

# Seasonal variation in the carbon isotopic composition of desert plants

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## Summary

1. Carbon isotope discrimination ( $\Delta$ ) has been shown to be a valuable tool for long-term estimates of water-use efficiency, because this parameter is influenced by the ratio of intercellular to ambient  $\text{CO}_2$  levels, which is in turn related to water-use efficiency. The objective of this study was to examine variation in  $\Delta$ -values of field materials and the extent to which changes in  $\Delta$  might be interpreted as changes in water-use efficiency.

2. On-line discrimination observations with four desert species confirmed theoretically expected relationships between  $\Delta$  and the ratio of intercellular to ambient  $\text{CO}_2$  concentrations, a prerequisite to applying  $\Delta$ -values to water-use efficiency. Seasonal changes in  $\Delta$ -values were measured in 15 desert species over the course of two growing seasons in western Arizona.

3. Two major patterns appeared: in leaves of some species there were 2–3‰ changes during the year, whereas in others the changes in isotopic composition were less than 1‰.

4. When carbon isotope analyses are applied to water-use efficiency extrapolations, it has been assumed that leaf temperatures are equivalent among the species being compared. An error analysis of this assumption indicated a near-linear relationship between leaf temperature differential and the difference in isotopic composition necessary to distinguish differences in water-use efficiency. When leaf temperature differentials between plants were  $\leq 2.5^\circ\text{C}$ , a 1‰ difference in  $\Delta$  was sufficient to rank plants correctly and unambiguously with respect to water-use efficiency, indicating the extent to which changes in intercellular  $\text{CO}_2$  can offset possible differences in the evaporative gradient among plants when calculating water-use efficiencies.

5. As leaf  $\Delta$ -values among different species often range 4‰ on a given sampling date, carbon isotope discrimination appears to be a feasible approach for ranking relative water-use efficiency differences among arid-land plants.

*Key-words:*  $^{13}\text{C}/^{12}\text{C}$ , carbon isotope, desert ecology, Sonoran Desert, stable isotopes, transpiration efficiency, water stress, water-use efficiency

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## Introduction

In North American deserts, limited soil moisture may be the factor most restricting plant growth (Hadley & Szarek 1981; Ehleringer & Mooney 1983; Smith & Nowak 1990) and competition for this limited resource is thought to play an important role in structuring plant communities (Fonteyn & Mahall 1978; Fowler 1986). The ability of plants to adapt to limited moisture conditions is aggravated by the unpredictable nature of that precipitation (Ehleringer & Mooney 1983), which often results in extended periods through the growing season without appreciable moisture input or periodically 1 or

more years in succession with very little, if any, moisture input. Both the rate at which plants use soil moisture and the efficiency with which that moisture is used should be important determinants of plant fitness in desert conditions (Cohen 1970; Cowan 1982; Iwasa & Cohen 1989), yet little comparative information is available on long-term patterns because of past difficulties in acquiring such information for large numbers of species.

The measurement of carbon isotope composition ( $^{13}\text{C}/^{12}\text{C}$ ) has emerged as an approach to integrate ecophysiological processes, such as water-use efficiency, over time (Farquhar, Ehleringer & Hubick

1989; Rundel, Ehleringer & Nagy 1988). Carbon isotope discrimination ( $\Delta$ ) in  $C_3$  plants is related to photosynthetic gas exchange; because  $\Delta$  is in part determined by  $c_i/c_a$ , the ratio of  $CO_2$  concentrations in the leaf intercellular spaces to that in the atmosphere (Farquhar, O'Leary & Berry 1982; Farquhar & Richards 1984; Farquhar *et al.* 1989). This ratio,  $c_i/c_a$ , differs among plants because of variation in stomatal opening (affecting the supply rate of  $CO_2$ ), and because of variation in the chloroplast demand for  $CO_2$ . Of the models linking  $C_3$  photosynthesis and  $^{13}C/^{12}C$  composition, the one developed by Farquhar *et al.* (1982) has been the most extensively tested. In its simplest form, their expression for discrimination in leaves of  $C_3$  plants is:

$$\Delta = a + (b - a) \frac{c_i}{c_a} \quad \text{eqn 1}$$

where  $a$  is the fractionation occurring due to diffusion in air (4.4‰), and  $b$  is the net fractionation caused by carboxylation (mainly discrimination by RuBP carboxylase, approximately 27‰).

Since the carbon incorporated in leaves is assimilated over a considerable time period and under a range of environmental conditions, measuring  $\Delta$  provides a long-term average estimate of  $c_i/c_a$ , and therefore, is a long-term indicator of plant metabolism. While the expected relationship between  $\Delta$  and  $c_i/c_a$  has been supported for crop species and over various time scales (Farquhar *et al.* 1989), there are only limited data for native species (Farquhar *et al.* 1982; Brugnoli *et al.* 1988).

