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HISTORICAL VARIATIONS IN $\delta^{13}\text{C}_{\text{LEAF}}$ OF HERBARIUM SPECIMENS IN THE SOUTHWESTERN U.S.

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and Peter K. Van de Water^{1,4}

ABSTRACT.—The uncontrolled, global increase in atmospheric CO_2 concentration (ca 80 ppmv) and decline in $\delta^{13}\text{C}_{\text{air}}$ (ca 1.5‰) since industrialization provide experimental boundary conditions by which to assess physiological response of vegetation. To examine consequences of these global atmospheric changes in the southwestern U.S., 350 specimens of *Atriplex confertifolia*, *A. canescens*, *Ephedra viridis*, *Pinus edulis*, *P. flexilis*, *Juniperus scopulorum*, and *Quercus turbinella* of precisely known age spanning the last 150 years were acquired from 9 herbaria. Cellulose analysis of $\delta^{13}\text{C}_{\text{plant}}$ and estimation of isotopic discrimination (Δ) permitted calculation of water-use efficiency (A/g). The $\delta^{13}\text{C}_{\text{plant}}$ chronologies of *C*₄ *Atriplex* spp. show some promise as a reliable proxy for $\delta^{13}\text{C}_{\text{air}}$ because their mean trends approximate the known $\delta^{13}\text{C}_{\text{air}}$ chronology. However, the high variability would necessitate multiple samples at any time period to accurately represent the mean. The generally increasing A/g trends of the 5 *C*₃ species are particularly pronounced for *P. edulis* and, after the 1950s, for *J. scopulorum*, but less evident for *P. flexilis*, *E. viridis*, and *Q. turbinella*, evidencing possible differences in species response to rising CO_2 concentrations. The trends are statistically noisy, however, possibly due to complex microclimates, extreme seasonality, and great interannual variability typical of the southwestern U.S. Herbarium specimens, at least in the Southwest, may be less useful for precise detection of direct CO_2 effects on plant physiology than tree rings, where the variability can be constrained to a single individual over time.

Key words: carbon isotopes, herbarium specimens, leaves, southwestern USA, northern Arizona, atmospheric carbon dioxide, isotopic discrimination, ecophysiology.

Effective methodologies to better decipher past environments and corresponding ecophysiological responses of flora often include analysis of a variety of plant materials, e.g., tree rings (Leavitt and Long 1988, Bert et al. 1997, Feng 1998), plants preserved in sediments and woodrat middens (Marino et al. 1992, Van de Water et al. 1994), and historically archived plant samples (Woodward 1987). Leaves would seem to be a particularly useful substrate from which to extract such information because they are at the forward edge of plant-atmosphere interactions and because current plant stable-carbon isotopic fractionation models are based on leaves (O'Leary 1981, Farquhar et al. 1982). The availability of historical sequences of leaves of known provenance, grown in natural settings under changing atmospheric environments, could help resolve important questions regarding the capacity of plants to maintain "set point" regulation (homeostasis) of ecophysiological parameters (Wong et al. 1979, Polley et al. 1993, Ehleringer and Cerling 1995,

Marshall and Monserud 1996), e.g., internal CO_2 concentration (C_i), ratio of internal to atmospheric CO_2 concentration (C_i/C_a), difference between internal and external CO_2 concentrations ($C_a - C_i$), and intrinsic water-use efficiency, A/g (where A is the rate of photosynthesis and g is the rate of stomatal conductance).

Herbarium leaves offer promise for assessing plant environmental response over the last several hundred years, the last 200 of which have been characterized by profound global-scale changes in atmospheric chemistry. Fossil-fuel consumption and land-use changes have driven an atmospheric CO_2 concentration increase of ca 30% (from ca 280 to 360 ppmv; Barnola et al. 1995) and a corresponding ^{13}C depletion in atmospheric CO_2 ($\delta^{13}\text{C}_{\text{air}}$) from ca -6.5‰ to -8.2‰ (Friedli et al. 1986, Keeling et al. 1989, 1995; Fig. 1). Leaves archived in herbarium collections in Europe from this time interval have already been exploited to

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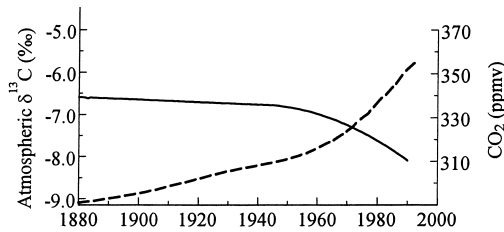


Fig. 1. Atmospheric changes in CO_2 (dashed line) and $\delta^{13}\text{C}$ (solid line) as determined from measurements of air trapped in bubbles of ice cores and direct atmospheric measurements (after Bert et al. 1997).

track effects of changing CO_2 as recorded in a stomatal density/stomatal index decline of ca 20–40% since 1800 A.D. (Woodward 1987, 1993, Peñuelas and Matamala 1990, Paoletti and Gellini 1993).

