

Temporal scaling of physiological responses from gas exchange to tree rings: a gender-specific study of *Acer negundo* (Boxelder) growing under different conditions

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Summary

1. Plant responses to water availability may change over time due to seasonality, inter-annual variation and developmental changes. Our past investigations demonstrated that male and female Boxelder (*Acer negundo*) exhibit differences in their responses to water availability. Here we use the genders as a model system to: (1) examine if cultural conditions have an effect on instantaneous gas exchange and time-integrated carbon isotope discrimination (Δ); (2) compare these physiological responses across a range of temporal scales (seasonal, annual, interannual); and (3) describe the responses of the genders at different ages to water availability.

2. Under well watered conditions, differences in instantaneous leaf gas exchange and Δ were observed between the genders at all developmental stages. Within a gender, Δ was similar throughout the growing season and between years, indicating a conserved physiological set-point despite changing growth conditions. When water stress was imposed, females exhibited greater reductions in photosynthetic rate and higher stomatal limitations to photosynthesis than males.

3. For well watered plants, a strong correlation existed between time-integrated intercellular leaf $[\text{CO}_2]$ calculated from $\Delta(\bar{c}_i)$ and c_i obtained from instantaneous gas exchange. However, when drought was imposed this correlation weakened in males and was non-existent in females.

4. This study indicates that instantaneous gas exchange and integrated Δ values can be used to characterize leaf-level responses to water availability when growth conditions are relatively constant. However, when water availability fluctuates, care must be taken in equating these measurements because changing conditions may decouple responses integrating different time-scales, and the degree of this decoupling may vary even at the subspecies (gender) level.

Key-words: age effects, carbon isotope ratio, cultural conditions, dioecious tree, temporal scaling, water stress

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Introduction

Understanding how the ecophysiology of plants changes at different developmental or life-history stages may offer insights into how and why ecosystem functions change as these systems age. Scaling plant processes over a wide range of temporal scales is necessary because factors such as net primary production, canopy architecture and water use may vary as a function of age

(Dawson & Ehleringer 1991; Clark 1993). Furthermore, carbon assimilation rate and water uptake may vary within (genotype and gender levels) and between species, and affect gas exchange at larger spatial scales. Such assessments of inter- and intraspecific diversity are also necessary for determining how competitive interactions within populations and communities can be influenced by changing resource availability.

Comparisons between instantaneous measures of leaf gas exchange and time-integrated measures of carbon isotope discrimination (Δ) explain how short-term responses of leaf-level performance relate to more integrated responses such as the life span of a leaf, an entire growing season and annual responses (Farquhar, O'Leary & Berry 1982; Ehleringer 1990; Osorio *et al.* 1998;

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Olivas-Garcia, Cregg & Hennessey 2000; Le Roux-Swarthout, Terwilliger & Martin 2001). Long-term physiological performance, measured as leaf Δ , can also be applied to simplify broad-scale models of stand-level or landscape-level carbon and water balance (Dawson & Chapin 1993; Ehleringer & Field 1993). Furthermore, tests need to be conducted to determine if instantaneous and more integrated physiological responses to water availability vary when plants are grown under more controlled cultural conditions (e.g. garden, large pots) compared with the field. This knowledge will allow us to determine if smaller-scale studies can identify physiological changes that will occur in ecosystems or over longer time scales.

Plant Δ measurements can integrate plant responses over varying time scales (Farquhar, Ehleringer & Hubick 1989; Adams & Grierson 2001; Dawson *et al.* 2002). Differences in Δ of C_3 tissues result from variations in the time-integrated ratio of internal to ambient CO_2 concentrations (c_i/c_a) within leaves caused by differences in stomatal regulation and photosynthetic rate (Farquhar *et al.* 1982; Geber & Dawson 1990; Ehleringer & Cerling 1995). These values are influenced by the availability of water and light (Ehleringer 1993; Zimmerman & Ehleringer 1990; Buchmann *et al.* 1996; Berry, Varney & Flanagan 1997; Warren, McGrath & Adams 2001); temperature (O'Leary 1981); humidity (Comstock & Ehleringer 1993); and salinity (Flanagan & Jefferies 1989; Walker & Sinclair 1992), and a host of other factors (Dawson *et al.* 2002). Differences in Δ have also been reported between genders of dioecious plants (Dawson & Bliss 1989; Dawson & Ehleringer 1993; Ward *et al.* 2002), and may also be related to life history and longevity (Donovan & Ehleringer 1991; Schuster *et al.* 1992a,b; Zacharisen *et al.* 1999).

Carbon isotope analysis is a powerful tool for studying woody species due to the availability of tissues produced over a range of different time scales (leaves, early wood, late wood, whole rings; Leavitt & Long 1986; Dawson *et al.* 2002; Jäggi *et al.* 2002). Such measurements will increase understanding of how physiological responses are scaled to longer-term growth and fitness parameters within a tree species (Dawson & Geber 1999). Furthermore, studying the physiological responses of trees to changing resource availability over long time scales, and the effects of these responses on growth and population structure, must be understood to predict larger-scale ecosystem responses.

From our previous investigations (Dawson & Ehleringer 1993; Ward *et al.* 2002) we identified large and significant differences in the physiological responses of male and female trees of Boxelder, *Acer negundo* var. *interior*, to resource availability. Therefore this species provides an excellent system for studying the effects of growing conditions and temporal scaling on subspecies components that are otherwise similar, yet also vary in response to water availability. Our objectives here were threefold: (1) to examine if growing conditions have an effect on instantaneous gas-exchange properties and time-integrated

internal CO_2 concentrations (c_i) calculated from Δ (designated as \bar{c}_i); (2) to compare these physiological responses across a range of temporal scales (e.g. seasonally, inter-annually); (3) to describe the responses of the genders at different ages to water availability.