Carbon isotope discrimination can also provide a comparative measure of instantaneous water-use efficiencies and long-term transpiration efficiencies among species (Farquhar *et al.* 1989); the relationship between  $\Delta$  and instantaneous water-use efficiency is illustrated by the following equations:

$$E = \nu g \quad \text{eqn 2}$$

and

$$A = (c_a - c_i)g/1.6 \quad \text{eqn 3}$$

where  $\nu$  is the difference in water vapour concentrations between the leaf and atmosphere divided by total atmospheric pressure,  $g$  is the leaf conductance to water vapour and 1.6 is the ratio of gaseous diffusivities of  $CO_2$  and water vapour in air. Since transpiration ( $E$ ) and net carbon assimilation ( $A$ ) share a diffusion pathway, the stomata, instantaneous water-use efficiency can be determined without an estimate of  $g$ ,

$$\frac{A}{E} = \frac{c_a \left(1 - \frac{c_i}{c_a}\right)}{1.6\nu} \quad \text{eqn 4}$$

and the relationship between  $\Delta$  and  $A/E$  is apparent; both are a function of  $c_i/c_a$ . Transpiration efficiency ( $W$ ) is the long-term estimate of  $A/E$ , corrected for

respiratory losses and possible stomatal water loss at night. Therefore determination of  $\Delta$  provides a long-term measure of  $c_i/c_a$  which can be used to estimate  $A/E$  or  $W$  provided the following assumptions are met: (1) evaporative demand,  $\nu$ , is equivalent and known for the species and environments compared and (2) plants and/or species compared do not differ in the fraction of the carbon gain which is lost through respiratory processes (Farquhar & Richards 1984). Neither of these conditions are likely to be entirely met in a field study of many species. Although  $\Delta$ -values of leaf tissue are unlikely to provide a direct, quantitative comparison of water-use efficiencies, they should provide a relative index for ranking water-use efficiencies among species. Furthermore, calculations can be made to determine the extent to which errors arising from differences in leaf temperatures among species being compared affect interpretation of carbon isotope composition data.

Using carbon isotope composition, we evaluated community-wide patterns for 15 common Sonoran Desert species growing in western Arizona. Carbon isotope and water potential data were collected through the growing season to evaluate the extent to which species exhibited similar response patterns to temporal differences in soil moisture availability. For many of the species in this study, photosynthesis occurred in both leaves and twigs (Ehleringer, Comstock & Cooper 1987), which allowed us to determine whether or not canopy-level specialization in water-use efficiency occurred in response to drought.

## Materials and methods

### FIELD SITES AND SAMPLE COLLECTION

Plants were sampled at a desert site approximately 9 km west of Oatman, Arizona (lat. 34° 57' N, long. 114° 25' W, 785 m elevation). The vegetation of this area is transitional between Mojave Desert southwestern portion and the Sonoran Desert Lower Colorado Valley portion (Shreve & Wiggins 1964). The species measured grew in sandy washes and alongside them or on adjacent slopes with shallow gravelly soils. Leaf and photosynthetic twig samples were collected from each species at each sample date. For each species, a single bulked sample was obtained collecting three shoots from each of three different individuals. The species sampled were all perennials and fell into two distinct groupings: those with both photosynthetic twig and leaf tissues and those with leaves as the only photosynthetic tissue. The photosynthetic-twig species included *Bebbia juncea* Benth., *Chrysothamnus paniculatus* (Gray) Hall, *Gutierrezia sarothrae* (Pursh) Rusby, *Hymenoclea salsola* T.&G., *Porophyllum gracile* Benth.,

*Psilostrophe cooperi* (Gray) Greene, *Salazaria mexicana* Torr., *Senecio douglasii* D.C., *Sphaeralcea parvifolia* Nels. and *Stephanomeria pauciflora* (Torr.) Nutt. The species having non-photosynthetic twigs included *Acamptopappus sphaerocephalus* (Harv. & Gray) Gray, *Ambrosia dumosa* Gray, *Ambrosia eriocentra* Gray, *Encelia farinosa* Gray and *H. monogyra* Torr. & Gray.

Long-term air temperature records for water-use efficiency calculations were not available for the Oatman site. Mean maximum air temperatures at Oatman were calculated using data from nearby sites in Arizona (Davis Dam, Kingman, Parker Dam and Yucca) and correcting for elevational differences by regression analyses. The  $r^2$  coefficients to describe the amount of the variation accounted for by regression analyses of temperature against elevation for any monthly period were generally greater than 80%.

