

Response to Drought and Heat Stress in Male and Female *Acer negundo* Inferred from  
Inter-annual Patterns of Radial Growth and  $\delta^{13}\text{C}$  Abundance in Tree-ring Cellulose

by

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## ABSTRACT

Dioecious plants often display sexual segregation in habitat preference and trait expression due to contrasts in reproductive costs. Females may be maladapted to environments with limited available resources, or habitats where resources are diminishing due to climate change. Reduced fitness in female individuals compared to males could lead to skewed sex ratios and reduce population fitness of dioecious species, including one of the most widely distributed dioecious tree species in North America, *Acer negundo*. The goal of this study was to evaluate how climate warming and drought may enhance sexual segregation in productivity and physiological stress in *A. negundo*. To address this goal, I measured radial growth and carbon isotope ratios ( $\delta^{13}\text{C}$ ) in tree-ring cellulose of 22-year male and female *A. negundo* trees growing in a common garden in Salt Lake City, UT. The trees were originally transplanted as one-year old cuttings from a nearby site that was 6.5 °C cooler than the common garden. I hypothesized that 1)  $\delta^{13}\text{C}$  would be lower (more negative) in late growth that is formed during the hottest months of the growing season in males than in females, and during years with no supplemental watering, indicating lower stress from heat and drought in males than in females. And 2) radial growth would be greater in males under warm, well-watered conditions and the addition of drought will exacerbate the difference between males and females. To test these hypotheses, cores were extracted from the main stem of nine male and nine female trees with an increment borer. Annual growth was measured on each core and cellulose was extracted to measure annual  $\delta^{13}\text{C}$  ratios. Males had a 0.63‰ lower mean  $\delta^{13}\text{C}$  than females in years after supplemental water had ceased ( $p = 0.03$ ) and a 4.12 mm wider radial growth compared to females while irrigated ( $p = 0.02$ ). Although

these data did not support my hypotheses per se, results nevertheless indicate that females are more likely to be maladapted to climate warming and drought to a greater extent than males. If so, a combination of drought and heat stress may have deleterious impacts on the population fitness of *Acer negundo* and other similar dioecious tree species.

## DEDICATION

I dedicate this thesis to women in STEM. For those in the past, made it possible for me to be here. Present, gave me the support and guidance throughout this process to help me reach the finish line. And to the future women in STEM, who will continue to push forward.

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## CHAPTER 1

### INTRODUCTION

Dioecious species are those that have male and female reproductive organs on separate plants. Dioecy occurs in approximately half of all angiosperm families (Heilbuth 2000), representing 7% of all angiosperm genera and roughly 6% of all angiosperm species (Chen *et al.* 2010). Dioecious species often play a key role in maintaining the stability of structure and function in terrestrial ecosystems (Xu *et al.* 2008), as many sex-specific relationships exist within ecosystems (Hultine *et al.* 2016). Therefore, phenotypic variation between males and females has notable impacts on ecosystem functions such as soil carbon fluxes and rates of nutrient transformation, as well as the composition of the plant, invertebrate, and microorganism communities associated with them (Hultine *et al.* 2016). Secondary sexual dimorphism - the differences between male and female plants in characteristics that do not involve sexual organs (Hultine *et al.* 2016) - can help each sex meet sex-specific resource demands associated with reproductive costs, thereby granting each sex with greater performance (Chen *et al.* 2010). Therefore, photosynthetic parameters such as stomatal conductance, net photosynthesis, and water use efficiency (the ratio of net photosynthesis to transpiration) are often different between co-occurring males and females. In high resource habitats, females are able to compensate for their higher reproduction costs of fruit development by constructing features that maximize carbon acquisition through photosynthesis (Hultine *et al.* 2016). Males are able to better endure less favorable habitats via enhanced resource use efficiency (Hultine *et al.* 2016). This leads to female biased sex ratios in resource rich habitats and male biased sex ratios in low resource (Dawson & Ehleringer 1993; Ward *et al.*, 2002). While differences in

reproductive costs often underpins spatial segregation of the sexes, it is important to note that under current climate conditions, the overall population sex-ratio does usually not deviate from one and the sexes are still close enough for reproduction to occur (Dawson & Ehleringer 1993). However, previous research on dioecious species found that under temperature warming or drought conditions, females often lost their competitive advantage of resource acquisition efficiency (Ward *et al.* 2002; Hultine *et al.* 2016). Specifically, in unfavorable habitats, females tend to express a higher stomatal sensitivity to water stress than compared to males (Hultine *et al.* 2016). Females may be maladapted to environments with limited available resources, or habitats where resources are diminishing due to climate change, which would lead to reduced fitness and stress survival compared to their male counterparts (Hultine *et al.* 2016), furthering skewed sex ratios and sexual segregation of dioecious species.

The global annual temperature has increased at an average rate of 0.08 °C per decade since 1880 and more than double that rate (0.18 °C) since 1981 (NOAA 2021). Climate change is causing an increase in heat and drought at a rapid rate, thus affecting an increasingly wider array of plant species, especially those in already arid or semi-arid climates (Wang, Vinocur & Altman 2003; Adams *et al.*, 2009). Plant populations that occur in the warm extremes of species ranges are more susceptible to changes in temperature (O'Neill *et al.*, 2008). In the drylands of the southwestern US, for example, climate change is disrupting recharge and runoff mechanisms and forecasts suggest that growing aridity will likely lead to reductions in both stream base flows and water tables (Stromberg *et al.*, 2009). In the face of climate change, riparian forests are among the most at-risk forests in semi-arid regions of North America (Stromberg *et al.*, 1993). Many

of the iconic and ecologically important tree species that make up riparian environments in the western US are dioecious, such as *Populus* spp. (cottonwoods), *Salix* spp. (willows), and *Acer negundo* (boxelder) (Hultine *et al.*, 2018). Stream flow reductions in riparian areas would alter the plant community from mesic canopies, such as the ones listed above, to more xeric shrub communities, resulting in diminished canopy cover and shorter canopies (Stromberg *et al.*, 2010). For example, foundation dioecious species of riparian forests, such as *Populus fremontii*, have rapidly declined in the last decade due to lower water tables and increased temperatures caused by climate change (Moran *et al.*, 2023). It is crucial to study dioecious species located in warmer and drier environments, like those of the riparian areas of the Southwest, as they are foundational to ecosystem function and community composition.

Riparian zones are transitional ecosystems between freshwater aquatic environments and terrestrial communities. They feature specialist ecological communities adapted to the proximity of water and provide important ecosystem services such as; critical habitat, structure, and function to streamside species (Rodríguez-Gonzalez *et al.* 2022), filtering pollutants, and stabilizing streambanks. The stability of riparian ecosystems in the Southwest and Intermountain west of the US depends largely on the dominant riparian trees that grow along streambeds within semiarid environments (Ward *et al.* 2002). In addition to land development, grazing, and other human-related activities, climate change is further threatening the decline of riparian forests in arid and semi-arid regions of North America (Hultine *et al.* 2018). The effects brought on by climate change such as increasing temperatures, higher evaporative demand, and earlier snow melt are causing stream discharge to decline in riparian areas of the western US, where plant

productivity and diversity are already restricted by the constraints of seasonal aridity (Hultine *et al.* 2018; Leung *et al.* 2004; Stewart *et al.* 2004; Barnett *et al.* 2008). Water stress has been shown to restrict the water and energy budgets more so in females than in males, such that males tend to have a less severe decline in photosynthetic gas exchange and growth rates (Xu *et al.* 2008). The increase in temperature and drought caused by climate change could exacerbate the already skewed sex ratios and sexual segregation dioecious species exhibit, causing extreme male biased sex ratios in resource limited habitats, and thus put dioecious species in riparian forests at greater risk of mortality and loss of fitness (Hultine *et al.* 2016).

*Acer negundo* (boxelder) is a dioecious riparian tree species found in the intermountain west of the United States. It serves as an exceptional illustration of secondary sexual dimorphism in terms of habitat preference, physiology, and growth (Dawson and Ehleringer, 1993; Ward *et al.*, 2002; Dawson *et al.*, 2004; Hultine *et al.* 2018). While *A. negundo* are highly sensitive to water stress (Smith *et al.* 1991), their response to water stress varies between sexes (Ward *et al.* 2002). In high resource habitats, females are able to compensate for their higher reproductive costs by developing features that maximize carbon acquisition through photosynthesis (Dawson and Ehleringer, 1993; Hultine *et al.*, 2007; Hultine. *et al.*, 2016). On the other hand, males are able to better endure less favorable habitats by having higher resource use efficiency (Dawson and Ehleringer, 1993; Hultine *et al.*, 2013). Therefore, dioecious plants tend to display sexual segregation due to the fact that female plants have higher reproduction costs, causing them to typically be located and outcompete male counterparts in high

moisture, nutrient rich habitats (Chen *et al.*, 2010), while males tend to dominate non-streamside, xeric habitats (Dawson and Ehleringer, 1993; Ward *et al.*, 2002).