Materials and methods

EXPERIMENTS

Several different sets of data were collected during this study.

1. Data were collected to assess the effects of cultural conditions on leaf gas exchange and Δ (equation 2) for male and female trees at the peak of the growing season (mid-July). Plants were growing under three cultural conditions (field, garden, pots) for 3 years. Field trees were mature adults (40+ years old); pot- and garden-grown trees were 2–4 years old and raised from vegetative propagation. The same genotypes were used for all three cultural conditions, and the same trees were measured each year. All measurements were made on well watered plants.
2. Data were collected to determine if there were within- and between-season changes in physiology, leaf gas exchange and leaf Δ . Measurements were taken three times during each growing season and for the three cultural conditions (field, garden, pots). Leaf environmental and developmental effects were controlled for by always sampling recently matured leaves in full sun that reflected recent growing conditions. These measurements were taken in early June, mid-July and late August for 3 years. Garden- and pot-grown trees were well watered and field trees were exposed to natural conditions. Leaf-to-air mole fraction vapour gradient (v) and leaf temperature were monitored continuously as described by Dawson & Ehleringer (1993).
3. Data were collected to determine the effects of drought on tree performance. This was assessed by measuring instantaneous intercellular CO_2 concentration (c_i) calculated from leaf gas exchange and the time-integrated intercellular CO_2 concentration calculated from Δ (\bar{c}_i , equation 3) for trees growing in 40 l pots. One-year-old trees were droughted by withholding irrigation from replicate trees of each genotype and allowing leaf water potential (Ψ_l) to decline to below -1.15 but never lower than -2.25 MPa. This drydown occurred very rapidly for potted plants (within days), but occurred more slowly for garden trees (2 weeks). Once plants had reached the drought stage (when Ψ_l had declined), instantaneous leaf gas exchange was determined on well watered and droughted trees. The leaves measured were then collected for Δ analyses once they had been dried. $A-c_i$ curves and leaf oxygen evolution were also measured on both sets of trees. Leaf Δ was also measured on field trees that occurred at

streamside habitats (wet) and non-streamside habitats (dry) to provide an integrated field measure of responses to differences in water availability.

4. Data were collected to determine the effects of age on gender-specific responses. Here the data from our current field studies and garden- and pot-grown culture were combined with those from our previous studies to compare the effects of time-integrated measurements (leaf Δ and wood cellulose Δ) and the duration of water treatments (short term; life span) on male and female Boxelder of different ages.

PLANT MATERIAL AND GROWING CONDITIONS

Stem cuttings were taken from 19 genotypes (nine female and 10 male genotypes) of field trees when they were leafless and dormant during the winter. New roots were formed \approx 2 months later in a medium of 1 : 1 vermiculite and perlite, and the plants were allowed to grow and establish in 5 l pots for 3 months before being transplanted into 40 l pots. While plants were growing in pots they were fertilized biweekly with half-strength Hoagland's solution and grown outdoors under natural conditions [28–40 °C, relative humidity 12–40%, photosynthetically active radiation (300–700 nm) 900–1850 mol m⁻² s⁻¹]. A subset of these trees was planted in a common garden (University of Utah, Salt Lake City) in the fall of the second growing season. Sampling of these trees began the following year. The measurements for the present study were obtained from adult trees in the field (Red Butte Canyon Research Natural Area, directly above the garden site; Ehleringer *et al.* 1992) and from 2–4-year-old, vegetatively propagated trees in pots and in the common garden over three growing seasons (measurements taken early June to late August).

LEAF SAMPLING

The same leaves were used for all gas-exchange and isotope comparisons. For time-series comparisons such as those

shown in Fig. 1, the most recently matured leaves growing in full-sun conditions were measured, which reflected the most recent growing conditions. Leaves are produced continually from May to September in *A. negundo*, and new leaves take 16–23 days to complete development. Leaves were sampled at the same age, but were exposed to different growth conditions throughout the growing season and between years. Total nitrogen concentrations in leaves were determined as in Dawson & Ehleringer (1993). Stomatal densities were determined on replicates of the same leaves. Here, seven to nine replicate leaves were removed from the plant and a plastic impression was made immediately with spray-on polystyrene. The plastic impression was removed and viewed at 300 \times under a Nikon R8 microscope (Tokyo, Japan) where the size of the viewing field was known. The numbers of stomata per field were then counted as in Dawson & Ehleringer (1993). Twenty fields per leaf were counted and these measures used to obtain numbers of stomata per mm².

MEASUREMENTS OF INSTANTANEOUS LEAF GAS EXCHANGE

Gas exchange was measured (A , g , A/g = carbon uptake relative to water loss, termed intrinsic water-use efficiency) in the field with a portable photosynthesis system (LI-6200, LiCor, Lincoln, NE, USA), and in the laboratory with an open-flow system following the methods outlined by Dawson & Ehleringer (1993). Rates of leaf oxygen evolution were determined using an oxygen electrode photosynthesis system (Hansatech, LD2/2, Norfolk, UK) in the gas-phase configuration (after Walker & Leegood 1993) on the same leaves where a 2 cm² leaf disk was excised from the central portion of a terminal leaflet. The CO₂ source was a 5% solution of sodium bicarbonate. Previous investigations by Flanagan & Jefferies (1989) demonstrated that measuring rates of O₂ evolution using this protocol eliminates stomatal limitation on photosynthesis by overcoming problems of CO₂ diffusion through stomata.

