

Responses of *Acer negundo* genders to interannual differences in water availability determined from carbon isotope ratios of tree ring cellulose

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Summary Understanding the responses of riparian trees to water availability is critical for predicting the effects of changes in precipitation on riparian ecosystems. Dioecious *Acer negundo* L. (box elder) is a common riparian tree that is highly sensitive to water stress. Earlier studies indicated that the genders of *A. negundo* respond differently to water availability, with males being more conservative in their water use than females. To assess the potential effects of changes in precipitation on the sex ratio of riparian trees, we extended earlier studies of *A. negundo* by analyzing responses of male and female genotypes to interannual differences in water availability in a common garden. We measured growth of tree rings and used stable carbon isotope analysis of tree ring alpha-cellulose to integrate physiological responses to annual water treatments. During dry years, male and female trees exhibited similar growth and physiological responses. However, during wet years, females exhibited higher growth rates and lower carbon isotope ratios (indicating less conservative water use) than did males. Furthermore, we found that male trees exhibited similar stomatal behavior (inferred from integrated carbon isotope ratios) whether years were wet or dry, whereas females did not exhibit a consistent response to changes in water availability. We predict that with increasing precipitation and soil water availability, the representation of females will be favored because of shifts in the competitive interactions of the genders. Such changes may affect the reproductive output of these riparian trees and may influence overall water flux from riparian ecosystems. In addition, this study demonstrates the utility of carbon isotope analysis for assessing long-term responses of tree populations to shifts in water availability.

Keywords: box elder, climate change, dioecious tree, precipitation, riparian ecosystems, sex ratio, water stress.

Introduction

Both the structure and biological diversity of riparian ecosystems are affected by stream flow regulation, stream diversion, water pollution and invasions by nonnative species (Smith et al. 1991, Grimm 1993, Busch and Smith 1995, Stromberg et al. 1996, Kolb et al. 1997, Sakai et al. 1999). Climate change is

expected to further affect the functioning of riparian ecosystems through changes in both the mean and variability of precipitation, which in turn, influences stream flow and groundwater levels (Cayan et al. 1999, National Assessment Synthesis Team 2000). Such changes are likely to affect both population and community structures of riparian vegetation (Dawson and Ehleringer 1991, Scott et al. 2000, Snyder and Williams 2000).

The stability of riparian communities in the Southwest and Intermountain West of the United States, in particular, is dependent on dominant riparian tree species that occupy a narrow corridor along streambeds within semiarid and arid landscapes. Riparian trees reduce soil erosion and provide a favorable habitat for fauna of reduced temperature and shade (Smith et al. 1991). Changes in precipitation abundance may alter the functioning of dominant tree species and therefore may have cascading effects on the composition and functioning of riparian and stream communities as a whole. Some climatic models predict that the Southwest and Intermountain West will experience increased interannual variability in precipitation in the future (IPCC 1996), with a possible increase in mean winter precipitation (National Assessment Synthesis Team 2000). This may increase water inputs to riparian ecosystems through increased stream runoff and groundwater recharge (Cayan et al. 1999). However, other models predict reduced precipitation for regions of the West (e.g., parts of the Rocky Mountains), coupled with higher temperatures that will increase evapotranspiration, reduce soil water content and perhaps greatly reduce stream flow (National Assessment Synthesis Team 2000).

Short-term studies have shown that riparian trees are particularly sensitive to water stress (Dawson and Ehleringer 1993, Dudek et al. 1998, Smith et al. 1998). However, little is known about the long-term impact of changes in water availability on the population genetics of riparian tree species. An assessment of the linkages between ecophysiology and population biology of riparian trees could facilitate more accurate predictions of water fluxes in riparian ecosystems.