More recently, $\delta^{13}\text{C}$ of herbarium leaves has been used in efforts to further expose and define the influence of these global atmospheric changes. Peñuelas and Azcón-Bieto (1992) measured a $\delta^{13}\text{C}_{\text{plant}}$ shift of ca -1‰ in the averaged isotopic composition of C_3 leaves of several species from herbarium collections that was consistent with fossil-fuel effects, and a decrease in plant isotope discrimination, Δ (the difference between atmosphere and plant $\delta^{13}\text{C}$; see Methods). The $\delta^{13}\text{C}$ trend from 2 C_4 species was less uniform but also suggested decreased discrimination. Woodward (1993) measured $\delta^{13}\text{C}$ in herbarium leaves for the 8 species of British trees initially sampled for stomatal density (see Woodward 1987). $\delta^{13}\text{C}_{\text{leaf}}$ generally declined during the past 250 years, though there was considerable scatter attributed to marked annual variations in weather. $\delta^{13}\text{C}_{\text{leaf}}$ decline may be influenced by the continual depletion of $\delta^{13}\text{C}_{\text{air}}$ and/or an increase in water-use efficiency (calculated as A/g , rather than instantaneous water-use efficiency, A/E , where E is the transpiration rate) from leaf responses to atmospheric CO_2 enrichment. Woodward (1993) calculated A/g from $\delta^{13}\text{C}_{\text{leaf}}$, using historical trends in $\delta^{13}\text{C}_{\text{air}}$ and C_a in the computation, and found that A/g increased 28% over the past century, most likely through increases in C_a . Beerling et al. (1993) also found that $\delta^{13}\text{C}$ declined from -24‰ to -30‰ in *Salix herbacea* leaves collected in England between 1819 and 1985. They felt the increasing Δ represented by the large $\delta^{13}\text{C}$ decrease was consistent with decreasing

stomatal density in *S. herbacea* found by Beerling and Woodward (1993) because stomatal density and Δ are both “surrogate” indicators of water-use efficiency, responding to increasing atmospheric CO_2 . Toolin and Eastoe (1993) also analyzed $\delta^{13}\text{C}$ of herbarium specimens and modern samples of C_4 *Setaria* grass species, their results falling within the range of other records of recent $\delta^{13}\text{C}_{\text{air}}$ changes. Most of these herbarium $\delta^{13}\text{C}$ and stomatal density studies have been characterized by limited numbers of leaf samples for individual taxa and few replicates for particular years or decades. Also, many herbarium studies do not present or plot measurements from individual taxa. Instead, they plot multispecies averages of actual measurements or relative differences to represent historical trends in stomatal density and $\delta^{13}\text{C}_{\text{leaf}}$ (Woodward 1987, 1993, Peñuelas and Matamala 1990, Peñuelas and Azcón-Bieto 1992, Paoletti and Gellini 1993). Kelly and Woodward (1995) advocate multispecies approaches in plant isotopic studies, which we also endorse in the sense of identifying and understanding differential species responses. Simple multispecies averaging, however, is likely to confound rather than enhance detection of leaf responses to environmental forcing because of different lengths of growing season, different above- and belowground architecture, different strategies for coping with drought, etc.

This study examines variations in $\delta^{13}\text{C}$ (and other related ecophysiological parameters) of leaves from several species representing different functional types (e.g., C_3 vs. C_4 , angiosperms vs. gymnosperms) and growth forms within a confined area of the southwestern part of North America since 1850 A.D. Because of the large number of samples analyzed (each time series represents ca 50 $\delta^{13}\text{C}$ measurements per taxon), the response of isotopic parameters to changing atmospheric composition can be better recognized and quantified.

METHODS

Herbarium specimens from northern Arizona and northwestern New Mexico were targeted for analysis (Fig. 2), with the majority of samples coming from either the Mogollon Rim or the Colorado Plateau physiographic provinces. A spreadsheet containing the age, geographic location, and isotopic results for each

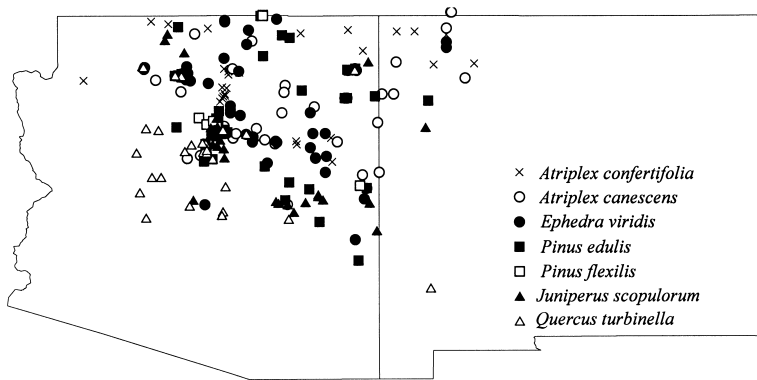


Fig. 2. Map of Arizona and New Mexico showing collection sites for herbarium specimens by species.

sample is available at http://www.paztcn.wr.usgs.gov/betancourt_table.html. The Rim is a topographic break oriented southeast to northwest between the Basin and Range Province (and Sonoran Desert) to the south and the Colorado Plateau to the north. The herbarium samples from the Rim come mostly from the deep valleys of the Verde River and its tributaries, which drain the Rim area south of Flagstaff. The Colorado Plateau consists of wind-swept mesas punctuated locally by volcanic terrain (e.g., the San Francisco Peaks near Flagstaff) and deeply incised, steep-walled canyons in nearly horizontal sedimentary formations.