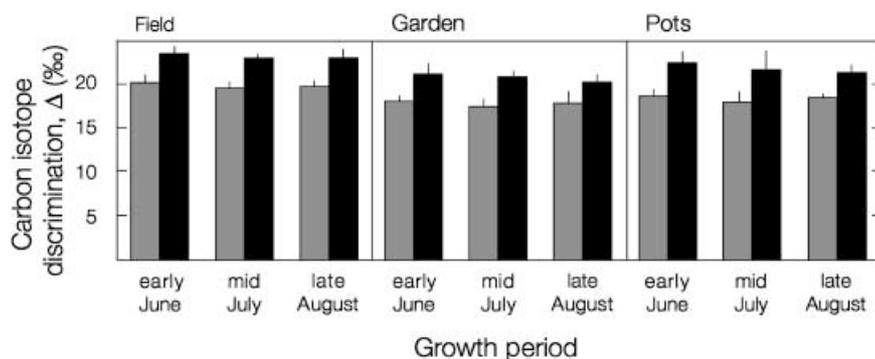


Fig. 1. Average leaf carbon isotope (¹³C/¹²C) discrimination (Δ) for male (grey bars) and female (black bars) trees of Boxelder (*Acer negundo*) during the early, mid- and late growing season, growing in the field, a common garden and 40 l pots. For leaf Δ all male–female comparisons were significantly different ($P < 0.01$), but no differences were detected between time points within the growing season or between cultural conditions. Data are averages (SD) taken over 3 years; no significant differences were detected (ANOVA, $P = 0.318$) between years therefore and the data were pooled.

MEASUREMENTS OF CARBON ISOTOPE RATIOS

Following gas-exchange measurements, leaves for carbon isotope ratio (δ) analyses were collected and immediately transported to the laboratory, where they were dried at 60 °C to a constant weight and then ground to 40 mesh to homogenize the tissues. A 3 mg subsample of the tissue was combusted for 6 h at 850 °C in sealed, evacuated Vicor tubes containing cupric oxide and silver foil, and then cooled to room temperature over a 12 h period (Ehleringer & Osmond 1989). CO_2 produced by combustion was purified cryogenically, then $\delta^{13}\text{C}$ was measured on either a Finnigan MAT delta E or delta S isotope ratio mass spectrometer. The instrument error (twice the standard deviation) associated with each observation was $\pm 0.1\text{‰}$, and the error between repeated analyses of the same ground tissue was less than $\pm 0.17\text{‰}$. The ratio of ^{13}C to ^{12}C (δ) in the leaf was calculated using delta notation:

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

where R is the $^{13}\text{C}/^{12}\text{C}$ and the standard was V-PDB. Then leaf carbon isotope discrimination (Δ) was calculated as:

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p) \quad \text{eqn 2}$$

where δ_a is the $\delta^{13}\text{C}$ of source CO_2 (assumed to be -8.0‰) and δ_p is the $\delta^{13}\text{C}$ of the plant material (from equation 1). Time-integrated intercellular CO_2 concentration (\bar{c}_i) was obtained by rearranging the equation for leaf carbon isotope ratio presented by Farquhar *et al.* (1982) to give:

$$\bar{c}_i = c_a(\delta_a - \delta_p - a)/(b - a) \quad \text{eqn 3}$$

where c_a was measured as $347\text{--}359 \text{ } \mu\text{mol mol}^{-1}$; a is the fractionation due to diffusion in air (4.4‰); and b is the net fractionation due to carboxylation (RuBP carboxylase, 27‰). For our carbon isotope analyses of wood cellulose we followed the procedures outlined by Ward, Dawson & Ehleringer (2002) for the purified (alpha) cellulose extraction and isotope analysis.

STATISTICAL ANALYSES

Differences between years or in the seasonal course of gas exchange and A/g were analysed by an analysis of variance for repeated measures (ANOVAR) where multiple measurements on a given plant constituted the repeated variables (Potvin, Lechowicz & Tardif 1990). Values of A/g were \log_e -transformed to conform to normality assumptions of the ANOVAR. In each analysis the main effect of gender was tested as a between-subjects effect. Seasonal, between-habitat and between-year variations were tested as both within-subjects and between-subjects effects (Potvin *et al.* 1990). Testing for differences in responses between genders, growing conditions (field, garden, pots), time of season, or between years was

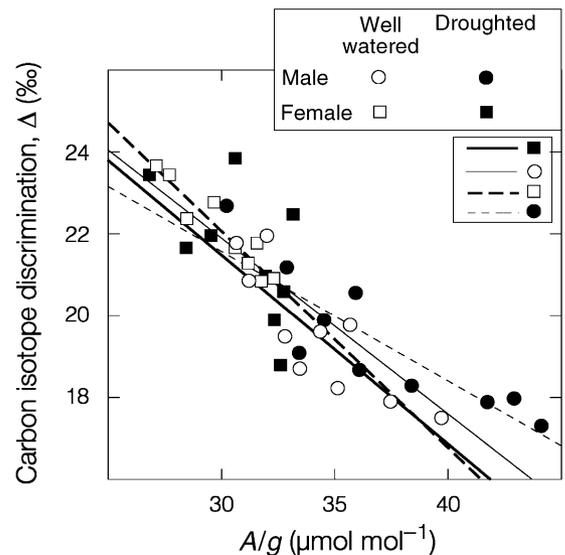


Fig. 2. Relationship between leaf carbon isotope discrimination (Δ) and intrinsic water-use efficiency (A/g) for male (circles) and female (squares) trees of *Acer negundo* grown in 40 l pots under well watered (open symbols, $\Psi_1 = -0.13$ to -0.72 MPa) and droughted (closed symbols, $\Psi_1 = -1.15$ to -2.25 MPa) conditions. Least-squares regression equations: male, well watered, $y = -0.462x + 35.380$, $r^2 = 0.770$; male, droughted, $y = -0.321x + 31.229$, $r^2 = 0.568$; female, well watered, $y = -0.527x + 37.908$, $r^2 = 0.893$; female, droughted, $y = -0.432x + 34.882$, $r^2 = 0.332$. Each point represents the mean for a single genotype.