#### ON-LINE DISCRIMINATION

On-line carbon isotope discrimination was measured using an open gas-exchange system (Ehleringer 1983) coupled to a vacuum line similar to that described by Evans *et al.* (1986). Calculation of gas-exchange parameters, including  $c_i$  values, were as described by von Caemmerer & Farquhar (1981). Air exiting the gas-exchange cuvette had a  $\text{CO}_2$  concentration of near  $350 \mu\text{l l}^{-1}$  and the diverted portion of that airstream was passed through the vacuum line at  $2\text{--}3 \text{ ml s}^{-1}$  and collected for 10–15 min. The carbon isotopic composition of the  $\text{CO}_2$  was then determined on an isotope ratio mass spectrometer (model delta S, Finnigan MAT, San Jose, California, USA). Instrument precision was  $\pm 0.01\%$ . Repeated subsampling of the same gas stream yielded a precision of  $\pm 0.1\%$ . No correction was made for the presence of  $\text{N}_2\text{O}$  in air samples, since calculation of the correction factor would have resulted in less than a 0.3% change in  $\Delta$ -values. Carbon isotope discrimination by leaves within the cuvette was determined using equations presented in Evans *et al.* (1986). The  $\text{CO}_2$  differential of gases entering and leaving the cuvette averaged approximately  $80 \mu\text{l l}^{-1}$  (precision  $\pm 0.5 \mu\text{l l}^{-1}$ ). Thus, the precision of our on-line carbon isotope discrimination estimate was approximately  $\pm 0.8\%$ . Potted plants used in the on-line discrimination measurements were grown from seed under greenhouse conditions and with supplemental high-intensity-discharge lighting to achieve irradiances approximately natural conditions. The species sampled using on-line measurements included *Encelia farinosa* (drought-deciduous perennial), *Geraea canescens* T.&G. (annual), *Gutierrezia sarothrae* (photosynthetic-twig perennial), and *Larrea tridentata* (DC.) Cov. (evergreen perennial). Variations in the  $c_i/c_a$  ratio among plants were achieved by either exposing leaves to changes in the

$v$ -value or exposing the roots to chilling temperatures while keeping  $v$  constant.

#### CARBON ISOTOPE DETERMINATION

Carbon isotope ratios ( $\delta^{13}\text{C}$ ) were determined separately on leaf and photosynthetic-twig tissues. Leaf or twig tissue was dried and ground to pass through a 40-mesh screen. A 2- to 3-mg subsample of the tissue was combusted for 6 h at  $850^\circ\text{C}$  to produce  $\text{CO}_2$  in sealed, evacuated Vycor tubes containing cupric oxide and silver foil, and followed by cooling slowly to room temperature over an 8-h period. The  $\text{CO}_2$  produced by combustion was purified cryogenically and the  $\delta^{13}\text{C}$  value of the  $\text{CO}_2$  was measured on the isotope ratio mass spectrometer. Again, the instrument error (defined as twice the standard deviation) associated with each observation was  $\pm 0.01\%$ , and the error between repeated analyses of the same ground tissue was  $\pm 0.11\%$  (1 SD). Carbon isotope discrimination values ( $\Delta$ ) were calculated from carbon isotope ratios (against the PDB standard) using a value for  $\delta_{\text{air}}$  of  $-8\%$  (Farquhar *et al.* 1989).

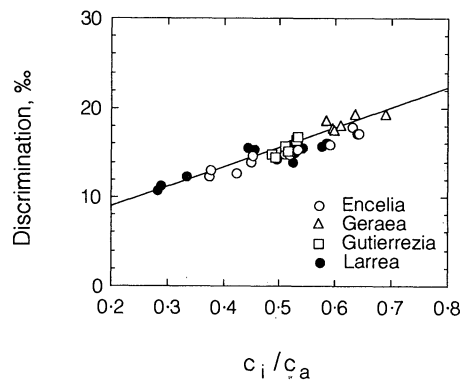
#### LEAF WATER POTENTIAL

Water potential was measured using a Scholander-type pressure chamber. Measurements were made on individual leaves when leaves were sufficiently large, and on short terminal twigs (with leaves attached) when leaves were very small. Measurements were made before sunrise on three to five plants.

## Results

#### ON-LINE MEASUREMENTS

The  $c_i/c_a$  ratio in leaves of plants of four desert species could be varied substantially when leaves were exposed to either changes in  $v$  or to root chilling (Fig. 1). Carbon isotope discrimination was measured simultaneously after leaves had achieved a steady-state  $c_i/c_a$  value. Over a broad range of  $c_i/c_a$  values, the measured values of carbon isotope discrimination agreed well with the theoretically expected relationship for  $\text{C}_3$  plants as described in equation 1 (Fig. 1). Regression analysis of the combined data set in Fig. 1 yielded values of 5.3 and 25.0% for  $a$  and  $b$  in equation 1, respectively. Given the precision of the on-line technique ( $\pm 0.8\%$ ), the calculated and theoretically expected values for  $a$  and  $b$  were not different from each other. These on-line gas-exchange data suggest that there is no reason to suspect that desert species behaved any different from theoretically expected relationships for  $\text{C}_3$  species and, therefore, that it is possible from field observations of  $\Delta$  in leaves to infer long-term  $c_i/c_a$  values.



**Fig. 1.** Observed carbon isotope discrimination from on-line gas-exchange measurements as a function of the simultaneously measured ratio of intercellular to ambient  $\text{CO}_2$  concentrations for *Encelia farinosa* (drought-deciduous shrub) ( $\circ$ ), *Gerarea canescens* (annual) ( $\triangle$ ), *Gutierrezia sarothrae* (winter-deciduous shrub) ( $\square$ ) and *Larrea tridentata* (evergreen shrub) ( $\bullet$ ). The line through the data represents the expected relationship based on equation 1.

