

Lawrence B. Flanagan · Craig S. Cook
James R. Ehleringer

Unusually low carbon isotope ratios in plants from hanging gardens in southern Utah

Received: 19 January 1997 / Accepted: 19 April 1997

Abstract Leaf carbon isotope ratios ($\delta^{13}\text{C}$) and photosynthetic gas exchange were measured on plants growing in hanging garden communities in southern Utah, USA. Hanging gardens are unusual, mesic cliff communities occurring where water seeps from the sandstone bedrock in an otherwise extremely arid region; there is very limited overlap in species distributions inside and outside these gardens. Solar exposure in hanging gardens varied with orientation and one of the gardens (Ribbon Garden) was shaded throughout the day. The leaf $\delta^{13}\text{C}$ values of plants in hanging gardens were significantly more negative than for plants from either nearby ephemeral wash or riparian communities. In Ribbon Garden, the observed $\delta^{13}\text{C}$ values were as low as -34.8‰ , placing them among the most negative values reported for any terrestrial plant species growing in a natural environment. Hanging garden plants were exposed to normal atmospheric CO_2 with an average $\delta^{13}\text{C}$ value of -7.9‰ and so the low leaf $\delta^{13}\text{C}$ values could not be attributed to exposure to a CO_2 source with low ^{13}C content. There was a seasonal change toward more negative leaf $\delta^{13}\text{C}$ values at the end of the growing season. The observed leaf $\delta^{13}\text{C}$ values were consistent with photosynthetic gas exchange measurements that indicated unusually high leaf intercellular CO_2 concentrations associated with the relatively low light levels in hanging gardens. Thus, extremely negative leaf $\delta^{13}\text{C}$ values would be expected if significant amounts of the seasonal carbon gain occur at light levels low enough to be near the light compensation point. Maximum observed photosynthetic rates varied with light levels at

each of the gardens, with maximum rates averaging 20.3, 14.6, and $3.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ at Double Garden, Lost Garden, and Ribbon Garden, respectively. Leaf nitrogen contents averaged 18.5 mg g^{-1} in species from the more shaded hanging gardens (Lost and Ribbon). When expressed on a leaf area basis, nitrogen contents averaged $117 \text{ mmol N m}^{-2}$ at Lost Garden and 65 mmol N m^{-2} at Ribbon Garden (shadiest of the two gardens). Leaf nitrogen isotope ratios averaged -2.3‰ (range of -0.7 to -6.1‰), suggesting that most of the nitrogen was derived from a biological fixation source which is most likely the *Nostoc* growing on the sandstone walls at the seep. These values contrast with leaf nitrogen isotope ratios of $5\text{--}9\text{‰}$ which have been previously reported for arid zone plants in nearby ecosystems.

Key words Photosynthesis · Desert ecology · Stable isotopes · $^{13}\text{C}/^{12}\text{C}$ · Water use

Introduction

Hanging gardens are unusual plant communities growing perched on cliff walls common in the red-rock sandstone country of southern Utah, United States (Welsh 1989). These mesic plant assemblages occur where water seepage is exposed on vertical canyon walls in the extremely arid regions of the Colorado Plateau. Such water seeps occur at the junction where a harder sandstone (often Kayenta Sandstone), relatively impermeable to the passage of water, is overtopped by a more porous sandstone (usually Navajo Sandstone) (Welsh and Toft 1981). Water that percolates through the porous sandstone collects at the surface of the impervious rock, or in bedding planes within the Navajo sandstone, and moves laterally until it is exposed at a canyon wall or is able to pass through the impenetrable material at a fault line (see Fig. 1). The wet surface created on the canyon wall provides a favorable microhabitat for these unusual plants to colonize. Initially plants occur only on the steep, wet face of the cliff. However, over time the

L.B. Flanagan (✉)
Department of Biology, Carleton University, 1125 Colonel
By Drive, Ottawa, Ontario, K1S 5B6, Canada
Fax: (613) 520-4497; e-mail: lflana @ ccs.carleton.ca

C.S. Cook · J.R. Ehleringer
Stable Isotope Ratio Facility for Environmental Research,
Department of Biology, University of Utah, Salt Lake City,
UT 84112, USA

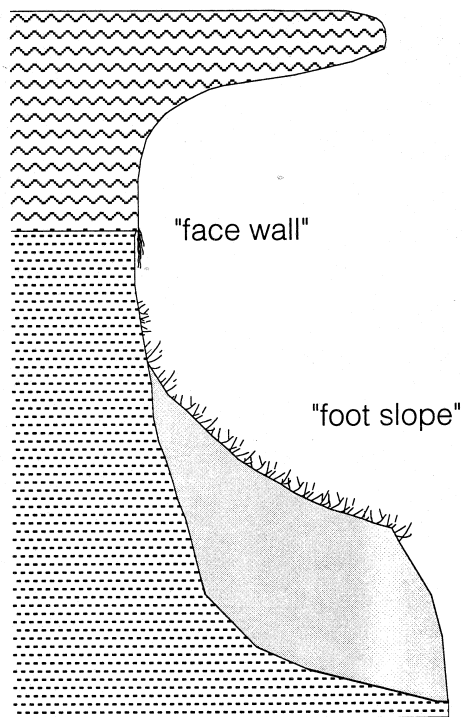


Fig. 1 Diagrammatic representation of the sandstone layers and plant distribution in the face and foot-slope portions of hanging garden communities in southern Utah, USA