often difficult because the assumption of compound symmetry in the covariance matrix was violated. In the absence of compound symmetry we performed a Huynh–Feldt corrected ANOVA for repeated measures. We chose this analysis because, in some cases, our sample sizes were small (testing for normality was difficult) and because the compound symmetry of the covariance matrix showed mild violation of Mauchly's criterion ($\epsilon = 0.9799 - 0.9607$). Our analysis of within-subjects effects are based on the Huynh–Feldt corrected significance levels. We performed both standard least-squares regressions and reduced major axis regressions on the data in Figs 2 and 3. No differences between regression methods were detected. Therefore the equations given in the figure captions are for standard least-squares regressions. The analysis performed on the $A-c_i$ curves follows the methods of Geber & Dawson (1997).

Results

COMPARISONS ACROSS CULTURAL
CONDITIONS (WELL WATERED)

Significant differences in A , g , c_i , A/g and Δ were observed between males and females (10 male and nine female genotypes; Tables 1 and 2). In general, genotypes with the highest gas-exchange rates (A and g), c_i and leaf Δ , and lowest A/g , were female trees. These patterns persisted regardless of the year when the data were obtained. We found no significant effects of cultural

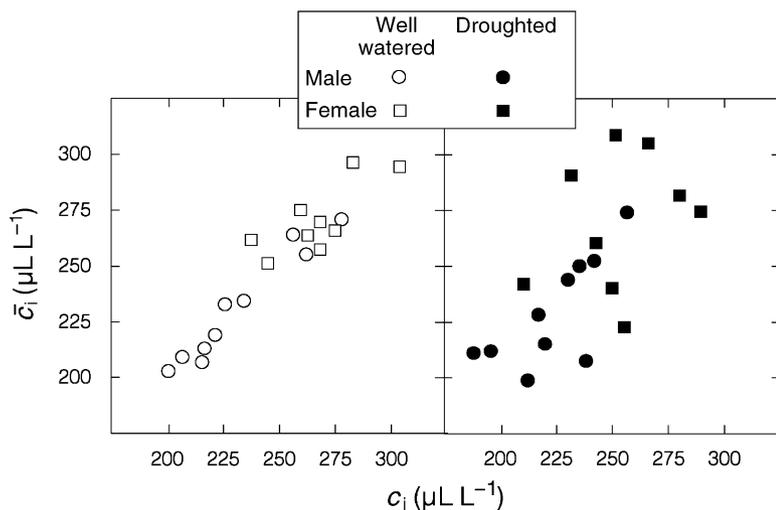


Fig. 3. Relationships between instantaneous intercellular CO_2 concentrations (c_i) determined from leaf gas exchange and time-integrated intercellular CO_2 concentrations calculated from equation 3 (\bar{c}_i) for male (circles) and female (squares) trees of Boxelder (*Acer negundo*) grown in 40 l pots under well watered (open symbols, $\Psi_1 = -0.13$ to -0.72 MPa) and droughted (closed symbols, $\Psi_1 = -1.15$ to -2.25 MPa) conditions during year 1. Least-squares regression equations: male, well watered, $y = 0.923x + 17.948$, $r^2 = 0.972$; male, droughted, $y = 0.846x + 40.629$, $r^2 = 0.503$; female, well watered, $y = 0.696x + 101.264$, $r^2 = 0.743$; female, droughted, $y = 0.369x + 176.273$, $r^2 = 0.083$. Lines have been omitted for clarity. Each point represents the mean for a single genotype.

conditions (field, garden, pots) on leaf Δ between male and female trees (Tables 1 and 2). However, c_i did differ between genders in the field, but not in the garden or in pots for each of the 3 years (Table 1).

SEASONAL AND INTERANNUAL EFFECTS (WELL WATERED AND NATURAL FIELD CONDITIONS)

Significant differences in leaf Δ were observed between males and females during early, mid- and late periods of the growing season (Fig. 1). Female trees had consistently higher leaf Δ compared with male trees irrespective of cultural conditions; coincident with this, instantaneous leaf-to-air mole fraction vapour difference and leaf temperature did show marked changes (data not shown) during a single growing season (and water availability in the field), but the relative ranking of males and females with respect to leaf Δ was the same for field-, garden- and pot-grown trees. Furthermore, no significant differences were detected within a gender between measurements made at different times during the growing season and during different years (Fig. 1). This occurred for leaves measured at the same age (recently matured), but at different times during the growing season.

INSTANTANEOUS VERSUS INTEGRATED LEAF MEASURES (WELL WATERED VERSUS DROUGHTED)

When water was withheld from half the pot-grown trees so that Ψ_1 declined to between -1.15 and -2.25 MPa (not

lower), the relationship between leaf Δ versus A/g shifted only slightly (there were no significant changes in the slopes of the lines in Fig. 2). Irrespective of whether genotypes had high or low leaf Δ under well watered conditions, their rank remained essentially the same for A/g when water was withheld. Genotypes with high Δ , A and g , and low A/g (females), generally had higher leaf N concentrations and higher abaxial stomatal densities than genotypes with the opposite set of traits (males), whether they were growing in the field, the garden, or in pots (Table 2; Dawson & Ehleringer 1993).

There were strong and significant differences in calculated \bar{c}_i (equation 3) among trees of different genders and habitat distributions in the field population (Table 3). Comparisons made across all 3 years and between streamside (wet) and non-streamside (dry) habitats showed that female trees (high Δ genotypes) always had a significantly higher \bar{c}_i than males (low Δ genotypes). When water was withheld from pot-grown trees so that Ψ_1 declined to between -1.15 and -2.25 MPa, strong and significant differences occurred for c_i (from leaf gas exchange) between water treatments and between the genders (Table 4). Interestingly, \bar{c}_i did not show the same pattern; significant differences were observed only between the genders, not between water treatments for pot-grown trees (Table 4).