We chose leaves of 7 species available from 9 herbaria to canvas 2 photosynthetic pathways: the C_4 pathway represented by the halophytes *Atriplex canescens* and *A. confertifolia*, and the C_3 pathway represented by the gymnosperms *Ephedra viridis*, *Pinus edulis*, *P. flexilis*, and *Juniperus scopulorum*, and the C_3 angiosperm *Quercus turbinella*. Herbarium specimens were selected to encompass a limited geographic and altitudinal range (a few degrees of latitude and longitude and less than ~ 1000 m difference in elevation) with a target of 50 specimens spanning 100–150 years for each species. For environmental uniformity, $\delta^{13}C_{\text{leaf}}$ studies should select leaves only from flowering shoots, with the assumption that they develop under a high irradiance from the outside of the tree crown (Woodward 1993). We note that botanists generally select flowering shoots in the field because most taxonomic studies require flowers. For convenience, botanists preferentially sample the outside of the plant's crown.

Four of our taxa (*A. confertifolia*, *A. canescens*, *E. viridis*, and *Q. turbinella*) are low shrubs less than 1.5 m tall; we are confident that most herbarium specimens from these species represent sun leaves from the top of the crown. The conifers (*P. flexilis*, *P. edulis*, and *J. scopulorum*) are relatively low (< 5 m) trees with open crowns and would have been sampled from the outside of the crown at 1.5–2 m heights. Desert scrub and woodland communities in this semiarid region are by nature open canopy; shading may be less confounding to leaf anatomy and physiology than in forested communities in more humid regions.

For *A. confertifolia* and *A. canescens*, we randomly selected 5 to 10 leaves from the herbarium specimens or from collections of previously detached leaves. Leaves were cut in half longitudinally along the central leaf vein, providing half for initial analysis and the remainder for replacement material if needed. We randomly chose 10 needles from multiple cohorts on single branches on *P. edulis* and *P. flexilis* herbarium sheets. One-third of the distal portion of each needle was removed and pooled into a single sample. The distal portions of 5 to 10 *J. scopulorum* twigs and 5 to 10 terminal joint segments from each *E. viridis* were likewise pooled. The *E. viridis* samples were cut in half perpendicular to their long axis. We removed 5 leaves for *Q. turbinella* from each sheet and cut them longitudinally along the central leaf vein. Leaf samples from living vegetation were collected in 1996 for some of the species from the Oak Creek Canyon area south of Flagstaff, Arizona.

The leaf material was milled to 20-mesh, soluble organic compounds were extracted with toluene and ethanol in a soxhlet extraction apparatus, and the remaining plant matter was delignified in a 70°C, acidified sodium chlorite solution (Leavitt and Danzer 1993) to obtain holocellulose. C_4 plant holocellulose was treated for 2 days with 10% HCl (after Baker 1952) to dissolve oxalates prior to combustion and analysis. The holocellulose was combusted with CuO in evacuated quartz tubes at 900°C for 2 hours and at 650°C for 2 hours (Boutton 1991). CO_2 was cryogenically collected and purified on a vacuum extraction line within 48 hours of combustion. It was then analyzed with a mass spectrometer (Finnigan Delta-S[®]), and $\delta^{13}\text{C}$ ($= [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}) - 1] \times 1000$) calculated with respect to the international PDB standard (Craig 1957). Repeated analysis of a spruce wood holocellulose standard with the C_3 samples gave a standard deviation of 0.13‰ ($n = 32$), whereas repeated analysis of a sucrose standard with the C_4 samples gave a standard deviation of 0.35‰ ($n = 24$). In this study the lower precision on the C_4 standard should not restrict interpretations because inter-plant variability turned out to be approximately 10 times higher.

Whole-tissue $\delta^{13}\text{C}$ values used in calculating the physiological parameters were obtained by subtracting 1.5‰ (Van de Water et al. 1994) and 2.6‰ (Van de Water 1999) from holocellulose $\delta^{13}\text{C}$ values for C_3 and C_4 plants, respectively. We estimate these conversion factors are accurate to about $\pm 0.5\%$. $\delta^{13}\text{C}_{\text{air}}$ and CO_2 concentrations (C_a) were derived from a spline fit of ice core and direct atmospheric measurements (Bert et al. 1997; Fig. 1) and were used to calculate discrimination (Δ), C_i/C_a , C_i , and A/g from the following relationships (Farquhar et al. 1982, O'Leary 1993):

$$\Delta(\text{‰}) = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}) / (1 + (\delta^{13}\text{C}_{\text{plant}}/1000)) \quad (1)$$

$$\delta^{13}\text{C}_{\text{C}_3 \text{ plant}} (\text{‰}) = \delta^{13}\text{C}_{\text{air}} - a - (b - a)C_i/C_a \quad (2)$$

$$\delta^{13}\text{C}_{\text{C}_4 \text{ plant}} (\text{‰}) = \delta^{13}\text{C}_{\text{air}} - a - (b_4 + b_3\phi - a)C_i/C_a \quad (3)$$

$$A/g = (C_a - C_i) / 1.6 \quad (4)$$

wherein a is the fractionation by diffusion into the stomata (4.4‰), b and b_3 are the fractionation caused by RuBP carboxylation (reported as ca 27–30‰; 27‰ and 29‰, respectively, are used in this study), C_i is the concentration of CO_2 in the intercellular leaf space, C_a is concentration of CO_2 in the air, b_4 is PEP-C

fractionation (–5.7‰, slightly temperature-dependent), ϕ is the fraction of CO_2 initially fixed in mesophyll by PEP-C that is decarboxylated in the bundle sheath cells but lost by diffusion before it can be fixed by RuBP, A is the assimilation rate, g is the leaf stomatal conductance to water vapor transfer, and 1.6 is the ratio of diffusivities of water vapor and CO_2 in air. Leakiness (ϕ) values range from 0.1 to 0.6, with no recorded values greater than 0.6 (Sandquist and Ehleringer 1995). For species subtypes, ϕ tends to be greater in dicots than among other C_4 subtypes (Farquhar 1983). In this study we used ϕ of 0.6 for all calculations involving C_4 species because in many cases lower ϕ values produced numerous unreasonable results such as $C_i/C_a > 1$ and negative A/g .

