurred among *F. americana* populations. Populations originating from the western edge of the range, where the climate is drier, have thicker leaves than populations from eastern locations (Figure 3-2). This correlation has been found in other tree species and is believed to be an adaptation to drought stress (Li et al. 2000; Li et al. 2004). The main effect of a change in leaf thickness is the concomitant change in the quantity of photosynthetic apparatus per unit leaf area, such that thinner leaves have less biochemical machinery and fix less carbon (per unit time) than thicker leaves (Fitter and Hay 2002). Because LMA was significantly correlated with stem circumference of *F. americana* populations in the common garden, this leaf trait may contribute to the differential growth of *F. americana* populations in the common garden at the edge of the species range.

Stomatal conductance (g_s) did not vary significantly among populations of *F. americana* in the common garden (Figure 3-3). This parameter provides a measure of the flux of water and CO₂ through the stomata of a leaf and is measured instantaneously with an infrared gas analyzer. Because this measurement can only

discern differences in g_s over the time span of a few minutes, it does not have the power to identify differences that may be present at other times during the growing season. In contrast, the measurements of foliar $\delta^{13}\text{C}$ suggest that significant differences in g_s do exist among populations in the common garden (Figure 3-5). This more integrative measurement of stomatal regulation indicates that there should be differences in g_s among populations, at least during the period of leaf formation, in the common garden. Presumably, more thorough testing would reveal differences in g_s among populations at critical times in the growing season.

Measurements of $\Psi^{\text{MD-PD}}$ and foliar $\delta^{13}\text{C}$ provide information about the water relations of trees over longer time periods than g_s . The measurement of $\Psi^{\text{MD-PD}}$ can be used to determine the relative daily water status of a tree, while the stable carbon isotope composition of leaves is a time-integrated index of the ratio of leaf internal to ambient CO_2 concentration (c_i/c_a) that can be used to infer photosynthetic WUE (Farquhar et al. 1989; Ehleringer 1991). Both the $\Psi^{\text{MD-PD}}$ and the foliar $\delta^{13}\text{C}$ data provide evidence that there is a correlation between the physiological responses of *F. americana* populations in the common garden and the climate at the population site of origin (Table 3-2; Figures 3-4, 3-5). These results indicate that eastern populations of *F. americana* from wetter climates are less conservative in their water use when compared with western populations that originate from drier climates, which are more conservative in their stomatal regulation. These differences impact the survival of each population in the drier climate of the common garden (Figure 3-6). The highest survival among the populations analyzed in this study was in those populations from

drier climates with more conservative water use. These results are consistent with several other tree species, including *Picea abies* (Norway spruce) and *Juglans nigra* (black walnut). In these species, populations more adapted to the local climate had higher growth and survival than populations from other areas of the species range (Bresnan et al. 1994; Oleksyn et al. 1998).

Although there is intraspecific variation in the morphology and water relations of *F. americana* populations, no intraspecific differences were found between genders of *F. americana*. Because there was also no significant difference in the stem circumference of male *F. americana* trees versus female trees in the common garden, it seems likely that there are few gender differences in *F. americana*. Intraspecific differences in water relations between genders have been identified for other tree species, such as *A. negundo* and *Populus tremuloides* (quaking aspen) (Wang and Curtis 2001; Ward et al. 2002). Ward and colleagues found that in wet years, the females of this species were less conservative in their water use than males. Such a process would have implications for tree reproduction in drier climates, but water relations of *F. americana* do not appear to be affected by gender.

Identifying the influence of morphology and water relations on the differential growth and survival of *F. americana* from different regions is an important step for understanding how this species will respond to climate change scenarios and for determining the level of genetic variation within the species for potential adaptive responses to future changes in inter-annual water availability. Western populations of *F. americana*, especially those from Nebraska, Kansas, and western Illinois, are the

most conservative in their water use and thus the most likely to survive the drier climate expected in the forest–grassland ecotone in the future. However, continued presence of the species in this area will be dependent on the rate of climate change and the potential for further adaptations that increase the conservation of water in these populations.