Dioecy occurs in 6–9% of higher plant species (Sakai and Weller 1999), and is most common in woody species, particu-

larly those that are wind-pollinated (Freeman et al. 1980). In a survey of plant communities of the U.S. Intermountain West and California, it has been estimated that an average of 12% of plant species and 32% of individuals in riparian woodland communities are dioecious (Freeman et al. 1980). Many dioecious species exhibit spatial segregation of the genders, with females occurring at higher frequency at high resource sites (e.g., streamside habitats), and males predominating at low resource sites (e.g., non-streamside habitats; Iglesias and Bell 1989). This pattern of segregation has been observed in the majority of dioecious plant populations studied, and presumably reflects adaptations to different reproductive costs in males and females (Freeman et al. 1976, Bierzychudek and Eckhart 1988, Dawson and Ehleringer 1993, Dawson and Geber 1999). Because segregation of the genders may be adaptive (Cox 1981, Eppley et al. 1998), changes in resource availability may change the population sex ratio and thereby the reproductive success of dioecious species exhibiting spatial segregation by gender. Furthermore, differential responses of males and females have been observed in *Populus tremuloides* Michx. in response to elevated CO₂ (Wang and Curtis 2001), and such differences may also occur in response to changing water resources.

Acer negundo L. (box elder), a dioecious maple, is an excellent indicator of water stress in riparian trees, which are generally sensitive to small changes in water availability (Smith et al. 1991). The response of *A. negundo* to water stress varies with gender. For example, it shows skewed sex ratios (ratio of males to females) throughout Utah, with females occurring most frequently on wet streamside sites, and males occurring more commonly on drier sites, although close enough to sites occupied by female plants for reproduction to occur (Table 1, Freeman et al. 1976, Dawson and Ehleringer 1993). Spatial segregation of the genders reflects the more conservative water use and slower growth of males compared to females (Dawson and Ehleringer 1993). Thus, females are likely to outgrow males near streams, whereas their lower frequency on drier sites may indicate that they are more susceptible than males to water stress.

Acer negundo is one of the most dominant tree species within North American riparian systems at mid-elevations, and its range extends from Montreal, Canada to southern California (Sargent 1965). Juvenile trees depend solely on precipitation or stream water for approximately the first 10–13 years, whereas older trees depend on groundwater (Dawson and Ehleringer 1991). Gender in this species is believed to be genetically controlled (Dawson and Geber 1999) and thus, sex-switching does not occur.

To assess the potential effects of interannual variation in precipitation on the sex ratio of *A. negundo* populations, we extended earlier studies by analyzing long-term responses of male and female genotypes to interannual differences in water availability in a common garden. We measured growth of tree rings over an 8-year period and used carbon isotope analysis of alpha-cellulose (Leavitt and Long 1986) to integrate physiological responses to annual water treatments. We hypothesized that males would have an advantage over females during dry

years as a consequence of a greater capacity for water conservation, whereas females would be favored during wet years because of a capacity for more rapid growth under wet conditions.

Materials and methods

Growth conditions in the common garden

Experimental trees of *A. negundo* var. *interior* (Britt.) Sarg. were propagated in a greenhouse from stem cuttings obtained from reproductively mature trees growing in Red Butte Canyon Research Natural Area (Ehleringer et al. 1992) in Salt Lake City, Utah. Dormant leafless cuttings were planted in a rooting medium of 1:1 vermiculite and perlite, and roots were produced within approximately 2 months. In 1988, the trees were transplanted to a common garden located at the base of Red Butte Canyon. Nine trees of each gender were planted and spaced to avoid shading from neighboring individuals. For each gender, three genotypes were represented that were replicated with three individuals each (female genotypes = 58, 1006, 1009; male genotypes = 1008, 1016, 1017). The trees were first exposed to an acclimation period between 1988 and 1991 when no measurements were taken. During 1992 and 1993 and from 1996 to 1999, the garden was exposed to natural dry conditions (summer rain ranged from 3.9 to 18.8 cm) with the exception of one watering to full saturation (root zone was flooded) during each growing season. During the 1994 and 1995 growing seasons, precipitation was supplemented by irrigation to full saturation every 21 days (summer rain for 1994, 2.5 cm; 1995, 8.3 cm). These treatments represented dry and wet years, respectively, and allowed for the effects of interannual variation in water availability on tree growth and physiology to be analyzed.