Third-order polynomial regressions do not have a mechanistic basis, but they were deemed flexible enough to identify trends in isotopic time series for this analysis and were especially useful to capture trends despite the scatter. These polynomials were sufficiently flexible to reveal isotopic changes in the last 50 years when exponential changes in atmospheric CO_2 and $\delta^{13}\text{C}_{\text{air}}$ have been most pronounced. Linear regressions of $\delta^{13}\text{C}$ with latitude, longitude, and altitude of each herbarium sample were employed to identify any geographical influences on each species.

RESULTS

Only 2 significant correlations of $\delta^{13}\text{C}$ with latitude, longitude, or elevation emerged, both at $P < 0.05$ with *J. scopulorum* (not shown). The 1st was a positive relationship of $\delta^{13}\text{C}$ with elevation ($r = 0.40$; regression slope = 0.0023% m^{-1}), and the 2nd was an inverse relationship with longitude ($r = -0.43$; regression slope = -0.034% $\text{degree longitude}^{-1}$). The relationship with longitude may be random, although the *J. scopulorum* sites generally are not at constant latitude but progressively lower latitudes moving eastward. Such a NW–SE alignment could imprint a signal of percent summer monsoon precipitation (versus annual total precipitation), which increases in the same direction. However, $\delta^{13}\text{C}$ is increasing toward the southeast, opposite of what might be expected if summer precipitation is increasing in that direction. The occurrence of depleted values at lower elevations

may result from a riparian association rather than from any overarching environmental gradients usually resulting in warmer, drier microclimate at progressively lower elevations. The distribution of $\delta^{13}\text{C}$ with elevation was strongly bimodal, with widely scattered values (range of -22‰ to -27.5‰) above 1700 m and depleted values (-24.5‰ to -27.5‰) below 1700 m. The most likely explanation is that below 1700 m *J. scopulorum* is restricted to riparian settings such as Oak Creek near Sedona, Arizona; trees in these settings were essentially being watered by permanent streams and would not reflect water stresses typical at lower elevations. Fortunately, sample elevations were distributed randomly throughout the *J. scopulorum* $\delta^{13}\text{C}$ chronology and would therefore be unlikely to force any trend in the chronology. Furthermore, in a separate study also on the Colorado Plateau, we measured $\delta^{13}\text{C}$ variations with elevation in modern populations of several species (including *P. edulis*, *E. viridis*, *A. confertifolia*, *A. canescens*). We found $\delta^{13}\text{C}$ variations with elevation to be largely nonsystematic (Van de Water 1999).

Figure 3 contains the leaf whole-tissue $\delta^{13}\text{C}$ chronologies for each species converted from holocellulose values. $\delta^{13}\text{C}$ values of *P. edulis* were constant through the time period, *P. flexilis* and to some extent *J. scopulorum* showed downward trends, and *A. confertifolia*, *A. canescens*, *Q. turbinella*, and *E. viridis* showed particularly pronounced $\delta^{13}\text{C}$ decreases after ca 1950. Discrimination (Fig. 4) increased markedly for *A. canescens* and *P. flexilis*. *Pinus edulis* exhibited clearly decreasing discrimination while the other species generally did not show a consistent long-term trend, although *A. confertifolia* and *E. viridis* exhibited an upturn after 1950 while discrimination of *J. scopulorum* declined. For A/g, *P. edulis* and *Q. turbinella* showed long-term increases, whereas *A. canescens* declined (Fig. 5). The other species showed limited long-term trends, although *P. flexilis* and *J. scopulorum* increased after 1950.

DISCUSSION

Altitude and Latitude Effects

Despite lack of significant correlations with altitude/latitude, there may yet remain altitude and latitude effects embedded in all of our isotopic results. For example, Körner et al. (1991) showed a $\delta^{13}\text{C}$ gradient of -0.036‰

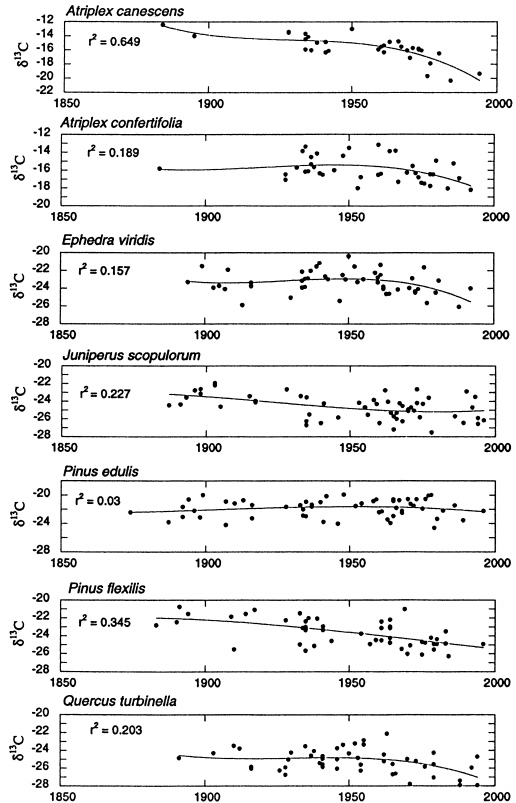


Fig. 3. Chronologies of herbarium leaf whole-tissue $\delta^{13}\text{C}$ for each of 7 species.

per degree of latitude for perennial herbaceous species growing across a span of 70° from the tropics to the poles. If this gradient is applied to the herbarium data in this study, which span at most 4° latitude, the latitude effect alone would make the most northerly samples only 0.14‰ more negative than the most southerly samples. Since this is well within our analytical error, any latitudinal influence should be negligible.

