David G. Williams · James R. Ehleringer

Carbon isotope discrimination in three semi-arid woodland species along a monsoon gradient

Abstract Leaf carbon isotope discrimination (Δ) was measured for three dominant, semi-arid woodland species along a summer monsoon gradient in the southwestern United States over a 2-year period. We tested the hypothesis that decreased humidity levels during the growing season along this gradient resulted in lower leaf Δ values. Sites of similar elevation along the transect were selected and the range in monsoon contribution to overall annual precipitation varied from 18 to 58%, while total annual precipitation differed by a maximum of only 25% across this gradient. Leaf Δ values in *Quercus gambelii* were negatively correlated with ω, a seasonally-weighted estimate of the evaporative humidity gradient, suggesting that stomatal conductance declined as transpiration potential increased. For two other trees that co-occurred along this gradient, *Pinus edulis* and *Juniperus osteosperma*, Δ remained relatively constant despite large variation in ω. These woodland species represent the full spectrum of responses of carbon isotope discrimination to increases in evaporative potential; that of decline where c_i/c_a (ratio of internal to ambient CO_2 concentration) and presumably stomatal conductance decrease, and that of constancy where whole plant internal adjustments allow c_i/c_a to remain stable.

Key words Carbon isotope discrimination · Humidity · Monsoon · Pinyon-juniper ecosystem

Introduction

In arid regions plant growth and photosynthesis are constrained by pronounced seasonal droughts and low atmospheric humidities (MacMahon and Schimpf 1981; Smith and Nowak 1990). Because both soil and atmospheric drought influence transpiration rates in similar ways, it can be difficult to distinguish their effects along complex climatic gradients. Indeed, debate continues on the exact stimulus-response mechanism for stomatal closure (Schulze 1986), what measure of humidity is sensed by plants (Grantz 1990; Aphalo and Jarvis 1991), or whether stomata respond to transpiration rate and not humidity (Mott and Parkhurst 1991; Monteith 1995). Irrespective of the exact mechanism, physiological adaptations governing water utilization by plants in arid regions are likely shaped by interactions between humidity *and* water availability.

An important physiological trait related to water use and stomatal behavior under drought conditions is the ratio of internal to ambient CO_2 concentration (c_i/c_a) of leaves (Farquhar et al. 1989). The c_i/c_a ratio represents the balance point between diffusion and biochemical constraints on photosynthetic carbon fixation and typically declines as stomata close with increasing drought stress (Farquhar et al. 1989). Carbon isotope discrimination against $^{13}CO_2$ (Δ) during photosynthesis is directly related to c_i/c_a as

$$\Delta = a + (b - a)\frac{c_i}{c_a}. \qquad \text{(Eq. 1)}$$

where a and b are fractionation factors associated with CO_2 diffusion into the leaf (4.4‰) and carboxylation (27‰), respectively. The Δ value forms the basis of a time-integrated measure of c_i/c_a in C_3 plants (Farquhar et al. 1989), recording in leaf biomass the balance between biochemical demand for CO_2 by the chloroplasts and CO_2 supply through the stomata. While Δ does not reflect actual rates of gas exchange, it can be regarded as a measure of the overall trade-offs among traits controlling photosynthesis and stomatal conductance. Since both water and CO_2 diffuse through the stomata, traits that determine Δ have important implications for transpiration, productivity, and plant survival in water-limited environments (Ehleringer 1993).