Collection and treatment of wood samples

At the end of the 1999 growing season (September), trees were harvested and a complete wood cookie was taken from each tree at 1 m above ground. The wood cookies were dried in a greenhouse for approximately 2 weeks, then scanned (Epson, Expression 636 scanner, Torrance, CA) at 1200 dpi. The images were imported into Photoshop 5.0.2 (Adobe, San Jose, CA) for analysis. The years corresponding to each ring were identified by inspection with a dissecting microscope, matching similar relative ring sizes across years, and using unique ring traits for marker years that allowed cross identification between trees. Ring widths were measured to the nearest 0.1 mm (Photoshop 5.0.2) on all four sides of the wood cookies and were averaged for each year. Relative growth rate of each ring (RGR_{ring}) was calculated with the equation:

$$\text{RGR}_{\text{ring}} = (1/W) dW/dt, \quad (1)$$

where W is the initial radius of the wood cookie (before the year in question) and t is time (= 1 between sequential years).

Individual wood samples were obtained across the full width of each ring for years corresponding to 1992–1999 using a Model 395 Dremel tool (Racine, WI) with a 1.6-mm drill

Table 1. Survey of *Acer negundo* sex ratios (males/females) and sample size (*n*) at streamside (within riparian corridor) and non-streamside (outside riparian corridor) habitats throughout Utah. North/central sites are from canyons surrounding Salt Lake City and data are from Dawson and Ehleringer 1993. Central and southern Utah sites are from J.K. Ward and R.E. Ward (Univ. of Utah, unpublished data 2000).

Location	Streamside	Non-streamside
<i>North/central Utah</i>		
Salt Lake City		
A. Red Butte Canyon	0.74 (356)	1.72 (237)
B. Parley's Canyon	0.75 (121)	2.25 (156)
C. Little Cottonwood Canyon	0.51 (157)	2.10 (152)
D. Mill Creek Canyon	0.66 (146)	0.74 (148)
E. Big Cottonwood Canyon	0.49 (100)	1.91 (131)
<i>Central Utah</i>		
Maple Grove (39.01866° N, 112.08910° W)	0.35 (31)	1.65 (53)
Maple Hollow (39.06201° N, 112.18156° W)	0.45 (29)	3.00 (4)
Beaver Canyon (38.25874° N, 112.54486° W)	0.83 (22)	1.40 (31)
<i>Southern Utah</i>		
Parowah Creek (37.77570° N, 112.83847° W)	1.20 (20)	2.10 (22)
Leeds Creek (37.29372° N, 113.41423° W)	< 4 available	3.50 (9)
Clear Creek (37.21475° N, 112.93798° W)	0.71 (29)	1.08 (27)
<i>Summary of Utah sites</i>		
North/central	0.65	1.62
Central	0.54	2.02
Southern	0.96	2.23
Overall	0.67	1.95 (1.61 if <i>n</i> < 10 removed)

bit. Samples from all four sides of the wood cookie corresponding to the same year were combined for analyses of carbon isotope ratios. Additional samples were obtained from latewood of 1994, early and latewood of 1995 and earlywood of 1996 by extracting wood samples along ring borders with a 1.6-mm drill bit. These samples were used to determine if the carbon isotope signature of photosynthetic products (storage) produced late in the previous year had an influence on the carbon isotope ratio of earlywood produced the following year, during a wet-to-wet year transition and a wet-to-dry year transition. On average, this procedure tested for the effects of the carbon isotope ratio from the previous year on the first 25% of ring growth occurring in the following year.

Samples of wood from each year between 1992 and 1999 (and late and earlywood samples) collected from all wood cookies were converted to alpha-cellulose (protocol modified from Leavitt and Danzer 1992) as follows. Lipids and resins were removed by extraction in a soxhlet apparatus containing a 2:1 toluene:ethanol mixture (48 h), followed by a 100% ethanol mixture (48 h). Samples were then boiled to remove soluble sugars and treated at 70 °C with three accumulating additions of 4 g of sodium chlorite and 2 ml of glacial acetic acid each day for 3 days to remove lignins and proteins. Samples were then treated with a 17% w/v solution of NaOH at room temperature for 1 h, resulting in holocellulose. Finally, samples were treated with a 10% solution of glacial acetic acid for 1 h at room temperature that resulted in the isolation of alpha-cellulose. Samples were dried at 75 °C for 48 h.