The effect of altitude on plant $\delta^{13}\text{C}$ is not fully understood and has been variously ascribed to temperature and partial pressures of CO_2 and O_2 ($p\text{CO}_2$ and $p\text{O}_2$; Körner and Diemer 1987, Körner et al. 1988, 1991, Morecroft and Woodward 1990, Kelly and Woodward 1995). Körner et al. (1988) found a $\delta^{13}\text{C}$ gradient of $+1.2\text{‰ km}^{-1}$ altitude irrespective of life form (trees, shrubs, forbs). This may be a consequence of lower C_i/C_a ratios at higher altitude (Körner and Diemer 1987, Körner et al. 1988). The mechanism suggested for the

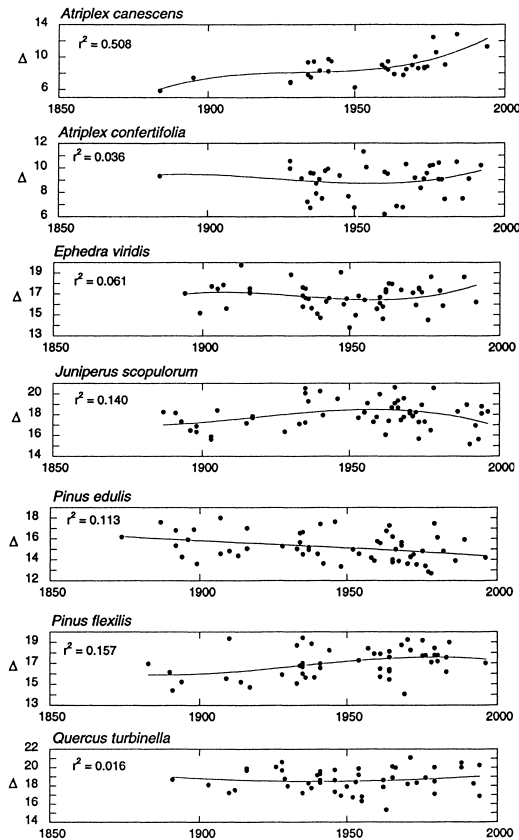


Fig. 4. Chronologies of herbarium leaf whole-tissue discrimination (Δ) for each of 7 species.

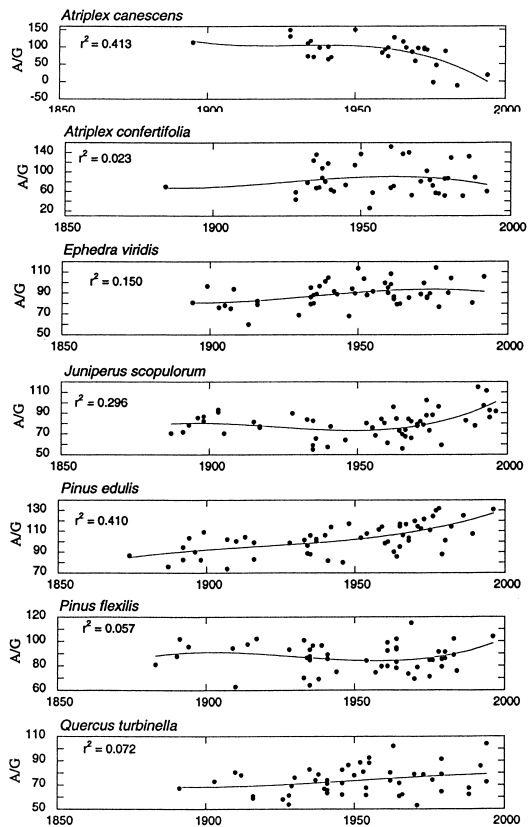


Fig. 5. Chronologies of herbarium leaf whole-tissue A/g (intrinsic water-use efficiency) for each of 7 species. These patterns would be identical to C_a-C_i plots.

decrease in C_i/C_a was higher carboxylation efficiency at higher altitudes. In contrast, Friend et al. (1989) determined that $\delta^{13}\text{C}$ either decreased or did not change at all with increasing altitude. Morecroft and Woodward (1990) found a similar relationship but concluded that, averaged over the globe, plants may show increasing $\delta^{13}\text{C}$ values with altitude although locally the slope could be negative, perhaps influenced by environmental factors such as soil water supply and water vapor pressure deficits. Kelly and Woodward (1995) reanalyzed data from Körner et al. (1988) and found that $\delta^{13}\text{C}$ actually does vary with life form, with more negative $\delta^{13}\text{C}$ values in taller plants. Kelly and Woodward (1995) concluded that differences in pCO_2 and pO_2 are sufficient to explain observed differences in carbon isotope composition. Vitousek et al. (1990), however, suggested that increasing leaf diffusion resis-

tance at higher elevations could cause the higher $\delta^{13}\text{C}$ values. Van de Water (1999) analyzed $\delta^{13}\text{C}$ of several taxa along 2 mountain gradients (1200–2600 m) in the southwestern USA. He found that most taxa exhibited either a slight decrease or no significant change in $\delta^{13}\text{C}$ with altitude. The depletion with altitude, compared to data sets elsewhere in the world, may be because isotopic discrimination decreases and A/g increases with water stress at more arid, lower elevations.