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The humidity environment of plants can be described by several different, but inter-related parameters. However, since transpiration is the product of stomatal conductance and v, the vapor pressure difference between leaf and air divided by total atmospheric pressure, v becomes the most important characterization functionally of a plant's humidity environment and one which has ramifications for the evolution of traits that influence c_i/c_a. The v value in any habitat or environment then could be characterized by v averaged over the year or growing season if we assume that leaf and air temperatures are similar. However, such an average does not take into account that plants have distinct seasonal periods of activity related to water availability. To address this, Comstock and Ehleringer (1992) developed a synthetic parameter ω which ranks habitats with respect to v adjusted for monthly water balance, where

$$\omega = \left[\frac{1}{p_{total}} \sum_{Jan}^{Dec} \left(e_{a,sat} \frac{P}{E_p} \right) \right] \Bigg/ \sum_{Jan}^{Dec} \frac{P}{E_p}. \qquad \text{(Eq. 2)}$$

P/E_p, the ratio of precipitation to potential evapotranspiration or precipitation sufficiency (Thornthwaite 1948), scales the potential activity of plants for a given month and p_{total} is total atmospheric pressure. The saturation vapor pressure ($e_{a,sat}$) calculated from monthly averaged temperatures, is a reasonable estimate of v if relative humidity is low and leaf temperatures are near ambient, assumptions usually met for microphyllous plants in arid regions. One very useful aspect of ω is that it can be calculated from simple climate records widely reported even at the most remote weather stations.

Comstock and Ehleringer (1992) found genetic variation for Δ among populations of the desert shrub *Hymenoclea salsola* grown in a common environment that was negatively correlated to the ω value at the sites of origin. The applicability of ω for other aridland ecosystems as a predictive measure of plant response has not been established. The purpose of this paper was to explore the use of ω as a tool for understanding physiological responses (Δ) to humidity for several woodland species of the pinyon-juniper ecosystem in the southwestern United States. This ecosystem was chosen for its broad geographic distribution across climates that vary in seasonal precipitation from predominantly winter-rain to predominantly summer-rain habitats. Because temperature and $e_{a,sat}$ are high in summer, sites receiving significant summer precipitation would tend to have high ω values compared to sites with dry-summer climates (Eq. 2). Observations using *Hymenoclea* lead to the prediction that along an increasing summer monsoon gradient in North America, plants should express continuous intraspecific variation for Δ, reflecting compensatory plant responses to variation in ω; Δ should decline (lower c_i/c_a) as summer precipitation and summer water availability increase. This seemingly counterintuitive response should be independent of absolute precipitation differences among sites.

Fig. 1 Locations of study sites along a monsoon precipitation gradient in the southwestern United States. *Pie charts* show average proportion of annual precipitation received during the monsoon season (July – September). Size of pie chart is scaled to represent average total amount of annual precipitation
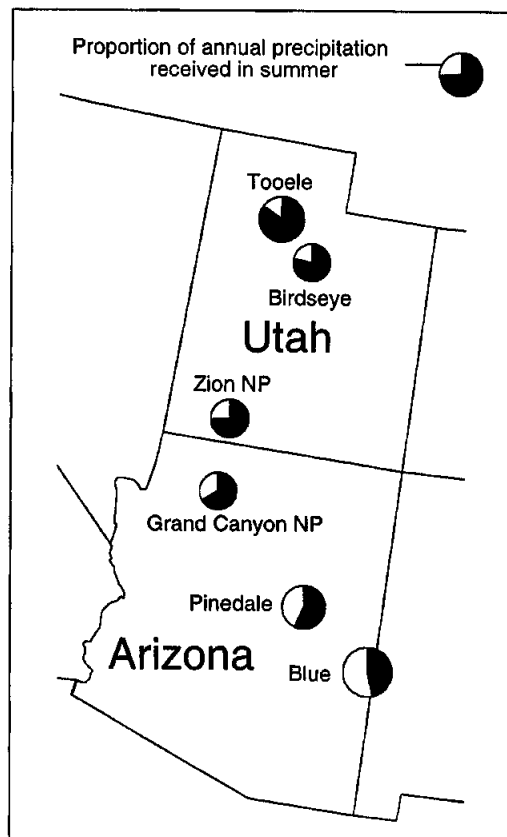
Table 1 Geographic and climatic information for study sites used to investigate responses of pinyon-juniper woodland species to climate gradients. Climate data are long-term averages from stations adjacent to field sites. Summer precipitation is that falling from July to September