Carbon isotope ratios ($\delta^{13}\text{C}$) of tree ring alpha-cellulose were taken as an indicator of time-integrated stomatal conductance, c_i/c_a (intercellular CO_2 concentration/ambient CO_2 concentration) and water-use efficiency (WUE) (Leavitt and Long 1986, Ehleringer and Cerling 1995). The offset between leaf and wood cellulose is generally in the range of 1–3‰, with leaves being isotopically lighter than wood (Leavitt and Long 1986). Carbon isotope ratios were determined for each subsample of alpha-cellulose (1.8–2.2 mg) using an elemental analyzer (Carla Erba, Model 1108, Milano, Italy) coupled with 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 δ notation:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1)1000, \quad (2)$$

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 $\pm 0.15\text{‰}$.

Data analysis

Responses of ring width and $\delta^{13}\text{C}$ were analyzed with a mixed model design with the response during the previous year treated as a covariate. Gender, genotype and water treatment were the main effects (discrete factors) and interaction terms included gender by water treatment, genotype by water treat-

ment and gender by year. Nested effects included genotype nested within gender and year nested within water treatment. The year (water treatment) variation was used as the error term for the water treatment effect and the genotype (gender) variation was used as the error term for the gender effect. Relative growth rate of rings (RGR_{ring}) was analyzed similarly, except that the covariate was not included because this measurement already accounts for previous size. For all analyses, Levine's test indicated that the variances were homogeneous.

In addition to these analyses, the effect of genotype was analyzed within individual years (although this increases the probability of Type I error) for ring widths and $\delta^{13}C$, because variation between years (natural rainfall, temperature) may have crossed threshold responses for male and female genotypes. These tests were conducted as one-way ANOVAs with genotype as the main effect, and individual differences among means were determined by Tukey-Kramer tests.

A linear regression was conducted between the $\delta^{13}C$ of individual trees averaged over all dry years and averaged over all wet years. This was done to determine if males and females exhibited variations in shifts in stomatal regulation between wet and dry years.

Linear regressions were also conducted between $\delta^{13}C$ and several wood growth parameters (ring width, RGR_{ring} and ring area around the circumference of the trunk) to determine if there was a relationship between stomatal regulation and growth over the 8-year experimental period and during wet and dry year treatments.

Results

Flowering and mortality

At the beginning of the measurement period (1992), all experimental trees had initiated flowering. Two of the three female trees of Genotype 1009 died during the first 2 years of drought treatment.

Gender responses

In response to dry years (1992, 1993 and 1996–1999), male and female trees exhibited no differences in ring width (Figure 1A). However, in response to wet years (1994, 1995), female trees had wider rings than males, resulting in a significant gender by water treatment interaction (Figure 1A). More specifically, female trees had 19% wider rings in 1994 and 25% wider rings in 1995.

Male and female trees exhibited similar RGR_{ring} during dry years (Figure 1B). During wet years, however, females had significantly higher RGR_{ring} values than males, resulting in a significant gender by water treatment interaction (Figure 1B). More specifically, female trees had 38% higher RGR_{ring} in 1994 and 30% higher RGR_{ring} in 1995.

The genders did not differ in alpha-cellulose $\delta^{13}C$ during dry years (Figure 1C). During wet years, female trees had significantly lower $\delta^{13}C$ than males, and a significant gender by water treatment interaction was detected (Figure 1C). On average, female trees had 0.67‰ lower $\delta^{13}C$ in 1994, and 0.52‰ lower $\delta^{13}C$ in 1995. In addition, no correspondence was found