#### SEASONAL DISCRIMINATION PATTERNS

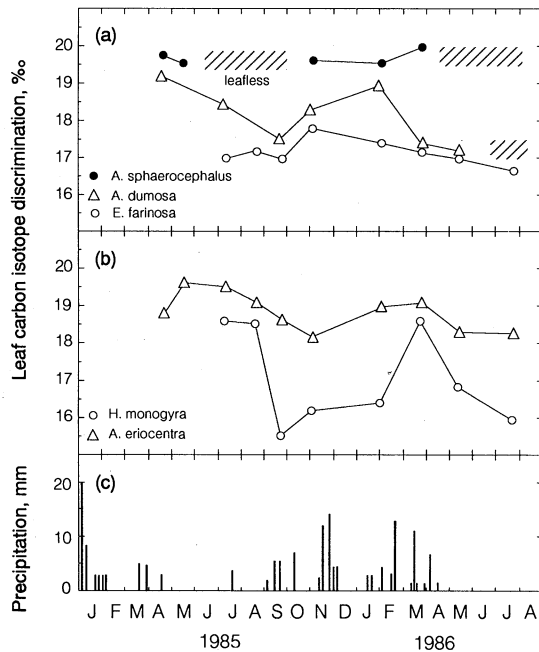
Maximum carbon isotope discrimination values ranged from a low of 17.8‰ in *E. farinosa* to a high of 21.0‰ in *Stephanomeria pauciflora* (Table 1). This range of values represents approximately a  $45 \mu\text{l l}^{-1}$  difference in intercellular  $\text{CO}_2$  concentrations (from equation 1). Minimum  $\Delta$ -values spanned an even larger differential, ranging from 14.6‰ in *Salazaria mexicana* to 19.5‰ in *Acamptopappus sphaerocephalus*. In Fig. 2, species are grouped by habitat. *Acamptopappus sphaerocephalus*, *Ambrosia dumosa*, and *E. farinosa* were slope species, while *Ambrosia eriocentra* and *H. monogyra* were wash species. Two distinct seasonal patterns of carbon isotope discrimination appear in the data (although data for only a limited subset are shown). For most species, there was a 2–3‰ seasonal change in carbon isotope discrimination (Table 1, Fig. 2). Yet for other species, such as *Acamptopappus sphaerocephalus* and *Encelia farinosa*, there were only small variations in  $\Delta$  through the growing season (Fig. 2). Seasonal  $\Delta$ -values tended to be highest in winter and early spring and lowest in autumn. Species with both photosynthetic twigs and leaves also exhibited pronounced seasonal fluctuations in their  $\Delta$ -values (Fig. 3). Variations of approximately 3‰ occurred in all photosynthetic-twig species (Table 1). The seasonal variation in  $\Delta$  among slope species was generally far less than that of wash species, especially so for those species with photosynthetic twigs. *Bebbia juncea* and *Stephanomeria pauciflora*, which obtain more than half of their annual carbon gain from twigs (Comstock *et al.* 1988), exhibited a seasonal cycle offset by several months from that of *C. paniculatus*, *H. salsola*, and *S. parvifolia*, which only obtain a quarter of their annual carbon gain from twigs

(Comstock, Cooper & Ehleringer 1988). Seasonal fluctuations in the  $\Delta$ -values of leaves and twigs for a given species were significantly correlated with each other for only three of the 10 species sampled, although the difference in leaf and photosynthetic twig  $\Delta$ -values were statistically significant in all species (Table 2). Some of this lack of statistical significance between tissue types may have been the result of limited sample periods, since correlation coefficients tended to be greater than 0.5 for half the species sampled. For *Gutierrezia sarothrae*, *Salazaria mexicana* and *Senecio douglasii*, leaf  $\Delta$ -values exhibited large seasonal fluctuations, whereas twig  $\Delta$ -values exhibited little change, resulting in the very low correlation coefficients (Table 2).

In 11 of the 15 species analysed, seasonal fluctuations in  $\Delta$ -values were not significantly correlated with pre-dawn leaf water potential (Table 1). The lack of a significant correlation may have arisen because data for a species from all sample periods were pooled together for each analysis. In some cases, this meant repeated sampling during the dry-down phase of a growing season (in which case a significant correlation would have been expected), whereas in other cases this meant that tissues were sampled immediately following rain events that trigger the new growing season, with possibly the bulk of the carbon derived from previous season's growth (in which case a significant correlation would not have been expected). Carbon isotope discrimination values tended to be greatest in the cooler winter and spring months. Yet when multiple regressions (incorporating both air temperature and water potential) were analysed, only for *Bebbia* was there any significant interaction between  $\Delta$  and temperature ( $P < 0.01$ ). In the other 14 species, there were no significant relationships between  $\Delta$  and temperature nor was temperature a significant covariate with water potential.