A key indicator of plant response to heat and drought is stomatal behavior. Under hot, dry conditions male *A. negundo* have been observed to have a significantly higher stomatal conductance ( $g_s$ ) than females (Dawson and Ehleringer, 1993; Dawson *et al.*, 2004; Hultine *et al.* 2013). A greater  $g_s$  allows plants to take up atmospheric  $CO_2$  ( $c_a$ ) into substomatal cavities ( $c_i$ ) at a higher rate during the day and is directly linked to plant productivity. However, higher  $g_s$  comes with the tradeoff of higher transpiration rates and reduced water use efficiency. One way plants survive drought is by maintaining a higher water use efficiency (the ratio of carbon assimilation through the stomata ( $A$ ) to water loss via transpiration ( $E$ )) as well as increasing stomatal sensitivity to water deficits (Dawson and Ehleringer, 1993; Cohen 1970, Cowan 1982, Passioura 1982, Geber and Dawson 1990, Johnson *et al.* 1990, DeLucia and Schlesinger 1991). Meaning in periods of drought,  $g_s$  will be more closely regulated to avoid losing water via transpiration. However, this can cause a tradeoff to occur between drought tolerance (shown via WUE patterns) and carbon uptake for growth and reproduction (Dawson and Ehleringer, 1993).

Carbon isotope ratios in plant tissues are a powerful tool for evaluating plant gas exchange patterns and stress (Farquhar *et al.* 1989). Specifically, the ratio of  $^{13}C$  to  $^{12}C$  (represented as delta ( $\delta$ )) in plant tissues reflect plant water budgets (water use efficiency, transpiration/stomatal conductance) and energy budgets (photosynthetic capacity) because these processes are directly linked to  $c_i/c_a$  and  $c_i/c_a$  largely governs carbon

isotope discrimination and hence,  $\delta^{13}\text{C}$  ratios (Farquhar *et al.* 1982). This relationship can be represented as:

$$\delta^{13}\text{C}_{\text{plant tissue}} = \delta^{13}\text{C}_{\text{atm}} - a - (b - a) c_i/c_a$$

where  $\delta^{13}\text{C}_{\text{atm}}$  is currently at -8.5‰,  $a$  is the fractionation coefficient caused by diffusion of  $\text{CO}_2$  through stomata (4.4‰),  $b$  is the fractionation associated with  $\text{CO}_2$  fixation by photosynthetic enzymes, primarily Rubisco (27‰), and  $c_i/c_a$  is the ratio of intercellular  $\text{CO}_2$  concentration to atmospheric  $\text{CO}_2$  concentration (Marshall *et al.*, 2007).

Enzymatic reactions, like carbon fixation, typically favor  $^{12}\text{C}$  in  $\text{C}_3$  plants, due to the strong preference of Rubisco to  $^{12}\text{C}$ . When plants are exposed to heat or drought stress, plants close their stomata to prevent water loss. If all things are equal, a lower  $g_s$  will reduce  $c_i$ , and Rubisco will start to fix relatively more  $^{13}\text{CO}_2$  molecules, thus increasing the  $\delta^{13}\text{C}$  in recently synthesized leaf sugars. The relationship between  $\delta^{13}\text{C}$  and a plant's water and energy budget can be described as:

$$A = c_i/c_a * g_s / 1.53,$$

$$E = g_s (L A V D),$$

$$\text{WUE} = A/E = c_i/c_a / [1.53(L A V D)]$$

where  $A$  is the net photosynthetic rate,  $E$  is transpiration rate,  $g_s$  is stomatal conductance,

1.53 is the ratio of diffusivities of water vapor and CO<sub>2</sub> in air (Campbell & Norman 1998), and leaf to air vapor difference (LAVD) is the difference in water vapor concentration between the interior of a leaf and its surrounding atmosphere (Farquhar & Richards 1984). Since  $c_a$  and LAVD can be assumed to be constant among plants being studied at a given site, plant  $\delta^{13}\text{C}$  and WUE can be linearly correlated (Marshall *et al.* 2007).

Analysis of tree rings provides a multiyear record of environmental drivers, which allows for the comparison of changes in physiological responses between past and current growing conditions, and in dioecious species, between male and female trees. The analysis of tree rings, therefore, can be used to evaluate if there is a significant sex-specific response to changing environmental conditions. The aboveground productivity of a woody plant is represented by radial stem growth that can be identified from individual growth rings (Babst *et al.*, 2014). The growth of tree rings is sensitive to many environmental and anthropogenic factors such as climate (Frank *et al.*, 2010), disturbances (Esper *et al.*, 2007), light and nutrient availability (Drever & Lertzman 2001), competition (Weber *et al.*, 2008), or forest management (Brienen & Zuidema 2006) and therefore can be used to create records ranging from a few years to several hundred years or longer. Physiological processes such as photosynthesis are regulated by the environment both before and after the growing season. This affects the final characteristics of tree rings; hence tree rings hold valuable information related to the energy, carbon, water, and nutrient budgets (Babst *et al.* 2014). Annual tree rings are comprised of early- and late-wood. Earlywood is made from sugars synthesized during the earlier part of the growing season (typically spring and early summer), and/or from the

previous growing season, whereas latewood is constructed from sugars synthesized during the latter part of the growing season (typically mid-summer to early fall). Seasonal and annual variations in water availability and temperature affect tree growth and carbon isotope discrimination (Livingston & Spittlehouse 1996), which is why studying both earlywood and latewood of tree rings will provide a more robust analysis of plant response to warming temperatures and drought compared to analyzing only inter-annual rings.

There have been previous studies on *A. negundo* analyzing  $\delta^{13}\text{C}$  of leaf soluble carbohydrates to infer short term gas exchange variability (Hultine *et al.* 2013) and studies analyzing sex-specific responses from tree-ring cellulose  $\delta^{13}\text{C}$  after implementing different watering treatments (Ward *et al.* 2002). However, research is lacking on long-term trends of sexual dimorphism in key traits related to climate sensitivity, in particular warming temperatures and drought. Given the trend of increasing temperatures and drought in the southwestern United States, there is a need to investigate the long-term differences of stress responses between the sexes of dioecious species where population survival may be at high risk of decline due to climate change. This research will provide valuable insight into the future reproduction and survival of a foundational dioecious tree species in the western US, *Acer negundo* Sarg. (boxelder).



## *Study Overview*

This investigation builds on previous research studying physiological contrasts between male and female *A. negundo* transplanted to a location with a significantly warmer mean annual temperature than the source population (Hultine *et al.* 2013). Water was provided in the common garden by a perennially flowing artificial stream system that delivered constant water to the *A. negundo* starting in 2005, before being discontinued after the 2010 growing season, leaving the trees entirely dependent on rainfall. The contrast in temperature between the source population and the common garden resembles the anticipated rise in average temperature expected over the next century in the southwestern US (Karl *et al.* 2009). My research compared the inter-active responses of male and female *A. negundo* trees to heat (derived from the 6.5 °C transfer distance from the source location) and drought stress, after the artificial stream was turned off. I compared the difference in annual radial growth, and  $\delta^{13}\text{C}$  in cellulose of tree rings from 2006-2014, and  $\delta^{13}\text{C}$  of early growth and late growth from 2006-2008. I hypothesized that 1)  $\delta^{13}\text{C}$  will be lower (more negative) in males than in females, in wood synthesized during the warmer part of the growing season (late growth) and during non-irrigated years (2011-2014), 2) radial growth will be greater in males under warm, well-watered conditions (2006-2010) and the addition of drought will exacerbate the difference between males and females (2011-2020) , and 3) differences in growth and  $\delta^{13}\text{C}$  will be greatest between sexes during the hottest and driest years. These hypotheses suggest overall that males will have less physiological sensitivity to heat and drought than the co-occurring females. This would provide further insight into how climate change could

further sexual segregation of dioecious species and potentially put their reproduction and survival at risk.