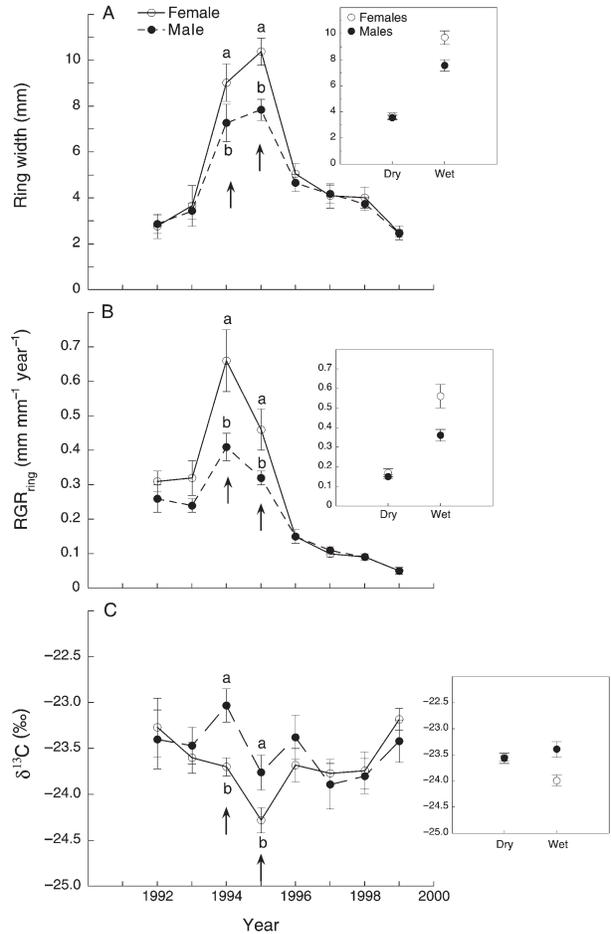


Figure 1. (A) Tree ring widths (mean \pm 1 SE) of male and female *Acer negundo* in response to wet and dry annual water treatments from 1992–1999. Wet years with consistent irrigation treatments are shown with arrows. Inset: Tree ring widths (mean \pm 1 SE) of male and female trees for all dry years and all wet years combined (female and male symbols overlay one another in the dry treatment). The gender by water treatment interaction was significant at $P < 0.002$, $F_{1,89} = 10.536$. (B) Relative growth rate of tree ring width (mean \pm 1 SE) of male and female trees in response to wet and dry annual water treatments from 1992–1999. Inset: Relative growth rate of ring width (mean \pm 1 SE) of male and female trees for all dry years and all wet years combined. The gender by water treatment interaction was highly significant at $P < 0.0001$, $F_{1,104} = 33.13$. (C) $\delta^{13}C$ (alpha-cellulose, mean \pm 1 SE) for male and female tree rings in response to wet and dry annual water treatments from 1992–1999. Inset: $\delta^{13}C$ of male and female tree rings (mean \pm 1 SE) for all dry years and all wet years combined (female and male symbols overlay for the dry treatment). The gender by water treatment interaction was significant at $P < 0.012$, $F_{1,89} = 6.51$.

between latewood of 1994 and earlywood of 1995 ($P = 0.69$ with linear regression, data not shown) and between latewood of 1995 and earlywood of 1996 ($P = 0.33$, data not shown). Thus, no carry-over effects of $\delta^{13}C$ between tree rings were detected in a dry-to-wet year or a wet-to-dry year transition.

Genotype responses

Male and female genotypes showed no differences in ring width during dry years (Figures 2A and 2B). Overall, during

wet years (1994 and 1995), female genotypes had higher mean ring widths than male genotypes, with only one exception (in 1994, the mean width of female Genotype 1006 was slightly lower than male Genotype 1008, Figures 2A and 2B). However, these mean differences between females and males during wet years did not translate into a significant genotype by water treatment interaction in the overall ANOVA model. When analyzing differences among genotypes within years, female genotypes had significantly wider rings than male genotypes ($P = 0.038$) in 1995, specifically because female Genotype 58 had such wide rings relative to male genotypes (Figures 2A and 2B).

Male and female genotypes exhibited similar RGR_{ring} during dry years (data not shown). During wet years, however, female genotypes exhibited significantly higher RGR_{ring} than male genotypes, resulting in a significant genotype by water treatment interaction in the overall ANOVA model ($P = 0.0001$; data not shown). In the individual analyses, there was a significant effect of genotype ($P = 0.0058$) during 1994 that was primarily attributable to female Genotype 1009, which exhibited higher RGR_{ring} than any of the male genotypes (data not shown).

In the overall ANOVA model, there was no significant genotype by water treatment interaction for $\delta^{13}C$ (Figures 2C and 2D). However, the within-year analyses indicated that during 1995, genotypes from different genders exhibited differences in $\delta^{13}C$ ($P = 0.021$), mainly because all female genotypes had

lower $\delta^{13}C$ relative to male Genotypes 1008 and 1017 (Figures 2C and 2D).