#### LEAF TEMPERATURE DIFFERENCES AND CALCULATED WATER-USE EFFICIENCIES

Separate from the climatological question of possible interactions between observed  $\Delta$ -values and seasonal temperatures (discussed above) is the question of how much does variation in leaf temperatures among species on a given sample date affect the actual  $A/E$  value. When comparing  $\Delta$ -values to estimate water-use efficiencies ( $A/E$  or  $W$ ), it is assumed that the leaf-to-air water vapour gradients are equivalent among species being compared (equation 4); this means that leaf temperatures must be equivalent among species being compared. In order to evaluate the impact of possible differences in leaf temperature ( $T_l$ ) on errors associated with estimating or comparing water-use efficiencies among species, two sets of calculations were made. These calculations addressed the impact of either changes in  $c_i/c_a$  or  $T_l$



**Fig. 2.** Seasonal leaf carbon isotope discrimination for common species occupying slope (a) and wash (b) microhabitats. (c) Distribution of precipitation at Needles, California (~20 km distant).

on  $A/E$  for any given sample period. That is, given that water-use efficiency is the ratio of two activities (equation 4), to what extent can changes in intercellular  $\text{CO}_2$  (numerator) offset possible differences in the evaporative gradient (denominator) among plants when calculating water-use efficiencies? Initial conditions assumed a leaf and air temperature of  $30^\circ\text{C}$ , a  $c_i/c_a$  value of 0.74 (a  $c_i$  of  $260\ \mu\text{l l}^{-1}$  in an environment of  $350\ \mu\text{l l}^{-1}$ ), and a relative humidity of 10%, although the results are robust enough to be generally applicable over the full range of annual temperature variation.

First, assuming that  $c_i$  remained constant, what difference would a change in  $T_l$  have on water-use efficiency? Under initial conditions, a leaf with a  $T_l$  of  $30^\circ\text{C}$  would have an  $A/E$  value of  $1.47\ \text{mmol mol}^{-1}$ . Decreasing or increasing  $T_l$  by  $2^\circ\text{C}$  and keeping  $c_i/c_a$  constant at a value of 0.74 would change  $A/E$  by 12–14% (Fig. 4). Deviations in leaf temperature were nearly proportional to changes in the expected differences in  $A/E$  over the short  $5^\circ\text{C}$  range, although over a broader range this relationship should be non-linear because of the exponential dependence of vapour pressure on temperature.

**Table 1.** Maximum and minimum carbon isotope discrimination values and correlations between carbon isotope discrimination and pre-dawn leaf water potential for different species throughout the year

Species	Tissue type	Maximum $\Delta$ (‰)	Minimum $\Delta$ (‰)		$n$
<i>Acamptopappus sphaerocephalus</i>	Leaf	20.0 (April)	19.5 (May)	0.276	4
<i>Ambrosia dumosa</i>	Leaf	19.1 (April)	16.8 (May)	0.197	5
<i>Ambrosia eriocentra</i>	Leaf	19.6 (May)	18.2 (November)	0.297	10
<i>Bebbia juncea</i>	Leaf	19.4 (May)	16.9 (November)	0.410	5
	Twig	17.6 (July)	15.6 (March)	0.683*	9
<i>Chrysothamnus paniculatus</i>	Leaf	20.8 (May)	17.8 (January)	0.389	10
	Twig	20.1 (May)	16.4 (July)	0.706*	10
<i>Encelia farinosa</i>	Leaf	17.8 (November)	16.7 (July)	0.537	7
<i>Gutierrezia sarothrae</i>	Leaf	20.0 (January)	17.2 (August)	0.717*	10
	Twig	18.5 (July)	15.6 (May)	-0.375	10
<i>Hymenoclea monogyra</i>	Leaf	18.5 (March)	15.5 (September)	0.358	8
<i>H. salsola</i>	Leaf	19.6 (April)	16.6 (November)	0.587*	10
	Twig	18.5 (January)	15.6 (March)	0.176	10
<i>Porophyllum gracile</i>	Leaf	20.6 (March)	17.9 (November)	0.084	6
	Twig	19.0 (May)	16.6 (July)	0.152	10
<i>Psilostrophe cooperi</i>	Leaf	20.8 (May)	18.0 (November)	-0.141	5
	Twig	20.0 (May)	16.5 (March)	0.000	10
<i>Salazaria mexicana</i>	Leaf	18.7 (April)	17.2 (May)	0.480	4
	Twig	18.8 (November)	14.6 (May)	0.000	9
<i>Senecio douglasii</i>	Leaf	19.0 (July)	16.0 (January)	0.375	7
	Twig	18.1 (May)	15.6 (April)	0.141	9
<i>Sphaeralcea parvifolia</i>	Leaf	20.6 (September)	18.2 (November)	0.126	9
	Twig	19.7 (January)	16.1 (September)	0.002	10
<i>Stephanomeria pauciflora</i>	Leaf	21.0 (May)	17.8 (November)	0.373	6
	Twig	19.6 (July)	17.1 (November)	-0.255	9

$n$  is the number of dates when tissues were available for sampling. An asterisk indicates that the correlation was statistically significant at the  $P < 0.05$  level; all other correlations are non-significant.  $r$  is the correlation coefficient ( $\Psi \times \Delta$ ).