## CHAPTER 2

### MATERIALS AND METHODS

#### *Site Description:*

The common garden was constructed on the University of Utah campus (40°45'39''N, 111°49'49''W, 1481 m) and has been described in detail by Hultine *et al.* (2013). *A. negundo* trees were established from cuttings obtained from reproductively mature trees growing in Red Butte Canyon Research Natural Area (Ehleringer *et al.* 1992) in Salt Lake City, Utah where the growing season temperature averaged 6.5 °C cooler than the common garden the *A. negundo* were transplanted into in 2000 (Hultine *et al.* 2013). An artificial stream system, which has been described in detail by Hultine *et al.* (2008), brought free-flowing water within one meter of each tree from 2006 until late 2010 when the water was shut off (Fig 1). Thirty-six cuttings were originally planted in a six-by-six grid with 3 m spacing between them, of which 33 (13 males and 20 females) survived the initial establishment phase of the garden (Hultine *et al.* 2013). In February and April 2022, 12 female and 10 male trees were tagged, marked with their sample ID, and cored. The remaining 11 trees that survived initial establishment to the common garden did not appear as healthy as the 22 selected and thus, were not used for this study.



**Fig. 1:** Artificial stream system at University of Utah common garden that brought free-flowing water within 1 meter of each *A. negundo* tree. Four channels, approximately 1 m wide and 0.2-0.25 m deep were crafted using a small backhoe in 2005. A 0.75-inch (1.905 cm) diameter PVC pipe was installed along the center of each channel, spanning their entire lengths to supply water. The PVC configuration included a T-junction at the starting point where the streams diverged, and a rejoining point near the endpoint where the streams merged. This design ensured that the garden received water from a single irrigation source. Water was dispensed from the PVC piping into the channels through 360° adjustable bubblers. The drainage system comprised two 50-liter catch basins that emptied into a large lowland area near Red Butte Creek.

*Tree Coring and Cellulose Extraction Preparation:*

To study carbon isotope abundance in tree rings, tree cores were collected in February and April 2022 using a 12 mm increment borer. The cores were taken from the east facing side of the largest stem of each tree at approximately 1 m from ground-level. Measurements of stem diameter and basal area were taken from every stem of each tree with a diameter tape, and bark thickness was measured with a cork borer. Each core was

packaged in a paper straw, taped closed, and labeled with the correct sample ID. Once dried, the tree cores were split in half in the longitudinal direction using a band saw. After being split, each core half was mounted on a concave wooden block using water soluble glue, with the rounded side of the core facing down to minimize sample loss during sanding. Mounted cores were left with parchment paper and a weight on top overnight to ensure they dried straight and flush to the mount. After 24 hours, the cores were sanded using progressively finer grit, starting with 120, 150, 400, 600, and finishing with 1000 grit to make the ring boundaries as clear as possible. After sanding, each ring boundary was marked with a pencil under a dissecting microscope. A total of 18 (9 male, 9 female) of the 22 cores were used due to some cores not having enough visible rings. Each marked core was scanned with 2400 dpi resolution to have a reference before the cores were prepped for cellulose extraction. The scans were printed, and every ring boundary was analyzed for a marker year which was used to cross date each ring with its correct corresponding year. The nine middle years of the core (2006-2014) were dissected on each ring boundary using a scalpel, and the three earliest of the years before the water was turned off (2006-2008) were subdivided in half to represent early growth and late growth, which allowed detection of intra-annual changes through time in relative  $\delta^{13}\text{C}$  values between sexes. It was assumed that early growth was from photosynthates synthesized from early spring and the previous fall while late growth represented photosynthates synthesized in late spring and summer (Szejner *et al.* 2016).

*Cellulose extraction:*

Tree-ring cellulose provides a cleaner signal of  $\delta^{13}\text{C}$  abundance than whole wood tissue. Thus, cellulose was extracted from the dissected rings, primarily following the protocol of Szejner *et al.* (2016). Wood samples were ground to a particle size that could fall through 20 mesh with a rotary grinder or by cutting into small pieces with scissors, which ended up being slightly larger than 20 mesh. The sample material was then loaded into ANKOM F57 filter bags with a porosity of 25  $\mu\text{m}$ . Starting with extraction of waxes, oils and resins, a 700 ml of a 1:1 toluene: ethanol solution was used to ensure all the ethanol would not evaporate overnight. The 1:1 solution ran through the samples for 24 hours in a Soxhlet apparatus having a 1000 ml flask capacity, followed by 95% ethanol for 24 hours. Samples were rinsed and boiled in DI water for 6 hours. After boiling, samples were bleached using 7 g of sodium chlorite in 500 ml of DI water and adding 1 ml of acetic acid to maintain a pH below 4 for 48 hours. The samples were then rinsed again in DI water to produce holo-cellulose for  $\delta^{13}\text{C}$  analysis. Filter bags were cut open, and >0.50 mg of cellulose was weighed out and stored in autoclaved vials. To ensure homogeneity of cellulose samples, all holo-cellulose fibers were separated by sonicating the material in 1 mL of chilled deionized water with 30 s of ultrasound by using a Hielscher UP200S ultrasonic probe (Hielscher Ultrasonics, Teltow, Germany) (Laumer *et al.*, 2009). Samples ranging from 0.12-0.16 mg of holo-cellulose were loaded in tin capsules using a Cubis® II Micro Balance (Sartorius, Göttingen, Germany). The samples were analyzed at the University of Arizona Environmental Isotope Laboratory using a Costech elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA) coupled to Finnigan Delta PlusXL isotope ratio mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) with a sample precision of  $\pm 0.1\%$ .

*Ring width data:*

Each ring width was measured using scanned images of the cores that were then uploaded to CooRecorder (Cybis Elektronik & Data AB, Saltsjöbaden, Sweden). After downloading the CooRecorder software, core scans (that were greater than or equal to 1600 dpi resolution) were uploaded by selecting the “open image file for new coordinates” option. Tree rings were then digitally measured by selecting the “measure ring widths or blue data” feature and clicking each ring boundary, starting from the outermost ring.

*Statistical Analyses:*

All analyses were conducted using R version 4.1.1. Residuals of  $\delta^{13}\text{C}$  and ring width data were assessed for normality using the Shapiro-Wilk test. Two separate two-way ANOVA's were conducted to test for the effect of year, sex, and their interaction on  $\delta^{13}\text{C}$  and ring width. Students t-tests were conducted to test for contrasts between male and female trees in mean  $\delta^{13}\text{C}$  during irrigation (2006-2010) and during post-irrigation years (2011-2014). In addition, Students t-tests were also conducted to test for differences in mean ring widths between males and females during irrigation (2006-2010) and post-irrigation (2011-2020). For each Student's t-test, treatment was subset into irrigation and post irrigation treatment, to compare the difference in  $\delta^{13}\text{C}$  or mean ring width between males and females under irrigation and after irrigation. Two additional Student's t-tests

were conducted to test for difference in mean  $\delta^{13}\text{C}$  between males and females in early and late growth from rings split in half from 2006-2008. An additional student's t-test was conducted by pooling the sexes together for early and late growth to test the difference in mean  $\delta^{13}\text{C}$  between growth (not sex).