Site	Latitude (°N)	Elevation (m)	Annual precip. (mm)	Summer precip. (mm)	Average July max temp. (°C)
Tooele, UT	40.5	1980	448	79	27.5
Birdseye, UT	39.9	1859	371	80	30.2
Zion NP, UT	37.2	2000	403	96	29.9
Grand Canyon NP, AZ	34.7	2120	367	123	29.3
Pinedale, AZ	34.3	1970	427	183	30.1
Blue, AZ	33.6	1980	490	286	30.1

Materials and methods

Six sites were chosen within the pinyon-juniper ecosystem along a transect from northern Utah to southeastern Arizona (Fig. 1). An effort was made to keep elevation, growing season temperature and total annual precipitation similar among the sites (Table 1) so that the effects of seasonal precipitation patterns could be more easily evaluated. The sites spanned a broad summer precipitation gradient and encompassed a large portion of the geographic range of three dominant woodland species, including the two evergreen gymnosperms *Pinus edulis* Engelm. and *Juniperus osteosperma* (Torr.) Little, and the deciduous angiosperm, *Quercus gambelii* Nutt.

Leaves of *Pinus*, *Juniperus*, and *Quercus* were sampled in August of 1993 and 1994 for carbon isotopic analyses. Leaves were collected from three exposed south-facing canopy locations and combined into one sample for each tree. Five randomly chosen adult individuals with basal diameters ranging from 11 to 18 cm were sampled for each species from each site, except that *P. edulis* was not present at the Tooele, Utah site and *J. osteosperma* was not present at the Blue, Arizona site. The 1992 and 1993 needle cohorts were sampled from *Pinus* in 1993 and 1994, respectively. Photosynthetic tissue from the top 2 cm of shoot apex was sampled for *Juniperus* and fully expanded leaves developed from the spring leaf-out were sampled for the winter-deciduous *Quercus*. Carbon isotopic composition was measured on finely-ground, oven-dried (70°C for 48 h) samples using an isotope ratio mass spectrometer (delta S, Finnigan MAT, San Jose, California; Ehleringer and Osmond 1989). Carbon isotope ratios ($\delta^{13}C$) were calculated relative to the Pee Dee belemnite (PDB) standard, and converted to carbon isotope discrimination values (Δ, ‰), using an atmospheric $\delta^{13}C$ value of −8‰ (Farquhar et al. 1989). We were confident in using a uniform −8‰ for an ambient CO_2 carbon isotope ratio in these relatively open, structurally heterogeneous forest canopies based on a limited number of atmospheric analyses from the region.

Monthly summarized climate data from 1991 through 1994 for each site were obtained from nearby National Oceanic and Atmospheric Administration (NOAA) reporting meteorological stations. Elevation was used in regression analyses to predict climate variables for several of the sites when nearby stations were not at the same elevation. ω was calculated using Eq. 1 and restricting growth to May through September. P/E_p was not allowed to exceed 1; monthly P in excess of E_p is assumed to be lost as runoff. When P/E_p is 1, plants potentially are fully active and there is no soil water limitation. Summer precipitation was defined as that falling between July and September, the period influenced by incursions of the monsoon air masses from the Gulfs of Mexico and California (Hales 1974). Pearson product-moment correlation coefficients were calculated using the JMP statistical program (SAS Institute 1994) to analyze responses of Δ to climate variations.