Individual tree responses

There was no correlation between wet and dry years for alpha-cellulose $\delta^{13}C$ of female trees (Figure 3A, also see Figure 1C inset, where females exhibited an overall mean shift in $\delta^{13}C$ between dry and wet years), indicating that females did not maintain a consistent stomatal response under conditions of changing water availability. Furthermore, females showed a lower range of inter-tree variation in $\delta^{13}C$ than males. Males had a near one-to-one relationship (slope = 0.91 ± 0.3) between alpha-cellulose $\delta^{13}C$ in wet and dry years, indicating that stomatal regulation changed little between wet and dry years (Figure 3B, also see Figure 1C inset where, overall, males exhibited similar $\delta^{13}C$ values between dry and wet years). Furthermore, males exhibited variation in $\delta^{13}C$ between the two wet years of 1994 and 1995 (Figure 1C), and these 2 years were averaged for the regression of $\delta^{13}C$ of individuals during wet versus dry years (Figure 3B). Because the overall average responses during these 2 years were quite different, the similarity between males during wet versus dry years was at first thought to be an artifact from the averaging of the two wet years. However, with an additional regression involving only individual male responses between the two wet years of 1994 and 1995, relative differences among males were maintained ($r^2 = 0.45$, $P = 0.05$), supporting the idea that

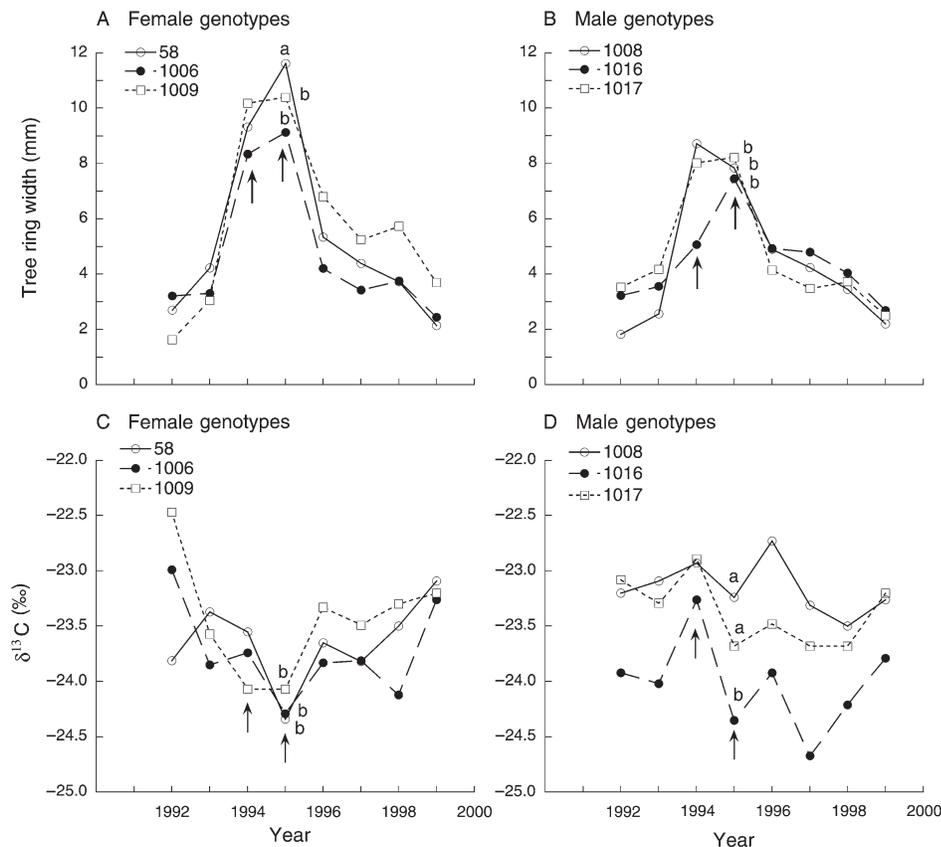


Figure 2. Tree ring widths (mean \pm 1 SE) of female (A) and male (B) genotypes and $\delta^{13}C$ from tree rings (alpha-cellulose, mean \pm 1 SE) of female (C) and male (D) genotypes of *Acer negundo* in response to wet and dry annual water treatments from 1992–1999. Wet years with consistent irrigation treatments are shown with arrows. In the overall ANOVA, no significant genotype by water treatment interaction was detected for tree ring width or for carbon isotope ratios. However, differences were detected between genotypes for individual analyses within years. For these analyses, different letters between genotypes within a year indicate significant differences at the $P < 0.05$ level.