**Table 2.** Correlations between carbon isotope discrimination ( $\Delta$ ) and the mean difference between leaf and photosynthetic twig  $\Delta$ -values

Species	$\Delta_{\text{leaf}} - \Delta_{\text{twig}}$	$n$	
<i>Bebbia juncea</i>	0.752	1.72*	5
<i>Chrysothamnus paniculatus</i>	0.733*	1.08*	10
<i>Gutierrezia sarothrae</i>	0.138	1.11*	10
<i>Hymenoclea salsola</i>	0.311	1.03*	10
<i>Porophyllum gracile</i>	0.933*	1.31*	6
<i>Psilostrophe cooperi</i>	0.834*	0.98*	5
<i>Salazaria mexicana</i>	0.002	1.69*	4
<i>Senecio douglasii</i>	0.114	0.47*	7
<i>Sphaeralcea parvifolia</i>	0.324	1.25*	9
<i>Stephanomeria pauciflora</i>	0.550	1.21*	6

An asterisk indicates that the correlation was statistically significant at the  $P < 0.05$  level; all other correlations are non-significant. The difference between paired leaf and twig  $\Delta$ -values was tested for significance using a Student's  $t$ -test.  $n$  is the number of sampling dates when both leaves and photosynthetic twigs were present for each respective species.

Second, if leaf temperatures differed between two plants, how much would  $\Delta$ -values have to differ so that  $A/E$  could be unambiguously ranked? Again, using a 30°C leaf as the starting point, decreasing or increasing  $T_l$  by 2°C would require that  $\Delta$ -values differ by more than 0.7–0.8‰. Further deviations in leaf temperature would result in nearly proportional changes in the expected differences in  $\Delta$  over a 4–6°C temperature range. That is, to differentiate unambiguously water-use efficiency rankings among species, a 1‰ difference in  $\Delta$ -values would allow one to reliably rank species if leaf temperatures differed by  $\leq 2.5^\circ\text{C}$  and a 2‰ difference would be required if leaf temperatures differed by 5°C.

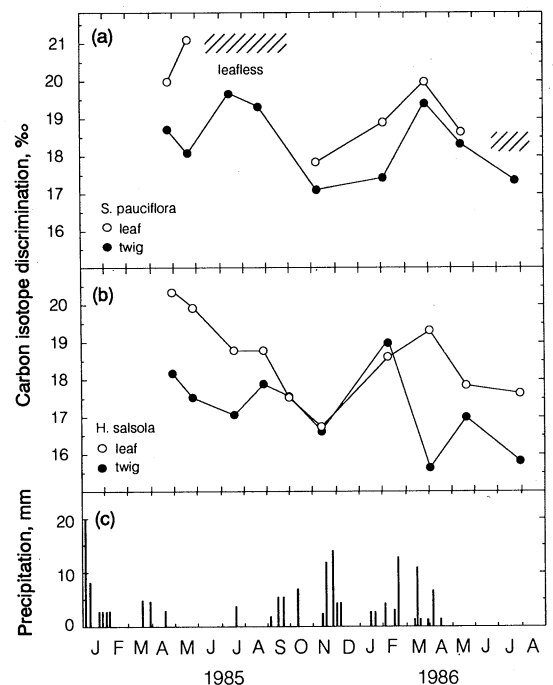
If  $\Delta$ -values measured on tissues produced at two times of the year were identical (as in *Acamptopappus sphaerocephalus* in Table 1), but air and leaf temperatures had changed between the two sampling dates, how much would absolute water-use efficiencies differ? This analysis is identical to the first question posed. That is, if  $\Delta$ -values were identical,  $A/E$  should change by approximately 5% °C<sup>-1</sup> (Fig. 4). In the case of *Acamptopappus sphaerocephalus*, where  $\Delta$ -values remained at approximately 20‰,  $A/E$  is calculated to have changed from 3.0 to 1.7 mmol mol<sup>-1</sup> between early winter and late spring sampling dates due only to changes in the evaporative gradient.

## Discussion

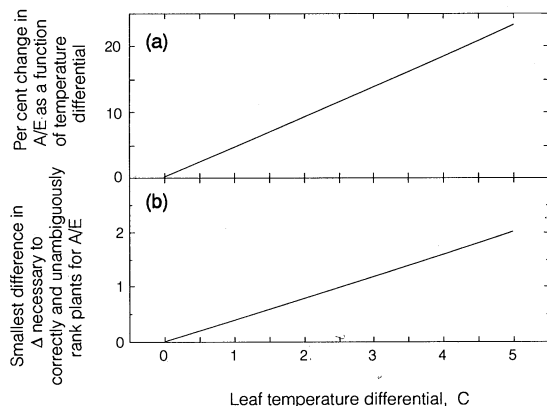
### $\Delta$ AND $A/E$

The fundamental assumption that leaf  $\Delta$ -values of desert species reflect  $c_l/c_a$  values was supported by gas-exchange observations, allowing inference of long-term  $c_l/c_a$  values from leaf  $\Delta$  observations. The assumption that leaf temperatures are equivalent