Results

Growing season ω and amount of summer precipitation for 1991 through 1994 tended to increase along the experimental transect from northern Utah to southeastern Arizona (Fig. 2). In 1992, high estimated leaf-to-air va-
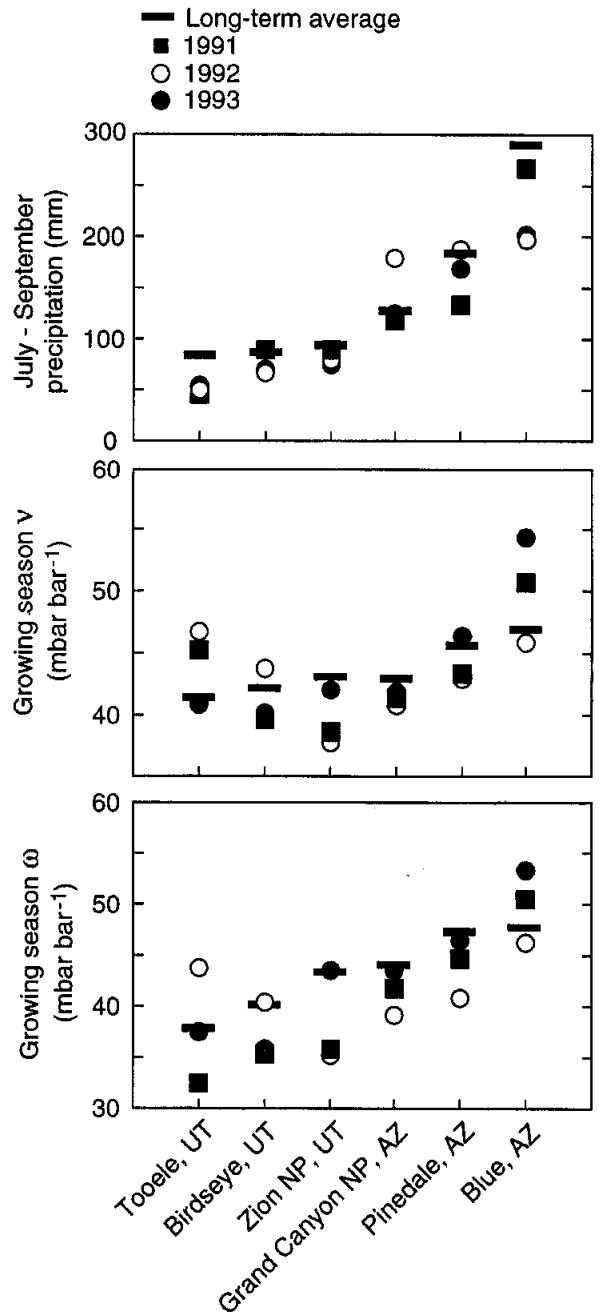

Fig. 2 Climate indices for the six study sites including values for 1991–1993 as well as long-term averages. Monsoon precipitation is defined as that falling between July and September (ν, vapor pressure difference between leaf and air divided by total atmospheric pressure, ω, parameter ranking habitats with respect to ν adjusted for monthly water balance)
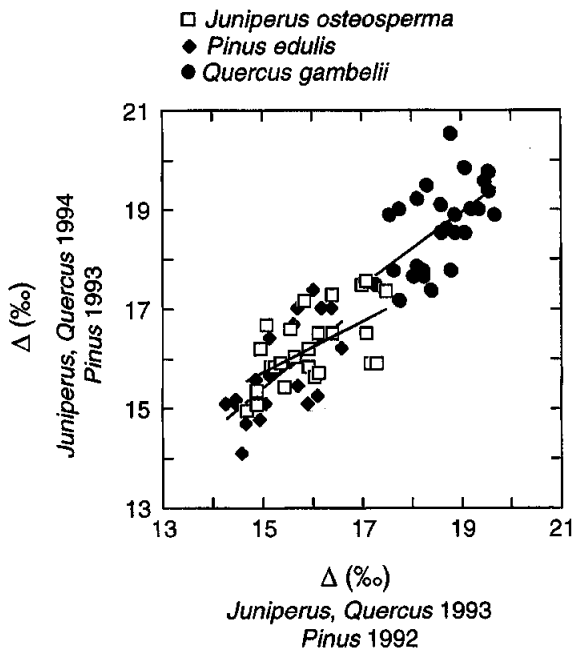
Fig. 3 Correlation between carbon isotope discrimination of leaves formed in consecutive years for three woodland species along the monsoon gradient. Data are for individual trees. Correlations within species are significant at the $P<0.05$ level