When the mean \bar{c}_i versus c_i concentrations for male and female genotypes were plotted, a strong and significant correlation for well watered plants was observed (Fig. 3, open symbols), but the same correlation was significantly weakened in male trees and was statistically non-existent in female trees when plants were subjected to droughted conditions (Fig. 3, closed symbols).

The $A-c_i$ curves indicated that females maintained higher A under well watered conditions, but experienced large reductions in A when water was withheld, resulting in lower A than in males under these conditions (Fig. 4). Furthermore, initial slopes of the $A-c_i$ curves were higher for females under well watered conditions, indicating higher Rubisco concentrations and/or activity in this gender in the absence of stress (Sage 1994). When drought was imposed, the initial slope of the $A-c_i$ curve and maximum rate of carbon assimilation were reduced significantly (Fig. 4). The shape of the curves also changed, especially for females (Fig. 4). Simultaneous measurements of O_2 evolution from the same leaves indicated that when droughted, plants were supplied with very high CO_2 concentration ($50\,000\ \mu\text{L L}^{-1}$), the dramatic shift in photosynthetic metabolism (assessed from the $A-c_i$ curves) was relatively less pronounced, particularly in females (Fig. 4, right). This indicates that photosynthetic capacity was strongly influenced by stomatal limitation under drought, and was a more dominant factor for females relative to males.

EFFECTS OF AGEING

Marked age effects were found. These showed that in young trees (2 years old) Δ was higher in females than

Table 1. Photosynthesis (A), stomatal conductance (g), internal leaf CO_2 concentration from instantaneous measurements (c_i), intrinsic water-use efficiency (A/g), and leaf carbon isotope discrimination (Δ) for male and female trees of Boxelder (*Acer negundo*) determined in three growing seasons in the field, a common garden and 40 l pots

Parameter	Sex	Field	Garden	Pots
Year 1				
A (mol m ⁻² s ⁻¹)	Male	9.8 (1.25)* ^a	9.3 (1.36)* ^a	10.1 (0.96)* ^a
	Female	13.9 (2.11)* ^a	14.3 (1.38)* ^a	15.1 (1.64)* ^a
g (mol m ⁻² s ⁻¹)	Male	0.2 (0.06)* ^a	0.3 (0.07)* ^a	0.3 (0.07)* ^a
	Female	0.4 (0.11)* ^a	0.5 (0.08)* ^a	0.5 (0.14)* ^a
c_i ($\mu\text{mol mol}^{-1}$)	Male	221 (24)* ^a	236 (19) ^a	227 (16) ^a
	Female	262 (13)* ^a	255 (26) ^a	259 (20) ^a
A/g (mol mol ⁻¹)	Male	40.7 (12)* ^a	37.4 (16) ^a	36.2 (13) ^a
	Female	31.6 (9)* ^a	29.10 (14) ^a	28.4 (11) ^a
Δ (‰)	Male	19.6 (0.37)* ^a	17.5 (0.38)* ^a	18.1 (0.40)* ^a
	Female	23.4 (0.33)* ^a	20.0 (0.55)* ^a	21.1 (0.25)* ^a
Year 2				
A (mol m ⁻² s ⁻¹)	Male	8.3 (1.05)* ^a	8.8 (1.22)* ^a	9.6 (1.25)* ^a
	Female	15.7 (1.76)* ^a	14.3 (1.35)* ^a	15.8 (2.03)* ^a
g (mol m ⁻² s ⁻¹)	Male	0.2 (0.04)* ^a	0.2 (0.07)* ^a	0.2 (0.05)* ^a
	Female	0.4 (0.09)* ^a	0.5 (0.14)* ^a	0.5 (0.17)* ^a
c_i ($\mu\text{mol mol}^{-1}$)	Male	209 (28)* ^a	222 (19) ^a	230 (31) ^a
	Female	271 (44)* ^a	259 (30) ^a	255 (42) ^a
A/g (mol mol ⁻¹)	Male	39.7 (10) ^a	46.2 (17) ^b	41.9 (9) ^b
	Female	37.3 (13) ^b	31.8 (15) ^a	33.7 (14) ^b
Δ (‰)	Male	19.3 (0.30)* ^a	17.4 (0.69)* ^a	18.2 (0.42)* ^a
	Female	23.6 (0.44)* ^a	19.9 (0.51)* ^a	22.2 (0.73)* ^a
Year 3				
A (mol m ⁻² s ⁻¹)	Male	10.3 (1.67)* ^b	11.0 (2.07)* ^b	11.3 (2.22)* ^b
	Female	17.2 (2.46)* ^b	16.8 (1.66)* ^a	17.0 (2.71)* ^a
g (mol m ⁻² s ⁻¹)	Male	0.2 (0.04)* ^a	0.2 (0.07)* ^a	0.3 (0.05)* ^a
	Female	0.5 (0.12)* ^a	0.5 (0.09)* ^a	0.6 (0.14)* ^a
c_i ($\mu\text{mol mol}^{-1}$)	Male	220 (17)* ^a	229 (31) ^a	233 (12) ^a
	Female	266 (34)* ^a	272 (18) ^a	260 (24) ^a
A/g (mol mol ⁻¹)	Male	44.7 (16)* ^a	50.2 (20)* ^b	45.9 (21)* ^c
	Female	33.6 (12)* ^a	35.8 (22)* ^b	29.8 (17)* ^a
Δ (‰)	Male	19.0 (0.27)* ^a	17.9 (0.36)* ^a	18.3 (0.41)* ^a
	Female	24.1 (0.34)* ^a	23.0 (0.45)* ^b	23.2 (0.56)* ^a

Values are mid-season (July) means (SD) for nine female and 10 male genotypes (five leaves per genotype). Significant differences ($P < 0.05$) between the genders are noted with an asterisk (*); differences among years are noted with different lettered superscripts. No significant differences were detected among field-, garden- or pot-grown trees.