Within a given species, herbarium samples from altitudes 1000 m apart were not uncommon, and in a few cases up to 1500 m. If a gradient of $+1.2\text{‰ km}^{-1}$ altitude is applicable to this study, then elevation could have some measurable influence on $\delta^{13}\text{C}$ results. We did not apply altitude corrections, however, because (1) for all species, elevations were randomly distributed throughout the range of

ages of the herbarium samples; (2) there were no significant correlations of $\delta^{13}\text{C}$ of herbarium specimens with elevation (except *J. scopulorum*); (3) other field evidence indicates either no trend or decreasing $\delta^{13}\text{C}_{\text{leaf}}$ with altitude in the southwestern USA (Leavitt and Long 1983, Lajtha and Getz 1993, Van de Water 1999); and (4) there are large uncertainties in size and magnitude of any universal altitude effect.

C₄ Plants and Atmospheric $\delta^{13}\text{C}$

The case has been made (Francey 1986, Marino and McElroy 1991, Marino et al. 1992) that analysis of C₄ plants would permit exact reconstruction of past $\delta^{13}\text{C}_{\text{air}}$ when $\phi = 0.37$ because $\delta^{13}\text{C}_{\text{plant}}$ would be independent of C_i/C_a and discrimination would be constant (4.4‰); thus, $\delta^{13}\text{C}_{\text{plant}}$ would only be a function of $\delta^{13}\text{C}_{\text{air}}$. Small (up to 20%) deviations from $\phi = 0.37$ would produce fairly accurate reconstructions of $\delta^{13}\text{C}_{\text{air}}$, even if C_i/C_a was poorly known. Despite the scatter, the mean *Atriplex* herbarium trends generally follow the trend of atmospheric $\delta^{13}\text{C}$ changes, except for the pronounced negative departure of *A. canescens* after 1960. Both species, however, exhibit considerable isotopic variability, with a range of 3–4‰ among values within any given time interval. Thus, in the worst case, the value from an individual plant would be ca 2‰ from the average. If such a value had been used as a proxy for $\delta^{13}\text{C}_{\text{air}}$, then a 2‰ error in inferred $\delta^{13}\text{C}_{\text{air}}$ would translate to a ca 20% error in A/g for a C₃ plant. Clearly, multiple sampling and averaging of herbarium specimens of the same age or, by extension, packrat midden samples, could substantially reduce this error. The $\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}$ offset is ca 8.3‰ (not shown). This 8.3‰ offset is much larger than ca 4.4‰ expected if $\phi = 0.37$, suggesting $\delta^{13}\text{C}_{\text{plant}}$ values of these specimens are not independent of C_i/C_a .

The scatter in the $\delta^{13}\text{C}$ data for these 2 C₄ plants may be related to factors that influence ϕ and/or C_i/C_a . For example, Buchmann et al. (1996) found that variable light levels and water availability influenced discrimination of C₄ grasses in controlled-growth experiments, which they attributed to changes in ϕ when assuming a constant C_i/C_a . Sandquist and Ehleringer (1995) studied *A. confertifolia* along a salinity gradient and noted that salinity may

influence ϕ by disrupting membrane properties or the “coordination” of C₃ and C₄ cycles in the mesophyll and bundle sheath cells (Peisker and Henderson 1992). With increasing salinity, leakiness (ϕ) increased and discrimination increased as much as 2‰, perhaps related to changes in bundle sheath permeability to CO₂ and/or biochemical activity of the enzymes rubisco and PEP-carboxylase. In contrast, Peisker and Henderson (1992) found that C₄ plants became isotopically heavier as salinity increased, inferring that stomata close at high salt concentrations with attendant decrease in C_i/C_a . There is no relevant information on specific salinity, light, or moisture conditions associated with individual herbarium specimens, and thus no corrections can be made for these factors. This lack of local plant micro-environmental information is an inherent drawback of these herbarium studies.

A leakage factor of 0.6 was arbitrarily used to calculate ecophysiological parameters such as A/g. Had a more conventional leakage factor been assumed, such as the 0.37 used by Marino et al. (1992) for *A. confertifolia*, then many unrealistic values would have resulted (e.g., negative A/g). Even with this high leakage factor, a few of the ecophysiological parameters calculated for herbarium *A. confertifolia* are unrealistic (see web spreadsheet). Additional uncertainty arises because the factor we used to convert the original cellulose $\delta^{13}\text{C}$ values to whole-tissue $\delta^{13}\text{C}$ for set-point calculations has an estimated error of about $\pm 0.5\%$, which might have been avoided if the whole tissue had been analyzed directly. However, even if this had been done, there would remain uncertainties about movement of mobile organic compounds in and out of the leaf that were not representative of the leaf photosynthates at the time of manufacture. Also, the PEP-C fractionation term b_4 has a slight temperature dependence (Sandquist and Ehleringer 1995) that could also influence the results.

In view of the unusually high ϕ assumed in order to produce generally realistic ecophysiological parameters, the greatest utility of these inferred parameters may be in their patterns and trends rather than their absolute values. Additionally, the *Atriplex* species have among the most negative $\delta^{13}\text{C}$ values of C₄ plants, probably attributable to their general leakiness (Farquhar 1983).