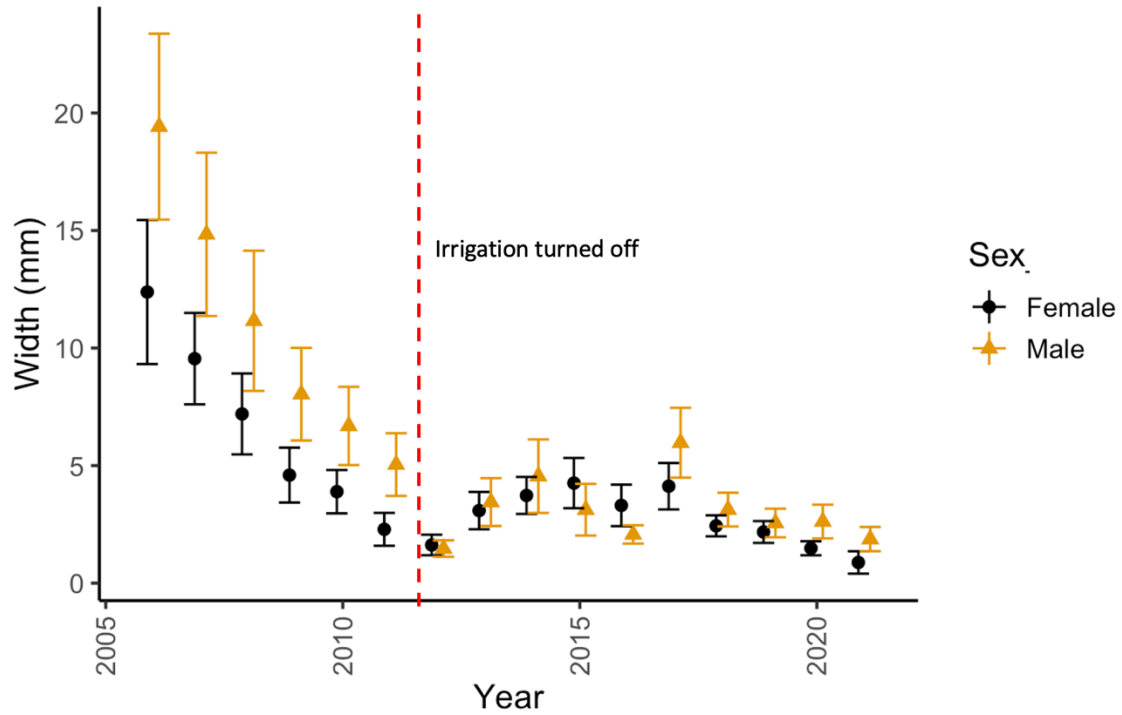
## CHAPTER 3

### RESULTS

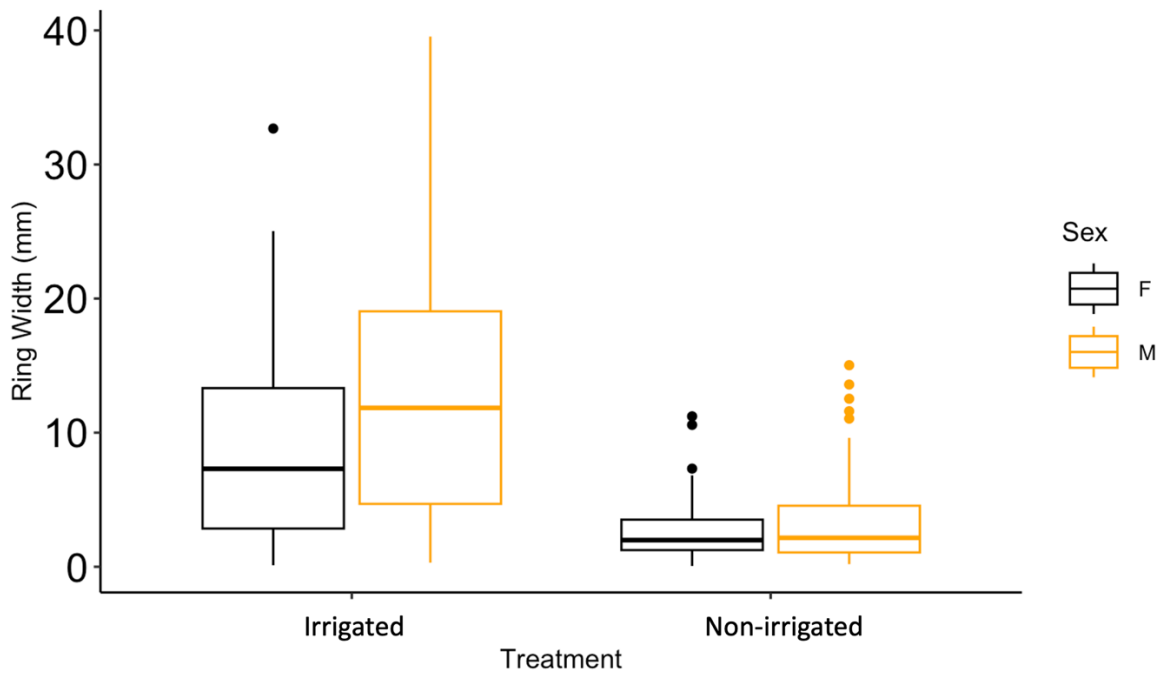
#### *Annual Ring Widths*

Over the 14 years in which ring widths were analyzed (2006-2020), mean annual radial growth was 7.47 mm / year (SE = 1.11) in males compared to 4.72 mm / year (SE = 0.86) in females ( $t = 4.61$ ,  $P = 0.0017$ ) (Fig 2). ANOVA results across all years showed that only time had a significant effect on ring width ( $F = 1.99$ ,  $p = 0.049$ ). However, when the years were pooled into irrigated and non-irrigated treatments differences across all years were a function of males having a 68% higher annual growth rate than females while irrigated: 13.05 mm (SE = 1.96) and 8.93 mm / year (SE = 1.46), in males and females, respectively ( $t = -2.42$ ,  $P = 0.017$ ). Once irrigation had ceased, mean annual growth dropped to 3.28 mm / year, or 25% of growth when irrigated in males, and to 2.75 mm / year, or 31% of growth when irrigated in females. No differences were detected between sexes after irrigation was ceased ( $t = -1.35$ ,  $P = 0.18$ ) (Fig. 3).





**Fig. 2** Mean annual ring widths in male and female *A. negundo* trees from 2006 to 2020. Error bars represent the standard error of the means. Two-way ANOVA results across all years showed only time had a significant effect on ring width, not sex nor their interaction ( $F = 1.99$ ,  $p = 0.049$ ).



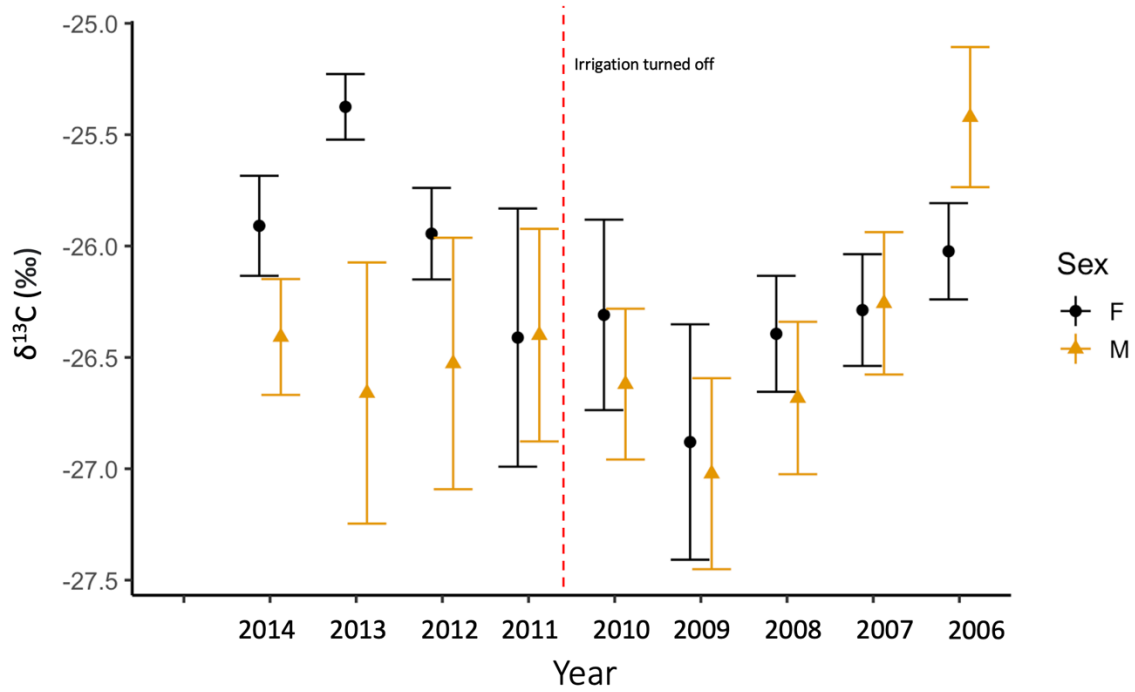
**Fig 3:** Box plot showing ring width data for males and females under irrigation and after irrigation was ceased. The line within the box represents the median, the edges of the box mark the 25th percentile (Q1) and the 75th percentile (Q3) of the distribution. The whiskers show the range of the data. Males had significantly greater growth under irrigation ( $t = -2.42$ ,  $P = 0.017$ ). No significant difference was detected between the sexes after irrigation was turned off ( $t = -1.35$ ,  $P = 0.18$ ).