Chapter 4

Summary

High levels of intraspecific variation in morphology, physiology, growth, and phenology are commonly observed in tree species with large ranges that cover different climatic, edaphic, and biotic regions (Donselman and Flint 1982; Geber and Dawson 1993; Abrams 1994; Aspelmeier and Leushner 2004). Tree physiology often changes along climatic gradients and may be driven by adaptation to temperature differences and/or differences in water availability throughout the species range. It is important to understand how ecologically important traits vary in natural populations because it furthers our understanding of the roles of natural selection, gene flow, and genetic drift in shaping levels of adaptation and in affecting the distribution and abundance of species (Jonas and Geber 1999). A better understanding of the natural variation in traits that affect growth and survival in plant species will improve models designed to predict future changes in species ranges that occur as a result of various climate change scenarios (Panek and Waring 1997; Iverson and Prasad 1999). It is also possible with this knowledge to identify which genotypes of a plant species may be the most successful in a given region under both current and future predicted climates.

Fraxinus americana L. (white ash) is an early successional species in the temperate deciduous forests of the United States. The natural range of *F. americana* is large, extending from Texas to Minnesota in the west and Nova Scotia to Florida in the east (Schlesinger 1990). This hardwood tree is often one of the first trees to

colonize abandoned fields and thus is important for forest regeneration across its range (Schlesinger 1990). Models designed to predict potential suitable habitat for tree species under future climate change scenarios indicate that *F. americana* will decrease its range as the optimum latitude for its habitat shifts north (Iverson and Prasad 1998).

Previous studies have found evidence for intraspecific variation in the cold tolerance of *F. americana* (Alexander et al. 1984) and in height and survival of various populations when grown in a common garden (Schuler 1994). The goal of my thesis research was to further investigate intraspecific variation in physiologically important traits in *F. americana* by examining 44 populations growing in a common garden at the western edge of the species range.

My research utilized a common garden of *F. americana* at the Nelson Environmental Study Area (University of Kansas) in Jefferson county, Kansas. The trees in the common garden were 30 years old and were grown from open-pollinated seeds collected from *F. americana* populations at 44 different locations throughout the species range. The study site is located along the western edge of the species range, where the average annual precipitation is 879 mm and the average annual temperature is 11.9°C. Because the influence of climate on the growth of trees is particularly discernible where trees grow in marginal environments (Puhe and Ulrich 2001), this study was performed in an ideal location for interpreting climatic effects on the growth of *F. americana* trees.

Stem circumference and survival varied significantly among the 44 *F. americana* populations in the common garden at the edge of the species range, in agreement with the findings of Schuler (1994). The differential stem circumference and survival among populations was correlated with latitude of population origination, and the highest stem circumference and survival was found in populations originating near the latitude of the common garden (39°N). A primary goal of my research was to identify possible causal mechanisms to explain this intraspecific variation in the growth and survival of *F. americana*.

The main objectives of my research were to (1) determine if there are differences in morphology, phenology, gas exchange, and stomatal regulation among *F. americana* populations, (2) determine if intraspecific variation in these traits affects growth and survival in the species, and (3) correlate intraspecific variation in *F. americana* with a corresponding climate variable in order to define the causal mechanism driving genetic divergence in the species. Morphology, phenology, and stomatal regulation varied significantly among the 44 *F. americana* populations in the common garden and were correlated with climatic differences in the locations of population origination.

The morphological parameter leaf mass per unit area (LMA) was analyzed in ten populations of *F. americana* from the common garden that originated from approximately 39°N, the optimal latitude for growth and survival in the common garden. LMA in these *F. americana* populations correlated ($R^2 = 0.58$, $p < 0.05$) with the mean annual precipitation at the location of population origin, indicating that

populations from drier areas had thicker leaves. Furthermore, LMA was positively correlated ($R^2 = 0.50$, $p < 0.05$) with stem circumference in these *F. americana* populations in the common garden.

Differences in phenology among *F. americana* populations in the common garden were measured as the average date of leaf emergence, which was converted into the average growing degree day ($^{\circ}\text{D}$) for leaf emergence in each population. The temperature sum required for leaf emergence in the spring was correlated ($R^2 = 0.47$, $p < 0.0001$) with latitude of origination for the 44 populations in the common garden. Populations originating from locations further north required higher temperature sums in the common garden before leaves emerged in the spring. Other studies have found that phenology can have considerable impacts on carbon gain and net ecosystem primary production (Cannell 1989; Myneni et al. 1997; Rousi and Puseenius 2005), and thus this variable may partially explain why northern populations exhibit less growth and southern populations have lower survival in the common garden.