Fig. 4 Relationships between population averaged carbon isotope discrimination (Δ) of leaves formed in 1992, 1993 and 1994 and growing season ω calculated for the year prior to leaf formation. *Error bars* are ± 1 SE of the mean

por pressure difference (v) in the summer dry region of northern Utah (Tooele and Birdseye) led to high calculations of ω at these sites. The years 1991 and 1993 were more typical of the long-term averages; ω increased linearly along the experimental transect in a manner similar to the long-term average for these sites.

Carbon isotope discrimination (Δ) ranged from low values near 15‰ in *Pinus edulis* to higher values of up to 19.5‰ in *Quercus gambelii* (Fig. 3). These woodland species maintained their relative ranking of Δ values between the two years, indicating that interspecific differences in time-integrated c_i/c_a were relatively stable. Intraspecific differences among individuals showed a similar tendency to maintain Δ rankings through time (Fig. 3); leaves formed in consecutive years were positively correlated among individuals in *Juniperus* ($r=0.55$, $n=27$, $P<0.01$), *Quercus* ($r=0.56$, $n=33$, $P<0.001$) and *Pinus* ($r=0.65$, $n=25$, $P<0.001$).

Of the three woodland species, only *Quercus* Δ values were significantly correlated to growing season ω values for leaves formed in both 1993 and 1994. This Δ vs. ω correlation was negative, indicating that c_i/c_a declined and intrinsic water-use efficiency increased in this species as the monthly-weighted vapor pressure gradients increased (Fig. 4, Table 2). *P. edulis* and *J. osteosperma*, however, exhibited no such decline in Δ with increased ω among sites (Fig. 4, Table 2).

The Δ values in Juniperus were significantly and positively correlated with summer precipitation in 1993 ($r=0.97$, $P<0.01$) and showed a similar tendency in 1994 ($r=0.70$, $P=0.19$) (Table 2). No consistent trends between Δ and either summer or total water year precipitation occurred in the other two species.
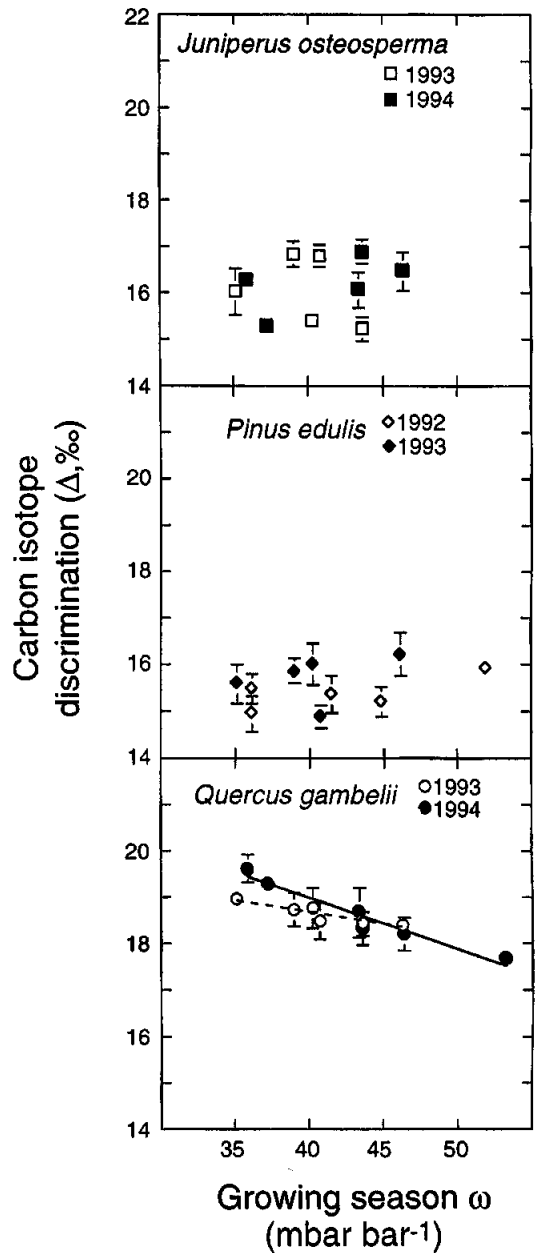
Discussion