Table 2. Average photosynthetic rate (A , mol m⁻² s⁻¹), stomatal conductance (g , mol m⁻² s⁻¹), intrinsic water-use efficiency (A/g , mol mol⁻¹), leaf carbon isotope discrimination (Δ , ‰), leaf nitrogen concentration (N_{leaf} , mmol m⁻²), and abaxial stomatal density (number mm⁻²) for male and female trees of Boxelder (*Acer negundo*) growing in the field, a common garden and 40 l pots

	A	g	A/g	Δ	N_{leaf}	Stomatal density
Male						
Field	9.5 (1.32)*	0.23 (0.03)*	41.7 (13)*	19.3 (0.31)*	129 (18.1)*	194 (27.7)*
Garden	9.7 (1.88)*	0.22 (0.06)*	44.6 (18)*	17.6 (0.53)*	141 (22.4)*	208 (22.2)*
Pots	10.6 (1.62)*	0.26 (0.05)*	33.2 (24)	18.3 (0.61)*	137 (28.0)*	183 (34.1)*
Female						
Field	15.6 (2.33)*	0.46 (0.10)*	34.2 (12)*	23.7 (0.40)*	175 (26.6)*	251 (33.8)*
Garden	15.1 (1.42)*	0.47 (0.07)*	32.2 (19)*	20.9 (1.7)*	193 (29.1)*	279 (51.6)*
Pots	16.0 (2.28)*	0.52 (0.11)*	30.6 (15)	22.2 (0.59)*	200 (33.9)*	262 (43.3)*

Values (SD) are for 10 male and nine female genotypes (five leaves per genotype were sampled) averaged over 3 years (Table 1). Significant differences between the sexes are noted with an asterisk (*, $P < 0.05$). No significant differences were detected among the three cultural conditions (field, garden, pots).

in males, regardless of cultural conditions and water treatments (Table 5). For trees 5–10 years old growing in the garden, gender differences were observed during wet years only; during dry years there were no differ-

ences between genders, as determined from integrated measures of wood cellulose Δ (Table 5; Ward *et al.* 2002). For trees older than 20 years Δ varied between the genders, indicating that even in old trees genders

Table 3. \bar{c}_i (11^{-1}) as a function of gender, year and habitat type

Year	Gender	Streamside	Non-streamside	Across habitats
1	Male	236 (10)*	216 (14)*	226 (12)*
	Female	289 (14)*	262 (15)*	275 (15)*
2	Male	232 (11)*	204 (17)*	219 (15)*
	Female	295 (17)*	250 (22)*	272 (18)*
3	Male	229 (14)*	196 (11)*	215 (12)*
	Female	302 (20)*	273 (14)*	286 (17)*

Values are means (SD) for five leaves taken from 15 trees per gender. Significant differences (*, Student's *t*-test) between genders occurred at $P < 0.0446$. No significant differences between years were detected.

vary in their physiological responses to water availability (Table 5). In summary, it appears that gender differences, as assessed from tree-ring Δ , were most pronounced in young trees and when water stress was less severe.

Discussion

GENDER RESPONSES TO WATER AVAILABILITY

Under well watered conditions, significant differences in instantaneous leaf gas exchange and leaf Δ were observed between the genders of Boxelder, with female trees having significantly higher *A*, *g*, and leaf Δ , and lower *A/g* (although not significant in all cases) than males (Tables 1 and 2). Dawson & Ehleringer (1993) showed previously that rates of gas exchange were greater

Table 4. Average calculated c_i (11^{-1} , mean and range) obtained from leaf gas exchange, and mean (SD) \bar{c}_i determined from carbon isotope ratios as a function of gender and water treatment (well watered versus droughted)

Treatment	Gender	c_i	\bar{c}_i
Well watered	Male	244 (201–283)* ^a	236 (12.8)*
	Female	257 (254–281)* ^a	269 (17.1)*
Water-stressed	Male	221 (178–258)* ^b	243 (18.4)*
	Female	276 (219–286)* ^b	281 (23.5)*

Ten male and nine female genotypes were used. Five individuals of each genotype growing outdoors in 40 l pots were measured during year 1. \bar{c}_i values are means (SD) for five leaves per individual. Significant differences ($P < 0.05$) between genders within a treatment are noted with an asterisk (*); differences between treatments within a gender are noted with different lettered superscripts.

for females than males under well watered conditions, and that females were more sensitive to drought. These patterns are consistent with the observations of others (Freeman, Klikoff & Harper 1976; Ward *et al.* 2002). High water use in female trees may explain why they are over-represented on wetter sites relative to males, as seen in other species (Dawson & Geber 1999).