among species and/or cultivars when  $\Delta$ -values are compared to evaluate possible  $A/E$  differences has not been examined by previous studies. Yet numerous studies have observed tight, significant correlations between  $\Delta$  and  $A/E$  (reviewed by Farquhar *et al.* 1989), leading one to expect that  $\Delta$  is a useful measure of  $A/E$ . One reasonable conclusion from these studies is that, as leaf temperatures among plants often did not differ by more than 2–4°C when leaves were actively photosynthesizing, then the impact of possible differences in  $v$  were minimal. In deserts, a number of studies have examined leaf temperature variation among species. The general conclusion is that leaf temperatures among species do not depart from one another by more than 2–3°C (Gibbs & Patten 1970; Smith 1978). Thus, if  $\Delta$ -values among leaves of desert species in the same environment differ by more than 0.7–0.8‰ (Fig. 4), it is reasonable to conclude that  $A/E$  values will also differ. There are, however, important situations in which plants with extremely high transpiration rates (Smith 1978; Althawadi & Grace 1986), large differences in leaf absorptance (Ehleringer & Mooney 1978), or extremes in the microclimate profile with height above the ground (Ehleringer 1985) have been compared. In those situations, differences in leaf temperature of up to 10°C have been reported, making it difficult to assume that  $\Delta$  and  $A/E$  would be inter-convertible under all comparative situations.



**Fig. 3.** Seasonal leaf carbon isotope discrimination for leaves and twigs of *Stephanomeria pauciflora*, a herbaceous species with photosynthetic twigs (a) and *Hymenoclea salsola*, a woody species with photosynthetic twigs (b). (c) Distribution of precipitation at Needles, California (~20 km distant).



**Fig. 4.** (a) The percentage change in water-use efficiency resulting solely from an incremental increase in leaf temperature. (b) The smallest difference in carbon isotope discrimination necessary to distinguish correctly and unambiguously water-use efficiencies of two different plants given the incremental changes in leaf temperature in (a). Both sets of calculations assumed that the initial temperature was 30°C, relative humidity was 10%, and that  $c_i/c_a$  was 0.74.

#### SEASONAL VARIATION IN $\Delta$

Mooney, Troughton & Berry (1974), Philpott & Troughton (1974), Szarek & Troughton (1976), Ehleringer (1988) and Toft, Anderson & Nowak (1989) reported a broad range of carbon isotopic composition values for North American desert plants. Despite the common perception that  $C_4$  plants are frequent in deserts, the vast majority of species possessed  $C_3$  photosynthesis. In the present work, it was only in the drier regions or regions with summer rains that CAM and  $C_4$  plants became more common. Limited seasonal-course carbon isotope data are also available. Troughton *et al.* (1977) observed that carbon isotope ratios ( $\delta$ ) increased (carbon isotope discrimination decreased) in several *Dudleya* species as soils dried out in spring. *Dudleya* shift between  $C_3$  and CAM photosynthetic modes, depending on drought, and thus their data are not directly comparable to those in this study. Smith & Osmond (1987) followed the isotopic composition in leaves and photosynthetic stems of *Eriogonum inflatum*, a short-lived  $C_3$  perennial in the Sonoran Desert. They observed an increase in carbon isotope ratios ( $\delta$ ) (carbon isotope discrimination decreased) through the growing season, analogous to the patterns reported in the present study. In similar studies, Lowdon & Dyck (1974) studying maple trees in a deciduous forest ecosystem and Smedley *et al.* (1991) studying grassland species in an arid-land steppe both reported a decrease in carbon isotope discrimination as the growing season progressed.

From previous studies, lower isotopic discrimination appears to be associated with plants growing under stress. Mooney *et al.* (1974) reported isotope values suggesting progressively lower  $c_i$  values for

plants growing in drier climates along transects through Baja California of North America and the Atacama Desert of South America. Ehleringer & Cooper (1988) reported that Sonoran Desert species growing on dry slopes discriminated less than species growing in the relatively wetter wash microhabitats, and in a study directly assessing water stress, Guy, Reid & Krouse (1980) showed a linear relationship between isotopic discrimination and water potential in two halophytic species. Thus, it was surprising that there was not a more clear pattern between  $\Delta$  and leaf water potential for the different species in this study. The most likely explanation is that the carbon used to produce new growth following rain is derived from stored carbon, which had been laid down at the end of the previous growing season. Therefore, plants at the beginning of the growing season exhibited what appeared to be a lower  $\Delta$ -value. From the data in Figs. 2 and 3, there was a lag between an increase in the carbon isotope discrimination by leaf tissues and the initial rain events, supporting the notion of a stored-carbon noise which made it difficult to evaluate directly the plants' response to water stress. However, when seasonal  $\Delta$ -values are combined with observations of canopy development by these same species (Comstock *et al.* 1988),  $\Delta$ -values were generally greatest before the periods of highest relative carbon gain. Comstock *et al.* (1988) noted that peak carbon gain of wash and slope species was offset  $\sim 2$  months, which is similar to the lag in  $\Delta$ -values in Fig. 2 and Table 1. Similarly, species with twig photosynthesis (largely species occupying wash habitats) had their greatest rates of carbon gain delayed into late spring as indicated in Fig. 3.