In previous studies spanning a range of species, leaf carbon isotope composition (expressed as either $\delta^{13}C$ or Δ) has been shown to decrease in response to drier atmospheric humidity levels (Winter et al. 1982; Madhaven et al. 1991; Rundel and Sharifi 1993; Sharifi and Rundel 1993). Such a pattern is anticipated as stomata close in response to increased evaporative gradients (Schulze 1986). Comstock and Ehleringer (1992) further showed that there was ecotypic variation in the relationship between carbon isotope discrimination and ω, a measure of the seasonal evaporative atmospheric humidity gradient,

Table 2 Correlations between carbon isotope discrimination and climate variables for leaves of woodland species sampled along a summer monsoon gradient in Arizona and Utah. Data are Pearson product-moment correlation coefficients (r) and significance

	Juniperus osteosperma	*Pinus edulis*	*Quercus gambelii*
1992 leaves			
Total precip. 1991	–	0.75ns	–
Summer precip. 1991	–	0.81ns	–
v 1991	–	0.84ns	–
ω 1991	–	0.70ns	–
Total precip. 1992	–	0.42ns	–
Summer precip. 1992	–	0.43ns	–
v 1992	0.89*	–	
ω 1992	–	0.93*	–
1993 leaves			
Total precip. 1992	0.88*	−0.20ns	−0.35ns
Summer precip. 1992	0.99***	−0.16ns	−0.40ns
v 1992	−0.26ns	0.33ns	−0.86*
ω 1992	−0.10ns	0.37ns	−0.91*
1993 leaves			
Total precip. 1993	−0.17ns	−0.42ns	0.49ns
Summer precip. 1993	0.97**	−0.08ns	−0.44ns
v 1993	0.77ns	0.20ns	−0.57ns
ω 1993	0.82ns	0.02ns	−0.41ns
1994 leaves			
Total precip. 1993	−0.21ns	–	−0.06ns
Summer precip. 1993	0.70ns	–	−0.91*
v 1993	0.43ns	–	−0.84*
ω 1993	0.56ns	–	−0.97**
Total precip. 1994	−0.53ns	–	−0.42ns
Summer precip. 1994	0.52ns	–	−0.53ns
v 1994	−0.90*	–	−0.33ns
ω 1994	0.53ns	–	−0.84*

$*$ $P<0.05$, $**$ $P<0.01$, $***$ $P<0.001$, ns correlation coefficient not significantly different from zero

consistent with the idea that plants originating from climates with increased evaporative gradients during the growing season had reduced Δ values. Thus, we expected significant negative relationships between leaf carbon isotope discrimination and humidity gradients in the three semi-arid woodland species investigated in this study. However, we were able to confirm such a trend only for one of the three species. While *Quercus gambelii* exhibited geographic changes in Δ values in response to the evaporative gradient, no such trend was observed in either *J. osteosperma* or *P. edulis*. One common link between *Quercus* and those species whose leaf carbon isotope composition has previously been shown to vary with humidity is that they are dicots. *Juniperus* and *Pinus*, both gymnosperms and already exhibiting low Δ values, did not respond further to changes in atmospheric humidity estimated on either seasonal or annual bases.