The leaf gas exchange parameters, light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s), were analyzed in two sets of *F. americana* populations in the common garden. In a latitudinal gradient (centered on a longitude of 89°W) of ten populations in the common garden, A_{sat} and g_s , did not vary significantly ($p = 0.35$ and $p = 0.25$, respectively). Similarly, there were no significant differences ($p = 0.80$) in g_s among the ten *F. americana* populations of the previously mentioned longitudinal gradient.

Stomatal regulation was analyzed through the comparison of the foliar carbon isotope ratios ($\delta^{13}\text{C}$) among *F. americana* populations, which is a time-integrated ratio of leaf internal to ambient CO_2 (c_i/c_a) concentration that can also be used to infer photosynthetic water use efficiency, WUE (Farquhar et al. 1989; Ehleringer 1991). When comparing the eleven *F. americana* populations in the common garden from the longitudinal gradient, higher foliar $\delta^{13}\text{C}$ and thus higher WUE were found in populations originating from the western edge of the range ($p = 0.0001$). These populations, when compared to others originating from along the same latitude, were also found to have the highest survival in the common garden ($R^2 = 0.45$, $p < 0.05$). This trend in foliar $\delta^{13}\text{C}$ in *F. americana* was correlated with the mean annual precipitation at the site of population origination ($R^2 = 0.81$). These differences in $\delta^{13}\text{C}$ provide a potential mechanism that may partially explain the differential growth and survival found among *F. americana* populations in the common garden at the edge of the species range.

Another objective of my research was to examine differences in water relations between genders in *F. americana*, a dioecious tree species, to determine if gender was another source of intraspecific variation for growth and survival in the species. However, no significant differences were found in the growth or water relations between genders in *F. americana*.

In conclusion, there was a great amount of genetically based differences in physiologically important traits among populations of *F. americana*. Intraspecific differences in morphology, phenology, and stomatal regulation were closely related to

the climate of the area of origination. The *F. americana* populations with the highest growth and survival at the edge of the species range originated from climates of similar annual temperature and precipitation to that of the common garden. The physiological responses of *F. americana* trees, which are still apparent after 29 years in a different climate, affect the ability of individual populations to grow and survive in novel climates.

Identifying the influence of temperature and precipitation on the growth and survival of *F. americana* from different regions is an important step for understanding how this species will respond to future climate change scenarios and for determining the level of genetic variation for potential adaptive responses within the species. Climate predictions for the next century indicate that temperatures will increase by 2–6°C and that precipitation patterns may become more variable in the *F. americana* species range (NAST 2000; McCarthy et al. 2001). This research indicates that several populations of *F. americana*, especially those from East Baton Rouge, LA, are extremely vulnerable to local extinction under predicted climate change scenarios and suggests that the species range will shift north in the near future.

Appendix

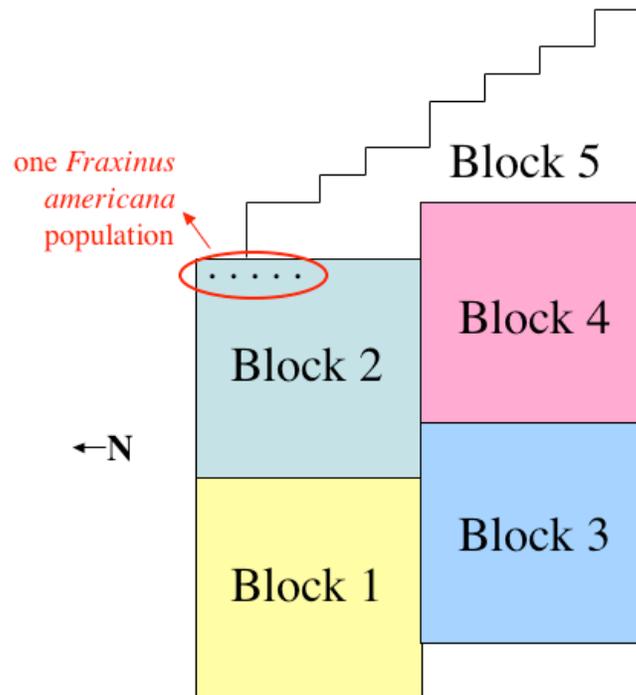


Figure 5-1. The experimental design of the *Fraxinus americana* common garden with each population represented by 25 trees (5 replicates in each of 5 blocks).