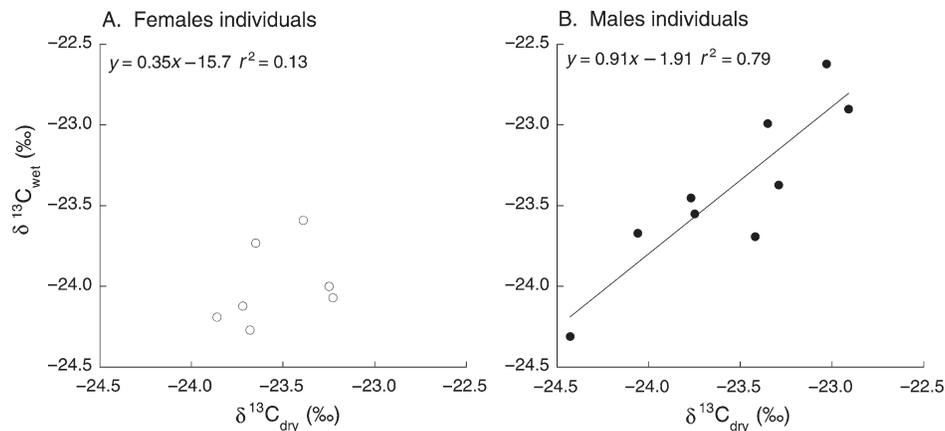


Figure 3. Values of $\delta^{13}\text{C}$ of tree rings (alpha-cellulose) for female (A) and male (B) individuals averaged for wet years (1994, 1995) versus dry years (1992, 1993 and 1996–1999). A linear regression was fit to the data for both genders and r^2 values are shown.

individual male trees did indeed maintain relatively constant stomatal behavior despite interannual differences in water availability.

Relationship between carbon isotope ratios and growth

There was only a weak relationship between the measured growth parameters and alpha-cellulose $\delta^{13}\text{C}$ using data for the entire 8-year experimental period (Table 2) and for wet and dry years analyzed separately (data not shown). For example, the P -value for the regression of $\delta^{13}\text{C}$ versus ring width across the 8-year period was low (0.016) for females, but the r^2 value was also low (Table 2), indicating that much of the total variation was unexplained by the model. For ring width of males and all other growth parameters (RGR_{ring} and ring area), P -values were high and r^2 values were low, indicating there was no direct correlation between stomatal regulation and aboveground growth (Table 2).

Discussion

During the 8-year common garden experiment we found that growth of *A. negundo* in dry years, whether on an absolute (tree ring width) or a relative basis (RGR_{ring}), was similar in male and female plants. Females, however, exhibited more rapid growth than males during wet years. Consistent with this finding, Dawson and Ehleringer (1993) found that vegetative ramets of *A. negundo* females growing in wet habitats along streamsidings had higher absolute growth rates than males. In contrast to the present study, Dawson and Ehleringer (1993) observed that males had higher seasonal growth rates than females in dry, non-streamside habitats, primarily because of the

lower growth rates of female than male reproductive ramets. Thus, the findings from both studies support the idea that reproductively mature females have a competitive advantage in wet streamside habitats, and during wet years in drier, non-streamside habitats. However, the results of the present long-term study fail to explain why males predominate on dry, non-streamside habitats. Perhaps females are more susceptible to water stress than males during early stages of development. To investigate this possibility, we plan to measure the physiological responses and mortality of male and female seedlings exposed to water stress. For this project, we are currently developing DNA markers to allow for the identification of gender in pre-reproductive trees.