#### *Irrigated and Non-irrigated $\delta^{13}C$*

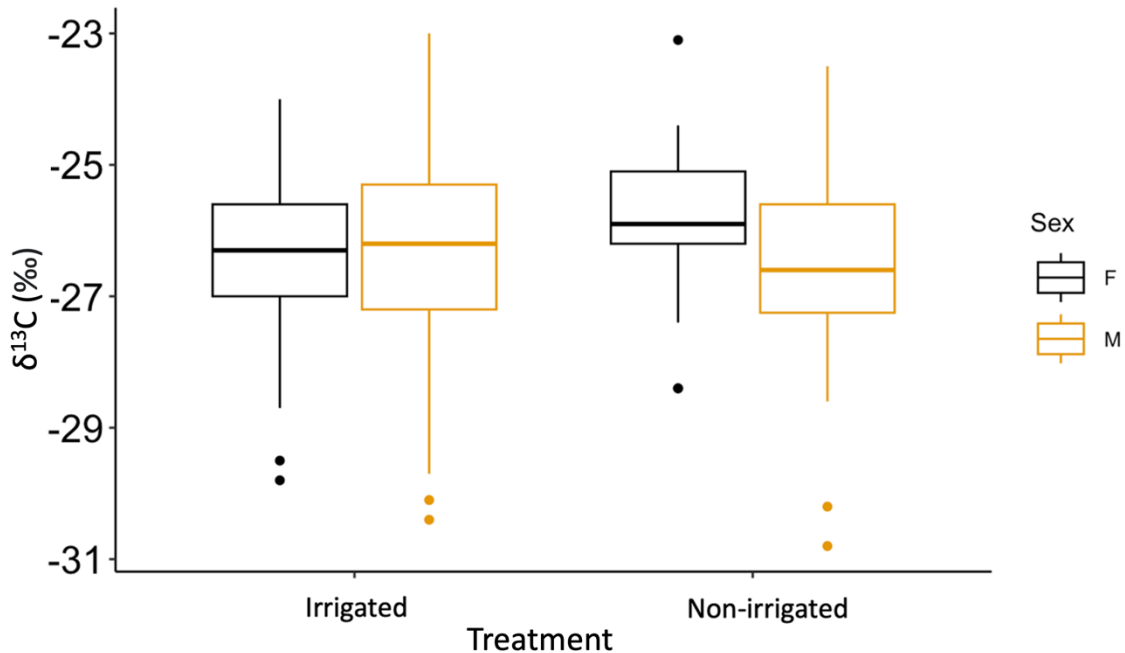
Carbon isotope ratios ( $\delta^{13}C$ ) were measured across 9 years (2006-2014) from female ( $n = 9$ ) and male ( $n = 9$ ) *A. negundo* tree-ring cellulose (Fig 4). ANOVA results across all years showed that sex and time had a significant effect on  $\delta^{13}C$  ( $F = 2.29$ ,  $p = 0.003$ ;  $F = 17.08$ ,  $p = 2e-16$ ). However, when the years were pooled into irrigated and non-irrigated treatments females and males had a mean  $\delta^{13}C$  that was nearly identical under irrigation:  $-26.24\text{‰}$  ( $SE = 0.14$ ), and  $-26.28\text{‰}$  ( $SE = 0.16$ ), respectively ( $t = -0.26$ ,  $P = 0.80$ ).

However, after irrigation ceased, males had a significantly lower mean  $\delta^{13}C$  than females

( $t = 2.24$ ,  $P = 0.03$ ), mean  $\delta^{13}\text{C}$  in females was  $-25.87\text{‰}$  ( $\text{SE} = 0.16$ ) while mean  $\delta^{13}\text{C}$  in males lowered to  $-26.50\text{‰}$  ( $0.23$ ) (Fig. 4).



**Fig. 4:** Mean annual  $\delta^{13}\text{C}$  of males and females from 2006-2014. Error bars represent the standard error of the means. Two-way ANOVA results across all years sex and time had a significant effect on  $\delta^{13}\text{C}$ , but their interaction did not ( $F = 2.29$ ,  $p = 0.003$ ;  $F = 17.08$ ,  $p = 2e-16$ ).



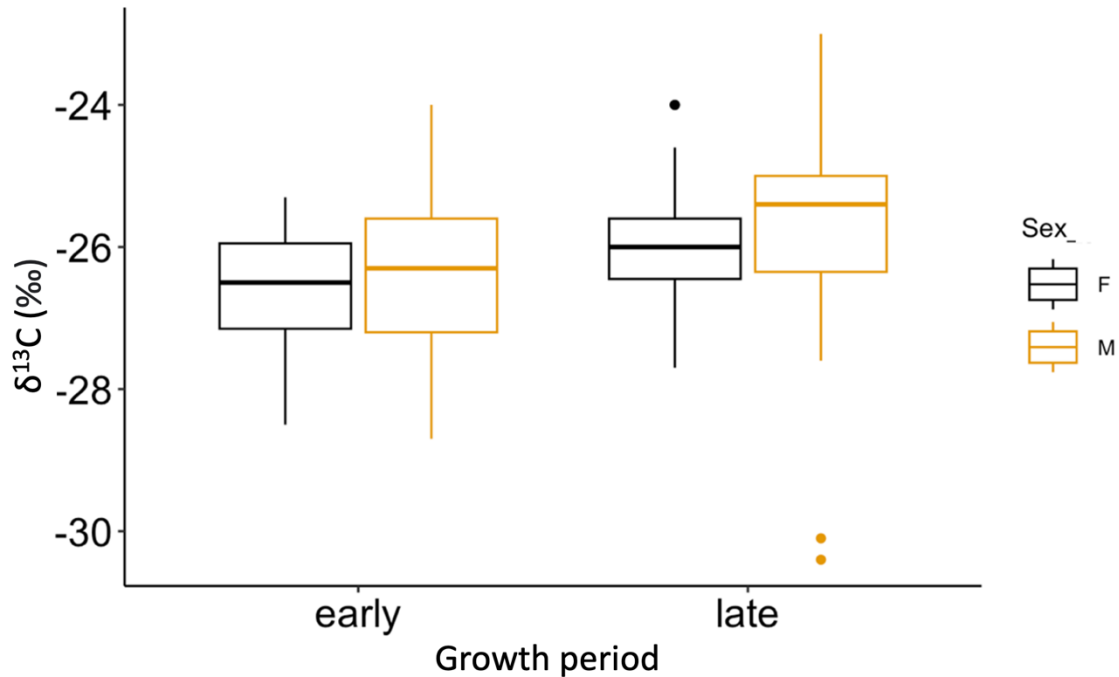
**Fig 5:** Box plot showing  $\delta^{13}\text{C}$  data of males and females under irrigation and after irrigation ceased. The line within the box represents the median, the edges of the box mark the 25th percentile (Q1) and the 75th percentile (Q3) of the distribution. The whiskers show the range of the data. There was no significant difference between males and females under irrigation ( $t = -0.26$ ,  $P = 0.80$ ). Males showed a significantly lower  $\delta^{13}\text{C}$  compared to females after irrigation had been turned off ( $t = 2.24$ ,  $P = 0.03$ ).

#### *Seasonal Patterns of $\delta^{13}\text{C}$*

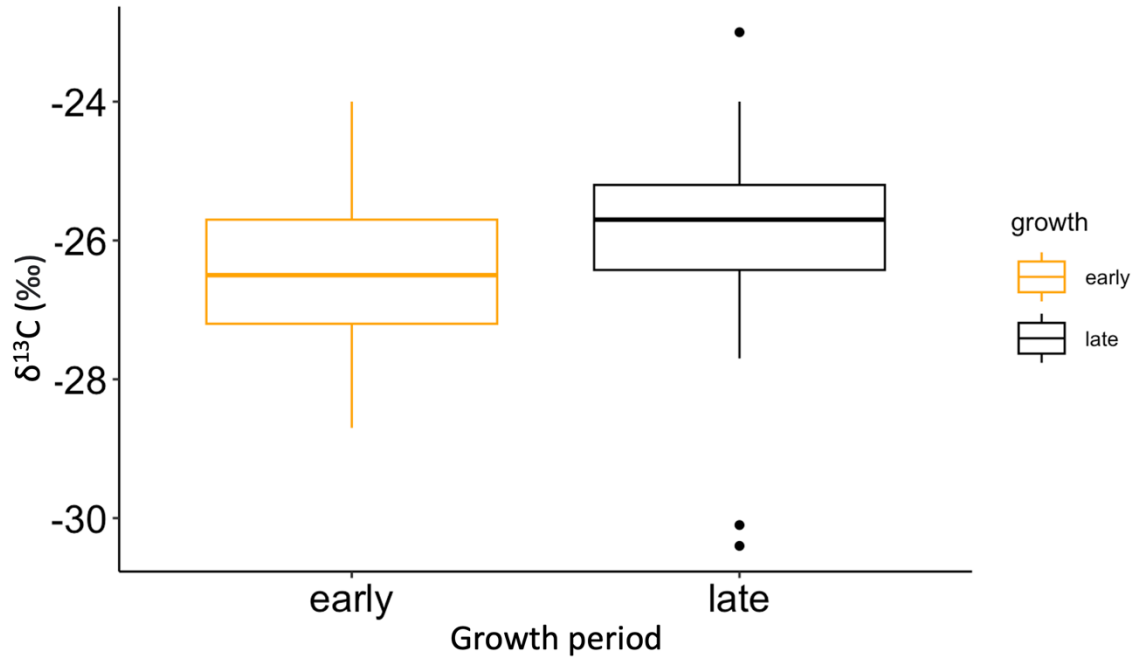
Between 2006-2008 I split the tree rings to examine seasonal trends in  $\delta^{13}\text{C}$  abundance.