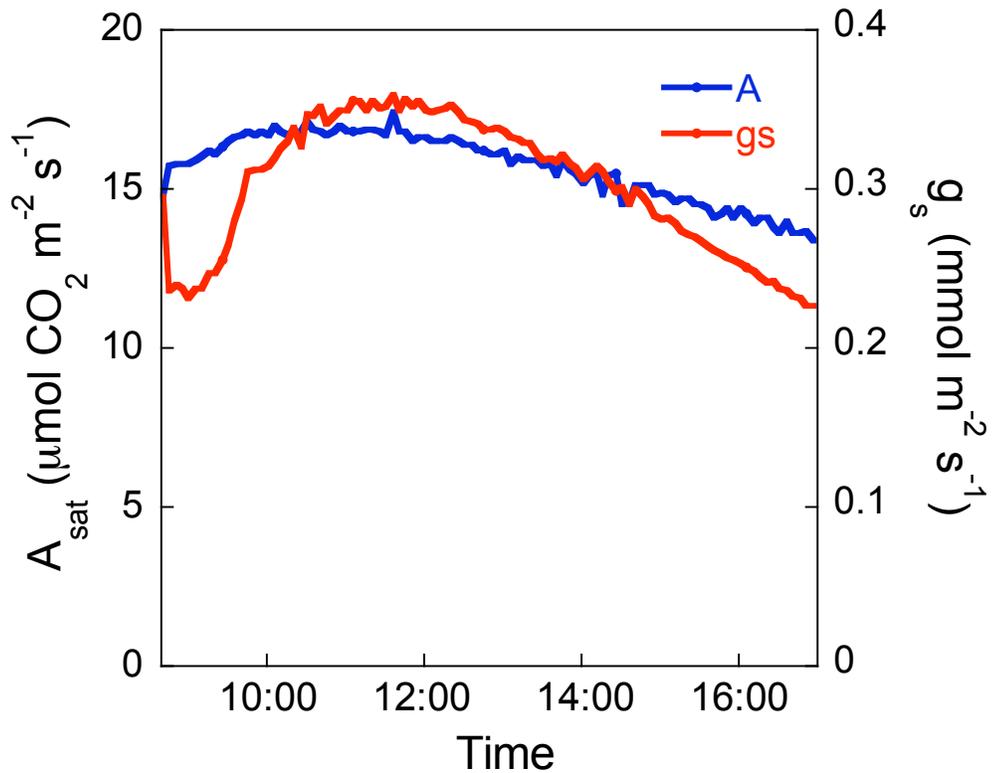


Figure 5-2. Light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) were measured on one intact *Fraxinus americana* leaflet from 0830–1700 h on 1 July 2005. Inside the gas exchange chamber, the CO_2 concentration was maintained at 380 ppm, the light level was $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and block temperature was 30°C . Measurements were recorded every five minutes. From this experiment, it was determined that the daily peak time for photosynthesis occurred between 1000 and 1400 h.