Alpha-cellulose $\delta^{13}\text{C}$ measurements of female and male tree rings did not differ during dry years, indicating that, on average, females and males maintained similar stomatal conductances under dry conditions. During wet years, however, alpha-cellulose $\delta^{13}\text{C}$ was lower in females than in males, which implies that females are less conservative of water, and have higher c_i values than males during wet years. Consistent with this finding, Dawson and Ehleringer (1993) observed transpiration rates in female *A. negundo* plants up to twice those of male plants. Thus, any change in population sex ratio can be expected to affect riparian ecosystem water fluxes.

In contrast to the results of the present study, Dawson and Ehleringer (1993) observed that the greatest differences in leaf $\delta^{13}\text{C}$ between male and female *A. negundo* plants were on dry, not wet, habitats. The difference between studies may have been a result of (1) differences in the time scale for the integration of leaf $\delta^{13}\text{C}$ (approximately 20 days versus 1 year), (2) variations in physiological acclimation to changes in water

Table 2. Values of P and r^2 from the regressions of $\delta^{13}\text{C}$ values versus several growth parameters including ring width, relative growth rate of rings (RGR_{ring}) and ring area around the circumference of the trunk (1 m above ground). Data from both wet and dry years were combined for this analysis.

	Ring width		RGR_{ring}		Ring area	
	P -value	r^2	P -value	r^2	P -value	r^2
Female	0.016	0.10	0.20	0.031	0.17	0.035
Male	0.51	0.06	0.31	0.015	0.98	0.00

availability across time (present study) versus space (Dawson and Ehleringer 1993 study), and (3) genetic differences between the material used in the two studies.

We found no correspondence between $\delta^{13}\text{C}$ and growth parameters, including tree ring width, RGR_{ring} and ring area for the 8 years combined and during separate analyses of wet and dry years. Others have also reported the absence of a strong correlation between cellulose $\delta^{13}\text{C}$ and growth of woody tissue (Donovan and Ehleringer 1994, Livingston and Spittlehouse 1996), which indicates the need for caution in equating $\delta^{13}\text{C}$ with growth. In this case, the lack of correlation may result from differences in biomass allocation between stems and roots, which may vary with plant age or in response to changes in water availability, or other environmental factors. However, variation in gas exchange of *A. negundo* (which is integrated by carbon isotope ratios) has been correlated with important components of fitness in previous studies (Dawson and Geber 1999). Thus, $\delta^{13}\text{C}$ measurements provide a useful indicator of plant water status and may aid in predicting the survival of male and female *A. negundo* under wet and dry conditions.

Comparing individual male and female trees, we found that females varied in cellulose $\delta^{13}\text{C}$ between wet and dry years, whereas males did not. This finding supports the concept that, on an integrated annual basis, males maintain the same “set point” (Ehleringer and Cerling 1995) for stomatal regulation and c_i/c_a whether the season is wet or dry, whereas females adjust their stomatal behavior in response to interannual differences in water availability.

Because of gender differences in physiological response to water, it is possible that rapid shifts in the sex ratio of *A. negundo* populations will occur in response to changes in regional precipitation. Such shifts could impact reproduction of the species. Furthermore, other dioecious and sub-dioecious species have also been found to exhibit gender differences in growth in response to water availability (e.g., *Salix arctica* Pall., Dawson and Bliss 1989; *Thymelaea hirsuta* (L.) Endl., El-Keblawy and Freeman 1999). Thus, climate-change related alterations in water availability may alter the sex ratio of other dioecious species as well. Sex ratio changes in *A. negundo* may increase water flux from riparian ecosystems because of more rapid transpiration by females than by males. In addition, females have taller, more open canopies relative to males (Dawson and Ehleringer 1993). Therefore, increased light availability in riparian corridors dominated by female *A. negundo* may increase the potential for invasion by other species.

We conclude that increased water availability may favor higher representation of females at the expense of males because of shifts in the competitive interactions of the genders. From these results it is predicted that the population structure of dioecious riparian trees may be highly affected by hydrologic shifts associated with changes in inter-annual variation of precipitation. This potential response may profoundly influence the functioning of riparian and stream communities, particularly in semiarid and arid regions.

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