There was no significant difference in mean  $\delta^{13}\text{C}$  between males and females for early growth ( $t = -0.67$ ,  $P = 0.51$ ), where mean  $\delta^{13}\text{C}$  was  $-26.38\text{‰}$  ( $\text{SE} = 0.26$ ) and  $-26.60\text{‰}$  ( $\text{SE} = 0.19$ ) for males and females, respectively (Fig. 6). Likewise, there was also no significant difference in mean  $\delta^{13}\text{C}$  between males and females in late growth ( $t = -0.45$ ,  $P = 0.66$ ), where mean  $\delta^{13}\text{C}$  was  $-25.79\text{‰}$  ( $\text{SE} = 0.29$ ) and  $-25.93\text{‰}$  ( $\text{SE} = 0.17$ ) for males and females, respectively (Fig. 6). While there was no significant difference in

$\delta^{13}\text{C}$  between males and females in early or late growth, there was a significant difference in  $\delta^{13}\text{C}$  overall between early and late growth ( $t = -2.65$ ,  $P = 0.0093$ ), where mean  $\delta^{13}\text{C}$  in early growth was  $-26.48\text{‰}$  and rose to  $-25.86\text{‰}$  in late growth (Fig. 6).



**Fig. 6:** Box plot showing  $\delta^{13}\text{C}$  data of males and females during early growth and late growth. The line within the box represents the median, the edges of the box mark the 25th percentile (Q1) and the 75th percentile (Q3) of the distribution. The whiskers show the range of the data. There was no significant difference in mean  $\delta^{13}\text{C}$  between the sexes in early growth ( $t = -0.67$ ,  $P = 0.51$ ) nor in late growth ( $t = -0.45$ ,  $P = 0.66$ ).



**Fig. 7:** Box plot showing  $\delta^{13}\text{C}$  of males and females when pooled together to see if there was a difference in mean  $\delta^{13}\text{C}$  between early and late growth overall. The line within the box represents the median, the edges of the box mark the 25th percentile (Q1) and the 75th percentile (Q3) of the distribution. The whiskers show the range of the data. Late growth had a significantly higher  $\delta^{13}\text{C}$  than early growth ( $t = -2.65$ ,  $P = 0.0093$ ).

## CHAPTER 4

### DISCUSSION

In resource rich habitats, like the streamside environments of riparian areas, *A. negundo* sex ratios are significantly female biased, while in drought-prone areas the sex ratios are significantly male biased (Dawson & Ehleringer, 1993; Ward *et al.*, 2002). The cause of these sex ratio patterns is thought to be associated with the higher cost of reproduction requiring greater resource uptake and growth than co-occurring males in high resource habitats (Hultine *et al.*, 2013). Previous research has found that physiological properties, such as stomatal conductance ( $g_s$ ) and carbon acquisition, were over 40% higher in mature female *A. negundo* than their male counterparts along a perennial stream in Red Butte Canyon (Dawson & Ehleringer, 1993). Moreover, female *A. negundo* growing alongside streams build larger canopies with greater leaf areas (Hultine *et al.*, 2007b) and have faster canopy and radial growth rates compared to males (Dawson & Ehleringer, 1993; Ward *et al.*, 2002). Such differences in resource acquisition, canopy composition, and growth provide streamside females with a competitive edge over streamside males and, possibly, over other tree species that share the same habitat, as thriving in these highly productive environments partially hinges on establishing canopy dominance and high resource uptake (Hultine *et al.*, 2013).

Results from a previous study using the same common garden site used in the present study found that under warmer growing conditions, females maintained a greater max mean  $g_s$  in cooler periods but displayed a greater reduction in  $g_s$  from May-August. Warmer temperatures and corresponding increased vapor pressure deficit (VPD) could result in females approaching their hydraulic limit as they compensate for their higher

reproduction cost of having to produce fruits and flowers between mid-May and mid-June (Hultine *et al.*, 2013). Results suggest that the sex ratio of male and female *A. negundo* could shift under global warming, becoming more male-biased even in habitats where water is not limited.

In this study I looked at male (n = 9) and female (n = 9) *A. negundo* trees that were transplanted to a common garden that was 6.5 °C warmer than the source site they were taken from. In combination with this heat treatment, a drought treatment was established as the constant irrigation that was supplied from 2006-2010 was ceased in late 2010. I hypothesized that 1)  $\delta^{13}\text{C}$  will be lower (more negative) in males than in females, in wood synthesized during the warmer part of the growing season (late growth) and during non-irrigated years (2011-2014) and 2) radial growth will be greater in males under warm, well-watered conditions (2006-2010) and the addition of drought will exacerbate the difference between males and females (2011-2020). My results indicate that females are likely to become more maladapted to climate warming than males, supporting earlier ecophysiology studies on *A. negundo*.

#### *Irrigated and Non-irrigated $\delta^{13}\text{C}$*

As expected, results indicated that the largest difference between male and female *Acer negundo* in the integration of  $\delta^{13}\text{C}$  was after irrigation had ceased (2010-2014), such that males were able to maintain a lower (more negative)  $\delta^{13}\text{C}$  than females. A previous study on male and female *A. negundo* in the same common garden (Hultine *et al.*, 2013) as well as a previous study that looked at *A. negundo* in their original source location (Dawson & Ehleringer, 1993), both found that females have a greater maximum mean  $g_s$



in the cooler seasons and under well-watered conditions. However, the same studies also found that females had a greater reduction in  $g_s$  in hotter and drier months, compared to co-occurring males. Moreover, it is known that plants that operate with a greater maximum  $g_s$  are more sensitive to water stress (Oren *et al.*, 1999; Ewers *et al.*, 2005). Previous studies corroborate results from this experiment showing that under heat and drought scenarios, females are edging towards the upper limits of their hydraulic capacity. These results could indicate a loss in population fitness if current climate change scenarios cause further sexual segregation of the sexes.

Results of mean  $\delta^{13}\text{C}$  under irrigation show no significant difference between males and females. Past research in the same common garden showed that  $\delta^{13}\text{C}$  of leaf soluble sugars was higher in females than males only in late summer (Hultine *et al.*, 2013). The fact that our results differed from the previous study may be due to the fact that I obtained  $\delta^{13}\text{C}$  from tree ring cellulose, thus I was unable to detect day-to-day variation in  $\delta^{13}\text{C}$  that may have been picked up from  $\delta^{13}\text{C}$  of leaf soluble sugars. However, I was able to detect some intra-annual variation in  $\delta^{13}\text{C}$  by comparing late and early growth of tree ring cellulose during the pre-drought period.

#### *$\delta^{13}\text{C}$ in early and late growth*

Investigating carbon isotope ratios of early- and late-growth is a useful tool to study the seasonal variations of specific physiological processes within a tree, such as carbon allocation (Kimak & Leuenberger, 2015). When I pooled sexes together, early growth had a significantly lower  $\delta^{13}\text{C}$  than late growth. This parallels other studies that have found  $\delta^{13}\text{C}$  to be lower during the early, cooler part of the growing season,

compared to the latewood, which is produced in the hotter and drier part of the growing season (Livingston & Spittlehouse, 1996). When I separated late and early growth by sex, there was no significant difference in  $\delta^{13}\text{C}$  between males or females in early or late growth measured from 2006-2008. While the lack of difference in  $\delta^{13}\text{C}$  between males and females in late growth contrasts my hypothesis, previous research found that variation in  $\delta^{13}\text{C}$  within years (seasonal variation) was highly variable and had no consistent relations (Vaganov *et al.*, 2009).