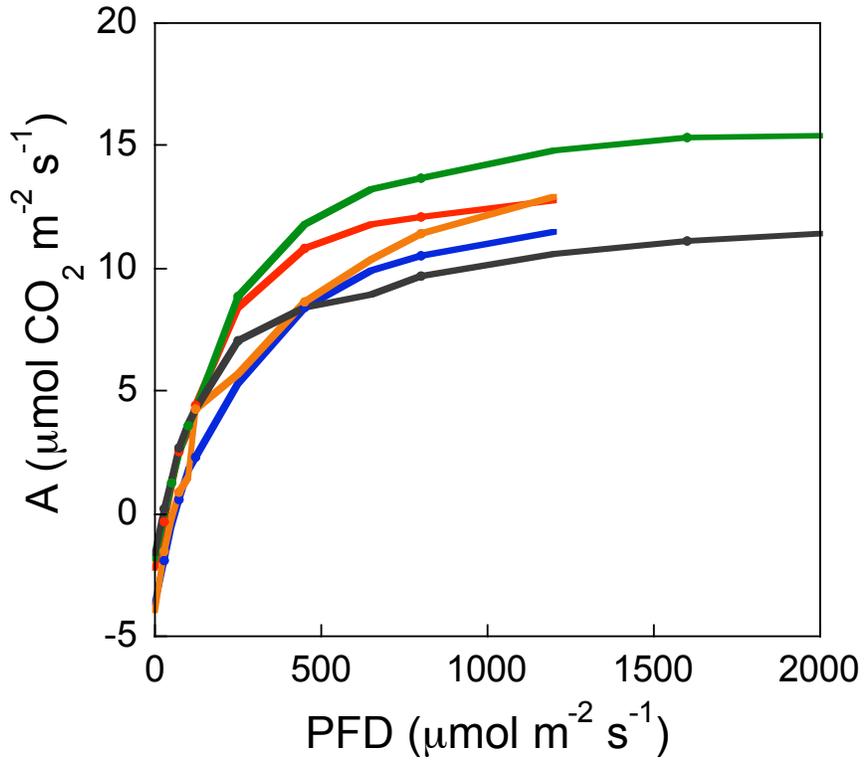


Figure 5-3. Photosynthetic rate (A) was measured on five intact *Fraxinus americana* leaves at a range of light levels on 24–27 June 2005. Colored lines represent leaves from different trees. While measurements were being recorded, the cuvette temperature was 30°C and the CO_2 concentration inside the cuvette was 380 ppm. From this experiment, it was determined the saturating light level for *F. americana* is 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

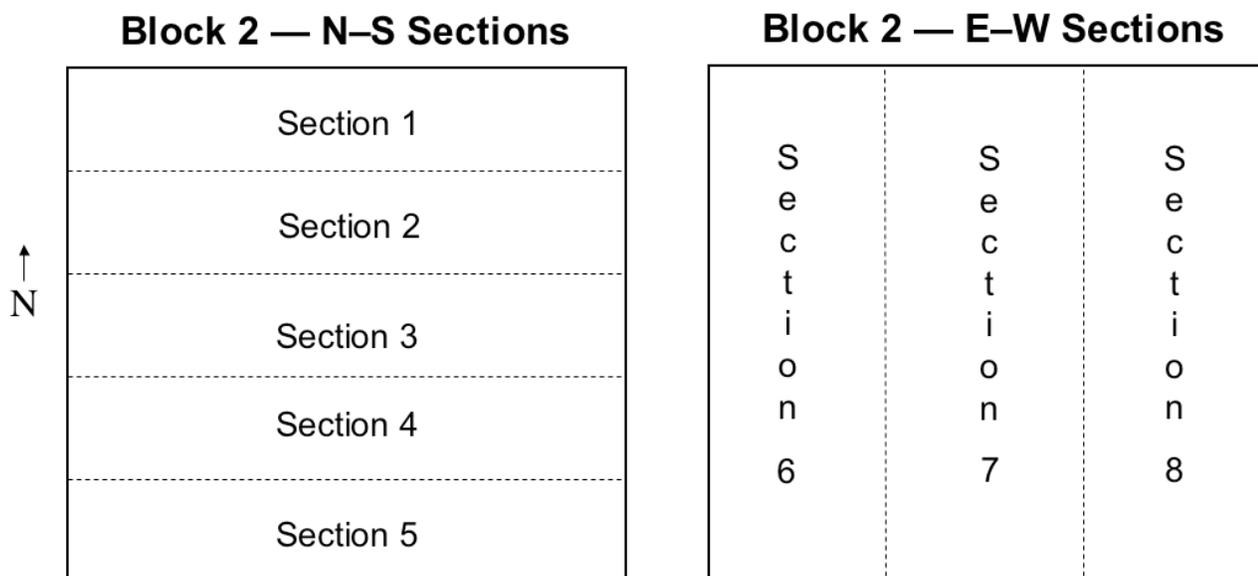


Figure 5-4. Block 2 of the common garden of *Fraxinus americana* trees was divided into sections in both the N–S and E–W directions in order to compare soil water content differences across the block. The six *F. americana* trees of approximately the same stem circumference that were growing outside the common garden are not pictured in the figure. Predawn water potentials (Ψ^{PD}) of leaves obtained on 20 July 2005 and 8 September 2005 in both the N–S and E–W sections were compared with Ψ^{PD} of leaves of the native Kansas population to determine if soil water content differed among these sections. One-way analyses of variance (ANOVA, $\alpha = 0.05$) with section as the main effect were used to statistically analyze the Ψ^{PD} of leaves from Block 2 and the Kansas population of *F. americana*. No significant differences in Ψ^{PD} among the N–S and E–W sections and the Kansas population were found on either 20 July 2005 or 8 September 2005 ($p = 1.00$, $p = 0.095$ for the N–S sections and $p = 0.098$, $p = 0.11$ for the E–W sections, respectively).