Read and Farquhar (1991) observed that under common growth conditions, leaf Δ values were negatively correlated to growing-season precipitation for a number of *Nothofagus* species from throughout the southern hemisphere. Their interpretation was that *Nothofagus* species from drier climates had evolved greater hydraulic conducting capacity, allowing stomatal conductance, c_i/c_a and Δ to remain comparatively high during drought periods. Consistent with this pattern, we observed that Δ values in *Quercus* were negatively related to monsoon precipitation. However, since the humidity gradient experienced by *Quercus* and quantified by ω is weighted by monthly P/E_p, the ratio of precipitation to potential evapotranspiration, it is not surprising to find that Δ should decline as summer precipitation increases. Total water year precipitation was similar among sites, but the period of potential plant activity across the gradient (defined by P/E_p) and the transpiration potential integrated over that period (defined by ω) varied. Correlations between Δ and humidity for *Quercus* were more robust when humidity was defined as ω than when defined as v and averaged over the growing season. Negative correlations between Δ and precipitation are more easily resolved when interpreted with respect to ω.

The pattern of constancy in Δ as ω increased for *J. osteosperma* and *P. edulis* suggests that these species rely on alternative mechanisms to control transpiration across a gradient in evaporative potential. In addition to having considerable plasticity for needle size and leaf phenology, both of these conifers are known to add late season wood when monsoon precipitation arrives. This morphological flexibility expressed over the whole shoot and leaf canopy could bring about dynamic adjustments in whole plant hydraulic conductivity, allowing transpiration to vary widely over our experimental transect while stomatal conductance and c_i/c_a remain unchanged. Indeed, total leaf area tends to be greater in pinyon-juniper stands of the Great Basin where ω is low than in similar stands in the southern Colorado Plateau where ω is high (Meeuwig 1979; Schuler and Smith 1988; Grier et al. 1992). The desert shrub *Larrea tridentata*, like *Pinus* and *Juniperus* in this study, has a stable pattern of Δ across environments ranging from the Sonoran to the Chihua-

huan Deserts despite that $\delta^{13}C$ could be altered on the short term by irrigation (Rundel and Sharifi 1993). Alterations in canopy architecture and hydraulic conductivity can buffer variations in environment that influence leaf water status and Δ (Meinzer 1993). Hydraulic conductivity in plants is positively associated with Δ, and both Δ and hydraulic conductivity increase under irrigation (Meinzer et al. 1993).

Whereas *Pinus* and *Juniperus* are morphologically flexible, *Q. gambelii* has a fixed pattern of wood growth and leaf phenology. Late season wood production may have relatively little consequence to overall hydraulic conductivity in this ring-porous oak and leaf area established early in the growing season does not change appreciably when summer rains arrive (David G. Williams, unpublished work). Response to changes in transpiration demand for the morphologically inflexible *Q. gambelii*, therefore, depends on stomatal adjustments giving rise to the measured decline in Δ along the monsoon gradient.

Stomatal and morphological adjustments, however, could occur in an integrated fashion to meet the transpiration demands across broad climatic gradients. *Hymenoclea salsola* apparently is intermediate with respect to its capacity to adjust leaf conductance and shoot architecture as humidity varies across its range (Comstock and Ehleringer 1992). *Hymenoclea* produces both photosynthetic stems and leaves, but increases production of stem tissue relative to leaf tissue in summer wet (high ω) compared to summer dry (low ω) climates (Comstock and Ehleringer 1992). Since photosynthetic stems operate at lower c_i/c_a than do leaves (Comstock and Ehleringer 1988), the net effect of altering stem and leaf tissue proportions was that canopy level Δ varied, but water-use efficiencies simulated from Δ values remained constant across the native sites of the *Hymenoclea* populations. In this sense, plants display a continuum of responses to humidity ranging from predominantly leaf gas exchange to whole plant morphological adjustments, and the expression of these responses could determine productivity and survival along broad humidity gradients. *Q. gambelii*, *P. edulis*, and *J. osteosperma* may represent the full spectrum of these potential responses. Whether stomatal or morphological adjustments predominate may depend as much on phylogentic constraint as on trade-offs that may exist between leaf level and whole plant level flexibility.