Although both *Atriplex* species show declining $\delta^{13}\text{C}$, particularly pronounced after 1940–1960, the *A. canescens* data points lie much closer to their fitted 3rd-order polynomial. The steep negative slope of *A. canescens* is much greater than the actual change in $\delta^{13}\text{C}_{\text{air}}$ of ca 1.5‰ over this time period. The *A. confertifolia* $\delta^{13}\text{C}$ slope more closely approximates the actual change in $\delta^{13}\text{C}_{\text{air}}$, but the scatter about the mean is much higher. Additionally, ecophysiological parameters such as A/g show little change for *A. confertifolia* but large changes for *A. canescens*. Although the certainty of the trends of the calculated ecophysiological parameters is limited by assumptions in equations 1–4 already discussed (e.g., the whole-tissue $\delta^{13}\text{C}$ vs. cellulose $\delta^{13}\text{C}$ correction factor), we are comparing trends of what are our best estimates.

C₃ Plants

C₃ plants in this study represented 4 gymnosperms (*P. edulis*, *P. flexilis*, *J. scopulorum*, and *E. viridis*) and 1 angiosperm (*Q. turbinella*). Mean $\delta^{13}\text{C}$ values for each species show *P. edulis* as isotopically heaviest (mean = -21.84‰ , $s = 1.26\text{‰}$) and *Q. turbinella* lightest (-25.17‰ , 1.34‰). *Juniperus scopulorum* was the lightest gymnosperm (-24.67‰ , 1.36‰), yet only 0.56‰ heavier than *Q. turbinella*. *Pinus flexilis* and *E. viridis* were intermediate, with mean $\delta^{13}\text{C}$ values of -23.68‰ ($s = 1.50\text{‰}$) and -23.32‰ (1.26‰), respectively. Previous comparisons of wood cellulose $\delta^{13}\text{C}$ have suggested gymnosperms are from ca 1‰ (Leavitt and Newberry 1992) to 3‰ (Stuiver and Braziunas 1987) ^{13}C -enriched compared to angiosperms, and *Q. turbinella* is indeed more ^{13}C -depleted than all the gymnosperms except *P. flexilis*.

The atmospheric $\delta^{13}\text{C}$ chronology shows a decrease as atmospheric CO_2 increases (Fig. 1), and most of the C₃ species also show various $\delta^{13}\text{C}$ declines, the most pronounced exception being *P. edulis* for which $\delta^{13}\text{C}$ remains constant. The constant *P. edulis* $\delta^{13}\text{C}$ is in sharp contrast to the *P. edulis* tree-ring results from this region by Leavitt and Long (1988) that show a downward $\delta^{13}\text{C}$ trend. This discrepancy may be a consequence of (1) tree rings more effectively integrating the influence of numerous crown microenvironments, (2) the selection of trees from more open woodlands

in the tree-ring study, and (3) different (and unknown) average microenvironmental conditions of the trees sampled for herbarium specimens.

Herbarium leaves of *P. flexilis* are the most spatially restricted of the 7 species used in this study, with all but 2 samples coming from the San Francisco Peaks near Flagstaff, Arizona. Additionally, because 40 of 50 samples were from a narrow 2600- to 3000-m elevational range, environmental conditions should have been uniform for this suite of samples. *Pinus flexilis* does show the clearest $\delta^{13}\text{C}$ declining trend, although there remains considerable scatter of up to ca 5‰ among samples of similar age. Furthermore, the *P. flexilis* $\delta^{13}\text{C}$ decline was 2–3 times greater than the ca 1.5‰ decrease in $\delta^{13}\text{C}_{\text{air}}$. Long- and short-term climate variability in north central Arizona may account for some of these isotopic characteristics.

As expected, greater scatter than that observed in the *P. flexilis* $\delta^{13}\text{C}$ chronology was found in the other 4 C₃ species whose provenance is more extensive. In addition to long- and short-term regional climate effects, individual microsite variability may exist, such as riparian associations of low-elevation *J. scopulorum* trees. Intra-canopy and inter-tree leaf $\delta^{13}\text{C}$ variability in the amount of 1–2‰ has been previously observed in *P. edulis* and juniper (*Juniperus* spp.; Leavitt and Long 1983, 1986). The leaf $\delta^{13}\text{C}$ variability with height in the canopy may be an effect of variable light and respired CO_2 concentrations at different heights below the canopy top (Medina and Minchin 1980, Garten and Taylor 1992).

There is the possibility that climate variability influences some of the chronologies in Figures 3–5. For example, in Figure 3 the apparent plateau of elevated $\delta^{13}\text{C}$ values for *A. confertifolia* and *E. viridis* coincides with mid-century, below-average precipitation in this region and what was possibly the worst drought in the Southwest during the past several hundred years (Meko et al. 1993, Swetnam and Betancourt 1998). In addition to the longer-term climate influences, short-term episodes such as El Niño may contribute to year-to-year variation, which might be lost if only the best-fit smoothed polynomial curves are examined.

Although an original goal of sample acquisition, samples could not always be found every 2 or 3 years through this century-long time

frame. The earlier part of this century has fewer samples in the chronology of each species, thereby providing unequal weighting of the best-fit polynomial curves. The reliability of the early part of many of the chronologies will therefore generally not be as high as the more recent portion of the curves.