As seen with females in this study, and in a variety of other herbaceous and woody species (Farquhar *et al.* 1982, 1989; Farquhar & Richards 1984; Hubick & Farquhar 1989; Condon, Farquhar & Richards 1990; Hubick & Gibson 1993), trees with high rates of net photosynthesis (*A*) and stomatal conductance to water vapour (*g*) had higher instantaneous intercellular CO_2 concentrations (c_i) and leaf Δ values (under both well watered and droughted conditions). Furthermore, female trees also had the lowest intrinsic water-use efficiencies (*A/g*) and higher abaxial stomatal densities and leaf N concentrations than male trees that had low leaf Δ , *A*, *g*, and high *A/g*. Because male trees had a low c_i and low *g* they were more conservative of water, leading to higher relative *A/g*. These traits confer a clear physiological benefit for droughted males and are consistent with them dominating dry, non-streamside habitats (Dawson & Ehleringer 1993; Ward *et al.* 2002). However, it is also likely that CO_2 concentration may have limited photosynthetic capacity in males, reducing potential growth, productivity (Dawson & Ehleringer 1993; Ward *et al.* 2002) and N-use efficiency, because N-expensive Rubisco may be limited by CO_2 . In relation to this, Ehleringer (1993) showed that differences in leaf Δ were ultimately related to productivity under well watered conditions and to survival under drought in the desert shrub *Encelia farinosa*. The Δ differences seen between male and female Boxelder may reflect the same contrasts. There is likely to be a complex relationship between *A/g*, absolute carbon gain and N-use efficiency. Evolutionarily, having higher *A/g* may not be so important for maximizing carbon returns on a finite water resource. Rather, higher *A/g* indicates a

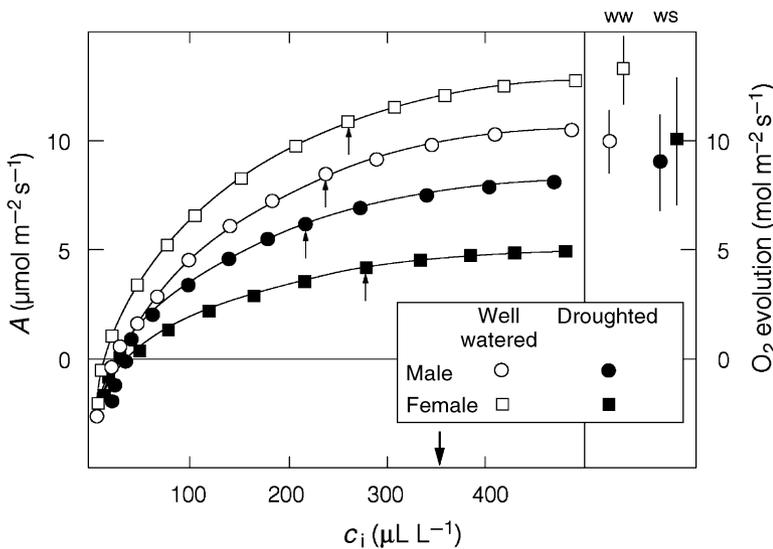


Fig. 4. Carbon assimilation rate (*A*) as a function of intercellular CO_2 concentration (c_i), and the rate of oxygen evolution (panel, right) for male (circles) and female (squares) trees of Boxelder (*Acer negundo*) growing under common garden conditions. Plants were either well watered (ww, open symbols) or droughted (ws, closed symbols) for 14 days, then measured. Arrows on the *A*- c_i curve show c_i at ambient $[CO_2]$ of ≈ 353 (± 5) 11^{-1} (arrow on *x*-axis).

Table 5. Summary of the response in carbon isotope discrimination (Δ) 'recorded' in leaf tissue and wood cellulose of female and male Boxelder (*Acer negundo*)

Plant age (years)	Culture	Treatment	Time scale of treatment	Tissue measured	Time integrated for Δ	Response for Δ	$\Delta_{\text{female}} - \Delta_{\text{male}}$	Reference
2	Pots	Wet	Days	Leaves	20 days	Females higher	3.88	Present paper
2	Pots	Dry	Days	Leaves	20 days	Females higher	2.47	Present paper
2	Garden	Wet	Weeks	Leaves	20 days	Females higher	3.34	Present paper
2	Garden	Dry	Weeks	Leaves	20 days	Females higher	2.11	Present paper
5–10	Garden	Wet	Growing season	Wood cellulose	Full growing season	Females higher	0.62	Ward <i>et al.</i> (2002)
5–10	Garden	Dry	Growing season	Wood cellulose	Full growing season	No difference	0	Ward <i>et al.</i> (2002)
20+	Field	Wet (streamside)	Life span	Leaves	20 days	Females higher	1.48, 4.37	Dawson & Ehleringer (1993)
20+	Field	Dry (non-streamside)	Life span	Leaves	20 days	Females higher	3.05, 2.87	first value, and present paper Dawson & Ehleringer (1993) first value, and present paper

Trees were of different ages and grown under different cultural conditions; levels of water availability (wet and dry) as noted. Field measurements made at non-streamside habitats may have involved mature trees that had access to groundwater and thus were not exposed to drought conditions (Dawson & Ehleringer 1991). All gender differences are at the $P < 0.05$ level.

more conservative water-use strategy. Conservative water use, in turn, may be critical if plants must allocate the carbon they do fix to competing demands such as growth (and competitive ability) and reproduction (fitness). For female trees with greater reproductive allocation and more chances for competition in streamside sites where they dominate (Dawson & Ehleringer 1993), being inefficient could put them at risk of running out of water which may, in turn, reduce carbon gain compared with males. If true, male trees would be favoured under water limitation, leading to strong selection for higher A/g and dominance in drier sites.