References

Aphalo PJ, Jarvis PG (1991) Do stomata respond to relative humidity? Plant Cell Environ 14:127–132

Comstock JP, Ehleringer JR (1992) Correlating genetic variation in carbon isotopic composition with complex climatic gradients. Proc Natl Acad Sci 89:7747–7751

Ehleringer JR (1993) Carbon and water relations in desert plants: an isotopic perspective. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego, pp 155–172

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW (eds) Plant physiological ecology. Field methods and instrumentation. Chapman and Hall, London, pp 281–300

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Grantz DA (1990) Plant response to atmospheric humidity. Plant Cell Environ 13:667–679

Grier CC, Elliott KJ, McCullagh DG (1992) Biomass distribution and productivity of *Pinus edulis-Juniperus monosperma* woodlands of north-central Arizona. For Ecol Manage 50:331–350

Hales JE (1974) Southwestern United States monsoon source – Gulf of Mexico or Pacific Ocean? J Appl Meteorol 13:331–342

Madhavan S, Treichel I, O'Leary MH (1991) Effects of relative humidity on carbon isotope fractionation in plants. Bot Acta 104:292–294

MacMahon JA, Schimpf DJ (1981) Water as a factor in the biology of North American desert plants. In: Evans DD, Thames JL (eds) Water in desert ecosystems (US/IBP 12). Dowden Hutchinson and Ross, Stroudburg, pp 119–171

Meeuwig RO (1979) Growth characteristics of pinyon-juniper stands in the western Great Basin (Forest Service Research Paper INT-238). United States Department of Agriculture, Washington

Meinzer FC, Goldstein G, Grantz DA (1993) Carbon isotope discrimination and gas exchange in coffee during adjustment to different soil moisture regimes. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant-carbon water relations. Academic Press, San Diego, pp 327–345

Monteith JL (1995) A reinterpretation of stomatal responses to humidity: theoretical paper. Plant Cell Environ 18:357–364

Mott KA, Parkhurst DF (1991) Stomatal response to humidity in air and helox. Plant Cell Environ 14:509–515

Read J, Farquhar G (1991) Comparative studies in *Nothofagus* (Fagaceae). I. Leaf carbon isotope discrimination. Funct Ecol 5:684–695

Rundel PW, Sharifi MR (1993) Carbon isotope discrimination and resource availability in the desert shrub *Larrea tridentata*. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant-carbon water relations. Academic Press, San Diego, pp 173–185

SAS Institute (1994) JMP user's guide, version 3 JMP. SAS Institute, Cary

Sharifi MR, Rundel PW (1993) The effect of atmospheric saturation deficit on carbon isotope discrimination in the desert shrub *Larrea tridentata* (creosote bush). J Exp Bot 44:481–487

Schuler TM, Smith FW (1988) Effect of species mix on size/density and leaf area relations in Southwest pinyon-juniper woodlands. For Ecol Manage 25:211–220

Schulze E-D (1986) Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil. Annu Rev Plant Physiol 37:247–274

Smith SD, Nowak RS (1990) Ecophysiology of plants in the intermountain lowlands. In: Pitelka LF, Osmond CB, Hudy GM (eds) Plant biology of the Basin and Range. Springer, Berlin Heidelberg New York, pp 179–241

Thornthwaite CW (1948) An approach toward a rational classification to climate. Geogr Rev 38:55–94

Winter K, Holtum JAM, Edwards GE, O'Leary MH (1982) Effect of low relative humidity on $\delta^{13}C$ value in two C_3 grasses and in *Panicum milioides*, a C_3-C_4 intermediate species. J Exp Bot 33:88–91