Based on previous research that attributed the difference in late growth  $\delta^{13}\text{C}$  between males and females to disparities in WUE (Hultine *et al.*, 2013), I had expected to observe a lower late growth  $\delta^{13}\text{C}$  in males versus females. Inter- and intra-annual variations in  $\delta^{13}\text{C}$  have been directly linked to the water balance and water use of trees (Livingston *et al.*, 1996), such that in arid and semi-arid ecosystems, greater WUE should correspond to higher  $\delta^{13}\text{C}$  due to a decline in  $g_s$ . Hultine *et al.* (2013), found female *A. negundo* in the same common garden site maintained a greater WUE than males throughout the year, but especially in late summer, because they have a greater decline in  $g_s$  from May to August than males, thus potentially increasing their latewood  $\delta^{13}\text{C}$ .

### *Ring Width*

As hypothesized, radial growth in males was significantly higher than in females under warm, but well-watered conditions. However, there was no significant difference in ring width between males and females once the irrigation had ceased (Fig 1). Moreover, males had a significantly lower  $\delta^{13}\text{C}$  than females once irrigation ceased, yet both sexes had relatively similar ring widths post irrigation. This supports previous evidence that

found females tend to express a greater stomatal sensitivity to water stress than males and therefore allocate less to growth than males in low resource habitats (Dawson & Ehleringer, 1993). However, these results also show that a combination of heat and drought stress appeared to have a significant deleterious effect on both male and female *Acer negundo*. It is important to consider when interpreting these results that these ring width data was not detrended to account for age-related contrasts in growth. This is because ring widths inevitably decline with age as a function of tree stems having a progressively larger cross-sectional area. Thus, detrending is usually necessary to accurately detect responses to environmental gradients. Once growth data is detrended, results could alter and show a difference between the sexes in annual absolute growth.

#### *Conclusions:*

Long-lived dioecious species, such as trees and woody shrubs, are particularly vulnerable to rapid shifts in climate due to their extended generation turnover times, limiting their capacity to adapt to changing environmental conditions (Sandel *et al.*, 2011; Corlett & Westcott, 2013). Such trees are vital to ecosystem structure and function across an array of environments. Tree ring analysis of growth and  $\delta^{13}\text{C}$  were used to examine long-term trends of sexual dimorphism in key traits related to climate change. This study aimed to better understand how dioecious species of riparian ecosystems will be affected by climate change, specifically in arid-semi-arid ecosystems as they experience increasing temperatures and drought and found that 1) under warmer conditions (while irrigation was still being supplied), males grew faster and 2) under the combination of heat and drought, female  $\delta^{13}\text{C}$  was higher (less negative). These results further support

the notion that males and females display sexual dimorphisms in secondary traits, such as resource allocation, due to their difference in reproduction costs. This study aligns with past research that suggests female plants are more likely to deploy a more isohydric strategy (have stomata that are highly sensitive to aridity) relative to males, potentially resulting in a higher probability of carbon starvation under stress (Hultine *et al.*, 2016). This means that females tend to express a higher stomatal sensitivity to leaf water potential than males, which is important as there is a clear relationship between chronic reductions in stomatal conductance and mortality (Hultine *et al.*, 2016). The findings from this study add to other work on dioecious species that suggests females will be more negatively impacted by climate change. Spatial segregation of the sexes is likely to increase under climate change, such that females lose their dominance in high resource habitats and males increase their dominance in lower resource habitats. This will likely lead to skewed sex ratios, which can have significant consequences on population dynamics and ecosystem structure. Skewed sex ratios reduce the effective population size and may lead to increased rates of inbreeding and the stochastic loss of beneficial alleles, causing cascading impacts on ecosystem structure and stability, as there are sex-specific relationships within ecosystems (Hultine *et al.*, 2016). For example, herbivores, pollinators, folivores, and gallers tend to prefer males over females, while frugivores, fungi, and other plants tend to favor females over males (Hultine *et al.*, 2016). In summary, skewed sex ratios in plants are closely linked to climate change, and these imbalances can have far-reaching consequences for plant populations, ecosystem dynamics, and the structure of dependent communities, favoring one plant sex over the other. These effects are more likely to manifest in species located in regions with high

rates of climate change and in long-lived species that struggle to adapt quickly to environmental shifts, such as dioecious species located in arid-semi-arid riparian environments. Further research on a broader scale of dioecious species, located in different environments is needed to better predict how climate change may affect dioecious species in terms of secondary trait expression, population structure, and ecosystem dynamics.

## REFERENCES

- Adams H.D., Guardiola-Claramonte M., Barron-Gafford G.A., Villegas J.C., Breshears D.D., Zou C.B., Troch P.A. & Huxman T.E. (2009) Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* 106(7063–7066).
- Ashman, T. L. (2000) Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism. *Ecology*. 81(2577–2591)
- Babst F., Alexander R.M., Szejner P., Bouriaud O., Klesse S., Roden J., Ciais P., Poulter B., Frank D., Moore D.J.P., Trouet V. (2014) A tree-ring perspective on the terrestrial carbon cycle. *Oecologia*. 176(307-322).
- Barnett T.P., Pierce D.W., Hidalgo H.G., Bonfils C., Santer B.D., Das T., Bala G., Wood A.D., Nozawa T., Mirin A.A., Cayan D.R., Dettinger M.D. (2008) Human-induced changes in the hydrology of the western United States. USGS Staff—Published Research. Paper 211
- Blasini D.E., Koepke D.F., Bush S.E., Allan G.J., Gehring C.A., Whitman T.G., Day T.A., Hultine K.R. (2022). Tradeoffs between leaf cooling and hydraulic safety in dominant arid land riparian tree species. *Plant, Cell, and Environment* 45(1-18).
- Brienen R.J.W., Zuidema P.A. (2006) Lifetime growth patterns and ages of Bolivian rain forest trees obtained by tree ring analysis. *Journal of ecology* 94(481–493).
- Campbell G., Norman J. (1998) *Plants and Plant communities. An Introduction to Environmental Biophysics* 2nd Edition, Springer-Verlag, New York. (223-246).
- Corlett, T. Westcott, D. A. (2013) Will plant movements keep up with climate change? *Trends in Ecology & Evolution*. 28, (482–488).
- Cernusak L.A., Ubierna N., Winter K., Holtum J.A.M., Marshall J.D., Farquhar G.D. (2013) Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist*. 200(950-965).
- Chaves M.M., Flexas J., Pinheiro C. (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany* 103(551–560).
- Chen L., Zhang S., Zhao H., Korpelainen H., Li C. (2010) Sex-related adaptive responses to interaction of drought and salinity in *Populus yunnanensis*. *Plant, Cell and Environment* 33(1767-1778).

Cohen D. (1970). The expected efficiency of water utilization in plants under different competition and selection regimes. *Israel Journal of Botany* 19(50-54).

Cowan I. R. (1982). Regulation of water use in relation to carbon gain in higher plants. Pages 589-613 in O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. *Encyclopedia of plant physiology (new series)*. Volume 12B. Physiological plant ecology. II. Water relations and carbon assimilation. Springer-Verlag, Heidelberg, Germany.

Dawson T.E. Ehleringer J.R. (1993) Gender-specific physiology, carbon isotope discrimination, and habitat distribution in Box-elder, *Acer Negundo*. *Ecology* 74(798–815).

Dawson T.E., Ward J.K., Ehleringer J.R. (2004) Temporal scaling of physiological responses from gas exchange to tree rings: a gender-specific study of *Acer negundo* (Boxelder) growing under different conditions. *Functional Ecology* 18(212–222).

DeLucia E. H., Schlesinger W.H. (1991). Resource-use efficiency and drought tolerance in adjacent Great Basin and Sierran plants. *Ecology* 72(51-58).

Drever C.R., Lertzman K.P. (2001) Light-growth responses of coastal Douglas-fir and western redcedar saplings under different regimes of soil moisture and nutrients. *Canadian Journal of Forest Research* 31(2124–2133).

Ehleringer, J.R., L.A. Arnow, T. Arnow, I.B. McNulty N.C. Negus. (1992) Red Butte Canyon Research Natural Area: History, flora, geology, climate, and ecology. *Great Basin Naturalist* 52(95–121).

Esper J., Buntgen U., Frank D.C., Nievergelt D., Liebhold A. (2007) 1200 years of regular outbreaks in alpine insects. *Proceedings of the Royal Society Biological Sciences* 274(671–679).

Evans, G.C. 1972. *The quantitative analysis of plant growth*. Blackwell Scientific, Oxford (734).

Ewers B.E., Gower S.T., Bond-Lamberty B., Wang C.K. (2005) Effects of stand age and tree species on canopy transpiration and average stomatal conductance of boreal forests. *Plant, Cell and Environment*, 28(660–678).

Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989). Carbon isotope discrimination and photosynthesis. *Annual review of Plant Physiology* 40(503-537).

Farquhar, G. D., Richards, R.A. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* 11(539-552).

- Farquhar, G.D., M.H. O’Leary, J.A. Berry. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9(121–137).
- Feng X. (1999) Trends in intrinsic water-use efficiency of natural trees for the past 100-200 years: A response to atmospheric CO<sub>2</sub> concentration. *Geochimica et Cosmochimica Acta* 63(1891-1903).
- Frank D.C., Esper J., Raible C.C., Buntgen U., Trouet V., Stocker J., Joos F. (2010) ensemble reconstruction constraints on the global carbon cycle sensitivity to climate. *Nature* 463(527–U143).
- Fu P., Griebinger J., Gebrekirstos A., Fan Z., Bräuning A. (2017) Earlywood and Latewood Stable Carbon and Oxygen Isotope Variations in Two Pine Species in Southwestern China during the Recent Decades. *Frontiers in Plant Science* 7(2050).
- Geber, M. A., Dawson, T.E. (1990). Genetic variation in and covariation between leaf gas exchange, morphology and development in *Polygonum arenastrum*, an annual plant. *Oecologia* (Berlin) 85(153-158).
- Heilbuth J.C., (2000) Lower species richness in dioecious clades. *The American Naturalist*, 156 (221-241).
- Hubick K.T., Farquhar G.D. Shorter R. (1986) Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Australian Journal of Plant Physiology* 13(803 – 816).
- Hultine K.H., Bush S.E., West A.G., Ehleringer J.R. (2007) Population structure, physiology and ecohydrological impacts of dioecious riparian tree species in western North America. *Oecologia*, 154(85–93).
- Hultine K.H., Jackson T.L., Burtch K.G., Schaeffer S.M., Ehleringer J.R. (2008). Elevated stream inorganic nitrogen impacts on a dominant riparian tree species: results from an experimental riparian stream system. *Journal of Geophysical Research Biogeosciences*, 113(G04025).
- Hultine K. H., Jackson T.L., Burtch K.G., Ehleringer J.R. (2013). Gender specific patterns of carbon uptake and water use in a dominant riparian tree species exposed to a warming climate. *Global Change Biology* 19(3390-3405).
- Hultine K.H., Bush S.E., Ward J.K., Dawson T.E. (2018) Does sexual dimorphism predispose dioecious riparian trees to sex ratio imbalances under climate change? *Oecologia* 187(921-931).



- Hultine K.H., Grady K., Wood T., Shuster S., Stella J., Whitham T. (2016) Climate change perils for dioecious plant species. *Nature plants* 2(16109).
- Johnson, D. A., Asay, K.H., Tieszen, L.L., Ehleringer, J.R., Jefferson, P.G. (1990). Carbon isotope discrimination: potential in screening cool-season grasses for water-limited environments. *Crop Science* 30(338-343).
- Kimak A., Leuenberger M. (2015) Are carbohydrate storage strategies of trees traceable by early–latewood carbon isotope differences? *Trees* 29(859–870).
- Leavitt, S.W. S.R. Danzer. (1992) Methods for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. *Analytical Chemistry* 65(87–89).
- Leavitt S.W. Long A. (1983) An atmospheric  $^{13}\text{C}/^{12}\text{C}$  reconstruction generated through removal of climate effects from tree-ring  $^{13}\text{C}/^{12}\text{C}$  measurements. *Tellus* 35B(92-102).
- Leung LB, Qian Y, Bian X, Washington WM, Han J, Roads JO. (2004) Mid-century ensemble regional climate change scenarios for the western United States. *Climate Change* 62(75–113).
- Livingston, N.J. D.L. Spittlehouse. (1996) Carbon isotope fractionation in tree ring early and latewood in relation to intra-growing season water balance. *Plant, Cell, and Environment* 19(768–774).
- Marshall, J.D., Brooks, J.R. Lajtha, K. (2007). Sources of Variation in the Stable Isotopic Composition of Plants. *Stable Isotopes in Ecology and Environmental Science* (Chapter 2).
- Moran M., Aparecido L., Koepke D., Cooper H., Doughty C., Gehring C., Throop H., Whitham T., Allan G., Hultine K.H. (2023). Limits of thermal and hydrological tolerance in a foundation tree species (*Populus fremontii*) in the desert southwestern United States. *The New Phytologist*, 10.1111/nph.19247. Advance online publication.
- NOAA National Centers for Environmental Information “Global Climate Report - Annual 2021.” Global Climate Report - Annual 2021 | National Centers for Environmental Information (NCEI), <https://www.ncdc.noaa.gov/sotc/global/202113#gtemp>
- O’Neill G.A., Hamann A., Wang T. (2008). Accounting for population variation improves estimate of the impact of climate change on species’ growth and distribution. *Journal of Applied Ecology*, 45(1040–1049).
- Oren R, Sperry JS, Katul G, Pataki DE, Ewers BE, Phillips N, Scheaffer KVR (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapor pressure deficit. *Plant, Cell, and Environment* 22(1515–1526).

Passioura, J. B. (1982). Water in the soil-plant-atmosphere continuum. Pages 5-11 in O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. Encyclopedia of plant physiology (new series). Volume 12B. Physiological plant. II. Water relations and carbon assimilation. Springer-Verlag, Heidelberg, Germany.

Rodríguez-González P. M., Abraham E., Aguiar F., Andreoli A., Baležentienė L., Berisha N., Bernez I., Bruen M., Bruno D., Camporeale C., Čarni A., Chilikova-Lubomirova M., Corenblit D., Čušterevska R., Doody T., England J., Evette A., Francis R., Garófano-Gómez V., ... Dufour S. (2022). Bringing the margin to the focus: 10 challenges for riparian vegetation science and management. *WIREs Water* 9(5), e1604.

Sandel, B., Arge L., Dalsgaard B., Davies R.G., Gaston K.J., Sutherland W.J., Svenning J.C. (2011). The influence of Late Quaternary climate-change velocity on species endemism. *Science* 334(660–664)

Sargent, C.S. (1965) *Manual of the trees of North America*. Dover, New York, (934)

Stewart I.T., Cayan D.R., Dettinger M.D. (2004) Changes in snowmelt run-off timing in western North America under a 'business as usual' climate change scenario. *Climatic Change* 62(217–232).

Stromberg J.C. (1993) Fremont cottonwood-Gooding willow riparian forests: a review of their ecology, threats, and recovery potential. *J Ariz Nev Academy Science* 26(97–111).

Stromberg J.C., Lite S.J., Dixon M.D. (2010) Effects of stream flow patterns on riparian vegetation of a semiarid river: Implications for a changing climate. *River Research and Applications* 26(712-729).

Szejner, P., Wright W.E., Babst F., Belmecheri, S., Trouet V., Leavitt S.W., Ehleringer, J.R., Monson R.K. (2016). Latitudinal gradients in tree ring stable carbon and oxygen isotopes reveal differential climate influences of the North American Monsoon System. *Journal of Geophysical Research Biosciences* 121(1978-1991).

Tjoelker M.G., Oleksyn J., Reich P.B. (1997) Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO<sub>2</sub> and temperature. *Tree physiology* 18(715-726).

Vaganov E., Schulze E., Skomarkova M., Knohl A., Brand W., Roscher C. (2009) Intra-annual variability of anatomical structure and  $\delta^{13}\text{C}$  values within tree rings of spruce and pine in alpine, temperate and boreal Europe. *Oecologia* 161(729–745).

Wang R., Chen S., Zhou X., *et al.* (2008) Ionic homeostasis and reactive oxygen species control in leaves and xylem sap of two poplars subjected to NaCl stress. *Tree Physiology* 28 (947–957).

Wang W., Vinocur B. & Altman A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218(1–14).

Ward J.K., Dawson T.E., Ehleringer J.R. (2002) Responses of *Acer negundo* genders to interannual differences in water availability determined from carbon isotope ratios of tree ring cellulose. *Tree Physiology* 20(339-346).

Weber P., Bugmann H., Fonti P., Rigling A. (2008) Using a retrospective dynamic competition index to reconstruct forest succession. *Forest ecology Management* 254(96–106).

Xu X., Peng G., Wu C., Korpelainen H., and Li C. (2008) Drought inhibits photosynthetic capacity more in females than in males of *Populus cathayana*. *Tree Physiology* 28(1751-1759).

Yin C., Peng Y., Zang R., Zhu Y. & Li C. (2005) Adaptive responses of *Populus kangdingensis* to drought stress. *Physiologia Plantarum* 123(445–451).