To improve our mechanistic understanding of stomatal regulation in response to water limitation and how these responses may affect more integrated measures, we studied the effects of excluding stomatal limitation on photosynthesis in males and females by saturating the leaf with CO_2 . This was necessary because stomatal densities are different in males and females (Table 2; Dawson & Ehleringer 1993). Different O_2 evolution rates between well watered (Fig. 4, left, ww) and droughted (Fig. 4, right, ws) trees can be interpreted as a shift in leaf photosynthetic biochemistry caused by leaf water deficit. Therefore the differences between the maximum rate of carbon assimilation shown by the asymptote of the $A-c_i$ curve and the maximum rate of O_2 evolution for a particular treatment and gender can be interpreted as a physiological change caused by stomatal closure (Fig. 4). Using these data, it is apparent that water stress caused maximum rates of carbon assimilation (assessed from the $A-c_i$ curves) to decline by $\approx 21\%$ in males and by $\approx 63\%$ in females. However, the O_2 evolution rates indicated that, for males, $\approx 50\%$ of this change was due to shifts in leaf biochemistry and 50% was caused by stomatal limitation; the corresponding figures for female trees were 30 and 70%, respectively. These results suggest that the variation in leaf Δ is probably influenced more by stomatal factors for females and non-stomatal factors for males, as previously discussed by Dawson & Ehleringer (1993).

GENDER RESPONSES TO THE EFFECTS OF AGEING

Gender differences in Δ are generally maintained with age (Table 5) in very young trees up to those >20 years old. The only exception was that we saw no gender differences for trees 5–10 years of age, assessed with wood cellulose Δ , growing in the garden during very dry years. Under severe and consistent drought gender differences may diminish unless trees can access other water sources as they do in the field (Dawson & Ehleringer 1991). Furthermore, when gender differences are observed, as is usually the case, females consistently have higher Δ than males. This indicates that females use more water relative to males for most of their life cycle, and where females are most abundant in the riparian zone the water flux from that zone will also be highest (Dawson & Ward 2004).

RESPONSES TO CULTURAL CONDITIONS

Absolute values and relative rankings of males and females did not vary between cultural conditions (field, garden, large pots) for the most of the physiological measurements (A , g , A/g , Δ) made under well watered conditions, suggesting that trees grown in pots may reflect responses occurring in the field. It should be noted, however, that the pots used in the present study were very large, which probably contributed to most measurements being similar between pots, garden and field. Trees had similar canopy properties (e.g. leaf area index and canopy/atmosphere coupling) among all cultural conditions and were not pot-bound in any case. Where small pots are used and pot-binding is an issue, greater differences between instantaneous and integrated measures for pot studies versus field measurements would be expected. This may be due to factors such as smaller soil volume for water acquisition, rapid onset of drought, altered canopy architecture, and possible negative feedbacks on photosynthesis (caused by downregulation of Rubisco) associated with pot-binding (Thomas & Strain 1991).

RESPONSES TO SEASONAL AND INTERANNUAL VARIATION

Leaf and air temperatures and leaf-to-air vapour gradients changed markedly during a single growing season, as they might in any ecosystem or with many species. Nevertheless, leaves of the same age collected for Δ analysis showed remarkably little seasonal variation. Furthermore, the relative rankings of females and males for leaf Δ did not change throughout a growing season, or between years. Taken together, these results indicate that leaf metabolic processes function within a relatively narrow range, what has now become known as the metabolic set-point (Ehleringer & Cerling 1995). Therefore leaf Δ can serve to integrate the responses of individual species for use in broad-scale models of stand- and landscape-level carbon and water balance (Dawson *et al.* 2002).

RESPONSES TO TEMPORAL SCALING (WELL WATERED VERSUS DROUGHTED)

Internal CO_2 concentrations calculated from instantaneous gas exchange and \bar{c}_i values from leaf $^{13}\text{C}/^{12}\text{C}$ ratios (equation 3) were measured on well watered and droughted trees growing in the field, garden, and in pots. For field measurements, wet sites were located at streamside habitats and dry sites at non-streamside habitats. Stream- and ground-water are more accessible to trees at streamside than in non-streamside habitats (Dawson & Ehleringer 1991). The \bar{c}_i varied significantly between genders and across habitat types in the field (Table 3). For plants measured in 40 l pots, instantaneous c_i varied between genders and water treatments (well watered and droughted; Table 4). However, time-

integrated c_i values (\bar{c}_i) varied only between genders, not between water treatments, which differed from the field results. This discrepancy was probably due to the rapid decline in water potential within potted plants (within 2 days) relative to the more constant responses in the field (long-term dry and wet habitats). In the case of pot-grown trees, instantaneous measurements reflected rapid adjustments to drought, whereas the effects of water stress were not evident in the \bar{c}_i values. This was probably because the period of low water availability was insufficient for photosynthetic products to be expressed the leaf Δ values from the drought, and also photosynthetic rates were lowered during drought (Fig. 4). It is also possible that leaf Δ may be influenced by biochemical fractionations that may occur in response to changing water availability (as in *Salix*; Le Roux-Swarthout *et al.* 2001).

Similarly, it was observed in pot-grown trees that the relationship between c_i and \bar{c}_i was very strong under well watered conditions, but not under drought. Under well watered conditions, c_i and \bar{c}_i were closely related because instantaneous and time-integrated measures were made under similar growing conditions. This is unlike the drought treatment, where most leaf development occurred when plants were well watered and was therefore reflected in \bar{c}_i , whereas instantaneous measurements reflected responses to current, low water availability. The result of this was a decoupling of c_i and \bar{c}_i values, the degree of this decoupling varying between males and females. Ward *et al.* (2002) found that the Δ of wood cellulose in males was maintained at a more constant value than that of females in response to shifts between wet and dry years. This helps explain why the c_i versus \bar{c}_i relationship remained stronger in males than in females when drought was imposed (Fig. 3). Overall, this suggests that temporal scaling of physiological responses may vary even at the subspecies level, and indicates the importance of understanding population-level responses to water availability when scaling to larger spatial and temporal scales.

This study indicates that both instantaneous gas exchange and integrated Δ values can characterize physiological responses to water availability when growth conditions are relatively constant. However, when water availability fluctuates care must be taken in equating these measurements, because changing conditions may decouple responses integrating different time scales. Furthermore, the degree of this decoupling may vary at the species level and even at the subspecies level.

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