Discrimination should increase if the CO_2 increase promotes elevated C_i/C_a ratios. Only *P. flexilis* exhibits increasing discrimination, while *E. viridis*, *J. scopulorum*, and *Q. turbinella* have no consistent long-term trends, and *P. edulis* discrimination actually decreases during the past ca 100 years. Discrimination can also be particularly susceptible to water availability such that drought conditions can reduce discrimination (Leavitt and Long 1988) as stomata tend to stay closed longer to reduce water loss. In addition to CO_2 partial pressures and water stress, other environmental factors such as irradiance, relative humidity, vapor pressure deficit, temperature, and nutrient content may influence C_i/C_a and therefore discrimination (Farquhar et al. 1982, Tieszen 1991). These environmental factors are typically related to rates of stomatal conductance and the chloroplast demand for CO_2 (rates of assimilation) that contribute to C_i/C_a differences (Ehleringer and Cerling 1995).

An anticipated consequence of elevated CO_2 is higher A/g. Woodward (1987) found an overall reduction in stomatal density from 1787 A.D. to the present and concluded that during pre-industrial times, when atmospheric CO_2 levels were only ca 270 ppmv, maintaining CO_2 uptake would have been costly in terms of plant water loss. Reductions in stomatal conductance or leaf area might have helped reduce water losses (Woodward 1993). Polley et al. (1993) demonstrated increases in A/g in growth-chamber experiments in which CO_2 was varied from subambient (180 ppmv) to double (700 ppmv) present CO_2 levels. Among herbarium species, A/g increased in *P. edulis* over the full period of record. For the other species, patterns were less pronounced, but *P. flexilis* and *J. scopulorum* exhibited increasing A/g ($\approx C_a - C_i$) since 1950–1960. A previous study with ancient *P. flexilis* leaves (Van de Water et al. 1994), however, found that both C_i and $C_a - C_i$ increased during deglaciation as atmospheric CO_2 increased ca 80 ppmv. A tree-ring $\delta^{13}\text{C}$ study with other modern conifers by Marshall and Monserud (1996) showed that

$C_a - C_i$ remained constant for Douglas fir, ponderosa pine, and western white pine as both C_i and the C_i/C_a ratio increased with rising CO_2 . The recent work of Feng (1998) for several western conifer species showed most exhibited increasing C_i , but $C_a - C_i$ trends in the 20th century were variable among species. Feng (1999) also found a trend of increasing water-use efficiency in these trees. Thus, a clear, common homeostatic physiological response for A/g does not emerge, even among conifers alone, although influences other than CO_2 changes may vary in the other studies. The leaf studies, however, represent many different trees, whereas the tree-ring studies analyze a few individuals through time.

Comparing overall A/g results for the 5 C_3 species, it is possible to identify differences among gymnosperms and angiosperms. In this study *P. edulis* has the highest average A/g compared to the other C_3 plants. This is consistent with an observed link of high A/g with plants that rely heavily on summer precipitation, a less stable source of water (Flanagan et al. 1992). *Juniperus scopulorum* and *Q. turbinella* have the lowest average A/g; *J. scopulorum* grows in more mesic environments (more moisture at the higher elevations), which may contribute to its lower A/g, and *Q. turbinella*, as an angiosperm, characteristically has lower $\delta^{13}\text{C}$ (and lower A/g by inference) than gymnosperms. In addition, it has been observed that plants with access to groundwater tend to have a lower A/g than plants using a more unstable source of water like summer precipitation in the Southwest (Flanagan et al. 1992).

CONCLUSIONS

There was high isotopic variability in the herbarium leaf samples. Analysis of a single C_4 *Atriplex* plant leaf is inadequate to serve as a proxy for atmospheric $\delta^{13}\text{C}$ at any point in time, but analysis of many leaf samples of the same age will more accurately represent $\delta^{13}\text{C}_{\text{air}}$, especially if more environmental variables are held uniform, i.e., light, salinity, etc. Application of C_4 proxy $\delta^{13}\text{C}_{\text{air}}$ results to prehistoric C_3 plant $\delta^{13}\text{C}$ would greatly benefit calculation of their ecophysiological parameters. The herbarium results suggest *A. confertifolia* better tracks atmospheric $\delta^{13}\text{C}_{\text{air}}$ than does *A. canescens*, although the scatter for *A.*

canescens seems to be lower where the values best track the atmospheric $\delta^{13}\text{C}$ prior to ca 1968 A.D. Among C_3 species, strategies for maximizing photosynthesis and minimizing water loss appear to be species specific. Over the past 100–150 years, only *P. flexilis* and *J. scopulorum* exhibited increasing A/g, while A/g for *E. viridis*, *J. scopulorum*, and *Q. turbinella* remained nearly constant.

Previous herbarium studies often present decadal or multidecadal averages, in some cases averaging across species; e.g., Peñuelas and Azcón-Bieto (1992) displayed only 4 data points for the last 240 years, each point representing a pooling of all the C_3 species analyzed in the study. It may be difficult to make appropriate conclusions when many species are averaged together from various sites. Even where the species can be held constant, inherent variability in herbarium data may make it difficult to detect and explain trends.

Intra-tree and intra-site variability in tree rings is much smaller than the variability observed in these herbarium samples, which may offer an advantage for some studies. An inherent problem with herbarium specimens is that they represent a single plant for which the year of collection is known exactly, but the exact year in which the leaves were produced is generally not. As a result, it is unlikely that it represents variability across populations. In fossil deposits, like packrat middens, a population of leaves from a single species probably represents several individual plants across decades (Van de Water et al. 1994). Midden leaves probably integrate spatially and temporally, whereas each herbarium sheet represents a single individual. It is clear that herbarium studies may provide some relevant information on gas-exchange properties in plants, such as dramatic increase in A/g of *P. edulis*. However, caution is required in interpreting previous and future isotopic measurements and physiological calculations made with herbarium specimens.

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