Table 5-1. Predawn water potentials (Ψ^{PD}) in Block 2 of the *F. americana* common garden

| Block 2 | Mean $\Psi_p \pm SE$ (July) | Mean $\Psi_p \pm SE$ (Sept.) |
|----------------|---|--|
| Section 1 | -12.71 \pm 7.6 | -9.54 \pm 10.6 |
| Section 2 | -12.59 \pm 10.6 | -9.76 \pm 17.9 |
| Section 3 | -13.78 \pm 10.7 | -4.81 \pm 5.2 |
| Section 4 | -9.24 \pm 7.2 | -6.60 \pm 6.5 |
| Section 5 | -16.03 \pm 7.4 | -5.65 \pm 5.0 |
| Kansas pop. | -15.87 \pm 10.3 | -14.60 \pm 13.7 |
| Section 6 | -12.38 \pm 9.0 | -7.03 \pm 10.0 |
| Section 7 | -13.13 \pm 11.1 | -7.26 \pm 8.0 |
| Section 8 | -12.90 \pm 8.2 | -7.30 \pm 13.2 |
| Kansas pop. | -15.87 \pm 10.3 | -14.60 \pm 13.7 |

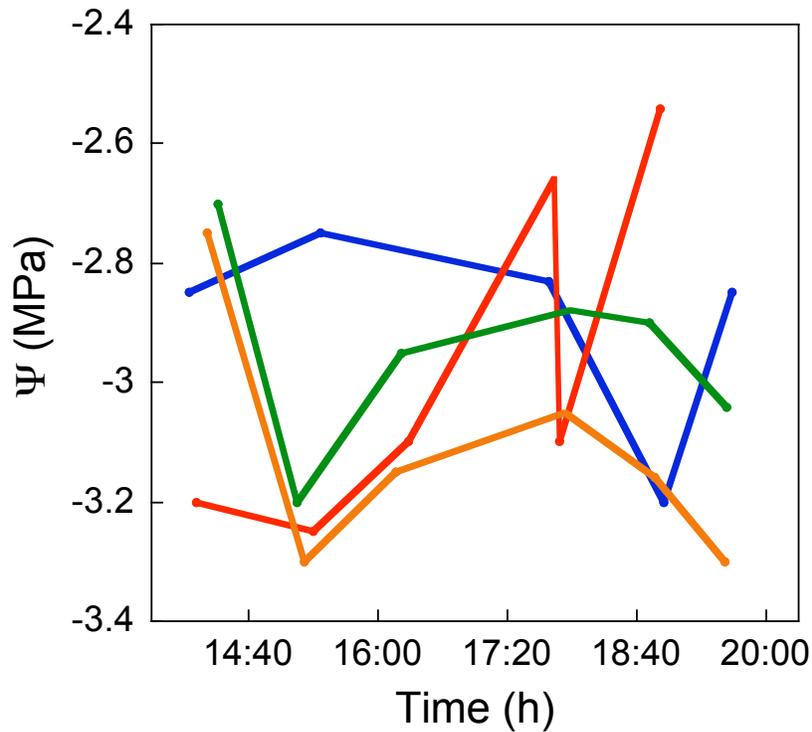


Figure 5-5. Changes in leaf water potential over time in four different *Fraxinus americana* individuals. Six leaves were collected at 1400 h on 8 July 2005 from the same bud, sealed in a plastic bag with a wet paper towel, and placed in a cooler. Leaves were brought back to the laboratory, and leaf water potential for each tree was measured at periodic intervals. Since there is no pattern in the changes in water potential of these four trees during the 5 h of this experiment, the sampling procedure utilized in this study for the measurement of leaf water potentials was considered appropriate.

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