#### $\Delta$ AND ADAPTIVE FORM

The three slope species represented two contrasting drought-deciduous patterns. *Acamptopappus sphaerocephalus* had high absorptance green leaves and maintained those leaves only during the wettest periods of the year. The  $\Delta$ -values remained essentially constant whenever leaves were present, suggesting that *Acamptopappus sphaerocephalus* leaves may have maintained a constant  $c_i/c_a$  ratio until water deficits appeared. Then, in response to that stress, leaves were shed. In contrast, both *Ambrosia dumosa* and *Encelia farinosa* have a reflective pubescent leaf covering, although the reflectance is much greater in *Encelia farinosa* than *Ambrosia dumosa*. These two species also had lower  $\Delta$ -values through the season, and thus apparently higher water-use efficiencies, than *Acamptopappus sphaerocephalus*. The reflective pubescence is expected to have decreased leaf temperatures of *Ambrosia dumosa* and *Encelia farinosa* below that of *Acamptopappus sphaerocephalus*, increasing possible  $A/E$  differences between the green- and grey-leaved species. This water-conserving pattern by grey-leaved species may

have contributed to their ability to persist longer into drought periods. Corresponding with this pattern, *Acamptopappus sphaerocephalus* is thought to be shorter lived than either *Ambrosia dumosa* or *Encelia farinosa*, and Ehleringer & Cooper (1988) have shown that  $\Delta$ -values were inversely related to plant longevity for desert shrubs.

There were consistent differences in the carbon isotope discrimination by leaves and twigs in photosynthetic-twig species. Carbon isotope discrimination by leaves was always greater than that of twigs, suggesting a lower stomatal diffusion limitation in leaves than twigs. Calculated intercellular  $\text{CO}_2$  values (from equation 1) for leaves and photosynthetic twigs differed by  $7\text{--}26\ \mu\text{l l}^{-1}$ . These data are similar to isotopic composition differences observed in photosynthetic twig species by Ehleringer *et al.* (1987) and to actual intercellular  $\text{CO}_2$  concentration differences measured by Comstock & Ehleringer (1988). In response to long-term drought, photosynthetic-twig species either abscise their leaves entirely or abscise leaves at a much greater rate than twigs (Fig. 3, Comstock *et al.* 1988). One interpretation of this response is that, during drought when water availability is decreased, these species shift the distribution of photosynthetic tissues (through abscission) so that a greater proportion of the carbon is gained by a more water-use efficient tissue.

#### INFERRED WATER-USE EFFICIENCY PATTERNS

Are the seasonal fluctuations in  $\Delta$ -values large enough to compensate for the expected temperature variation and its effect on  $\nu$  and therefore on water-use efficiency? As a first approximation towards addressing this issue, the seasonal  $A/E$  was calculated using observed  $\Delta$ -values and assuming that (1) leaf and air temperatures were equivalent and (2) midday relative humidity was 10%. These calculations indicate that changes in temperature (which affect  $\nu$ ) overwhelm any potential physiological response by the plant to maintain a constant  $A/E$  value (Fig. 5), even though plants appear to decrease  $\Delta$  as water availability declines. Undoubtedly plants will shift their diurnal patterns of transpiration in response to changes in air temperature and water availability, and this may ameliorate the annual variations in  $A/E$ . Yet these calculations show that over the course of the year, absolute water-use efficiencies of desert plants will be influenced more by changes in  $\nu$  than  $\Delta$ . The highest absolute water-use efficiencies occur during the coolest periods of the year when it is unclear why conservative water use would be critical to survival or to growth. Given the highly variable seasonal temperatures in deserts and the limits to which physiological changes in plants can occur, it may be that absolute water-use efficiencies provide little ecological insight, but that it is the relative water-use efficiency among plants that

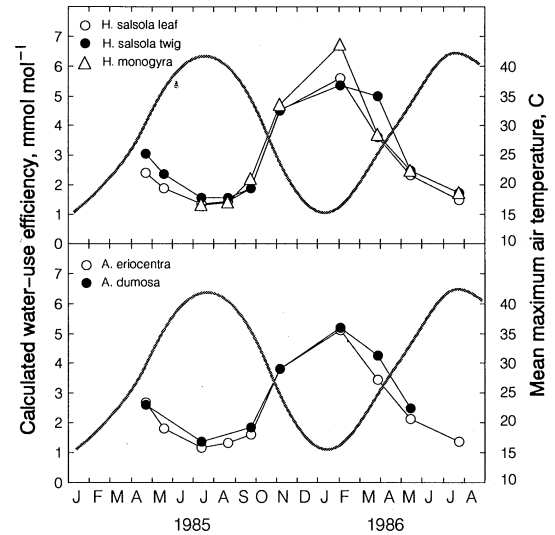


Fig. 5. Calculated absolute water-use efficiency values for carbon isotope discrimination data in Figs. 2 and 3 using mean maximum temperature data for Oatman, Arizona (sinusoidal grey line). (a) A comparison of two closely related wash-habitat species — one with (*Hymenoclea salsola*) and the other lacking (*H. monogyra*) photosynthetic twigs. (b) A comparison of two closely related species — one occupying wash habitats (*Ambrosia eriocentra*) and the other slope habitats (*Ambrosia dumosa*).

is the more relevant factor. In that regard, the observed 3‰ difference in  $\Delta$ -values during the spring growing season, should result in a greater than 20% difference in  $A/E$  and could be important for plant performance. If there are opposing growth and survival advantages associated with different relative water-use efficiency values, then natural selection would be expected to act on the variation in  $\Delta$  as it represents the differential response of plants when exposed to similar environmental conditions.

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