activity of plant roots aids erosion of the exposed cliff face to create a shaded alcove with a foot slope below that has a deeper alluvial profile. Relatively lush herbaceous plant growth can occur in alcove hanging gardens, providing a sharp contrast to the sparse, xeric plant communities normally found in the surrounding areas (Welsh 1989). In larger gardens, woody species can be prominent. Species composition in hanging gardens is also quite distinct from the surrounding vegetation. Although a few hanging garden plants are endemic, most plants are commonly associated with cooler and more mesic habitats characteristic of regions far from these arid zones (Welsh 1989). As a result of the physical development of a particular hanging garden (exposure, orientation, rate of water supply), there may be a large range in the microhabitat and in environmental conditions among hanging gardens. It is the combination of a distinct non-arid zone flora in a distinctly arid ecosystem, undoubtedly susceptible to changes in water availability and exposed to contrasting environmental regimes, that make hanging gardens of particular interest.

Our objective was to gain an understanding of the range of microenvironmental conditions experienced by plants in hanging garden habitats, of the in situ gas exchange characteristics of these plants, and of the carbon isotope ratios of leaf tissue [to obtain time-integrated measurements of the ratio of CO_2 assimilation to stomatal conductance and leaf intercellular CO_2 concen-

tration (Farquhar et al. 1989)]. We make comparisons among five hanging gardens with contrasting solar exposures along the Colorado and San Juan Rivers in what is now Lake Powell, four of which have been previously described (Welsh and Toft 1981; Welsh 1989).

Methods and materials

Study sites

The study was conducted at hanging gardens in Glen Canyon National Recreation Area, Utah, United States, along the Colorado and San Juan Rivers in what is now Lake Powell. We concentrated our studies in five hanging gardens that had distinctly contrasting solar exposures: Death Camus, Three, Double, Lost, and Ribbon (ranked from the sunniest and most exposed to the shadiest and most enclosed). With the exception of Lost Garden, these gardens have been previously described in some detail by Welsh and Toft (1981) and Welsh (1989). Thus, only a brief description of each garden is included below. Three Garden ($110^{\circ}53'30''\text{W}$, $37^{\circ}11'58''\text{N}$, 1182 m elevation) consists of three superposed alcoves that occur approximately 1.5 km north of the confluence of the Glen Canyon and San Juan arms of Lake Powell (Welsh 1989). We sampled plants from the upper alcove, where they grew at water seeps that occur in the bedding planes within the Navajo Sandstone. Double Garden ($110^{\circ}54'06''\text{W}$, $37^{\circ}13'8''\text{N}$, 1152 m elevation) is located approximately 1 km west-northwest of Three Garden. Plants in Double Garden grew in a linear array along the bedding plane at the base of the Navajo Sandstone. Lost Garden ($110^{\circ}51'36''\text{W}$, $37^{\circ}17'48''\text{N}$, 1152 m elevation) was similar in structure to Double Garden, and was located on the north side of Glen Canyon 1 km upstream from the junction of the Escalante River with the Colorado River. Death Camus Garden ($110^{\circ}52'16''\text{W}$, $37^{\circ}11'54''\text{N}$, 1273 m elevation) is located in the San Juan River arm of Lake Powell, where the Navajo Sandstone rises above the level of Lake Powell. Death Camus is a large alcove, approximately 100 m wide and 50 m high, that was dry in the upper portion but had water seeps along the base of the garden, which consisted of impervious Kayenta Sandstone (Welsh 1989). Ribbon Garden ($110^{\circ}51'36''\text{W}$, $37^{\circ}14'56''\text{N}$, 1182 m elevation) is located in a large alcove on the south side of Ribbon Canyon near the entrance of the canyon into Glen Canyon.

We also sampled the common plant species growing in an ephemeral wash habitat adjacent to Lost Garden and riparian plants growing along Ribbon Creek in Ribbon Canyon.

Environmental measurements

During 12–14 June 1990, diurnal environmental measurements were made in Lost and Ribbon Gardens. Air temperature was measured with fine-wire copper-constantan thermocouples, air relative humidity with a capacitance humidity probe (RH and Temperature Probe 207, Campbell Scientific Inc., Logan, Utah, USA), and photon flux density with a quantum sensor (LI 190SB, LiCor Inc., Lincoln, Neb., USA). Each sensor was connected to a data logger (CR21X, Campbell Scientific Inc., Logan, Utah, USA), which scanned the sensors every second and averaged the readings for 30-min intervals throughout the day.

Stable isotope sampling

Leaf samples for carbon isotope ratio and nitrogen isotope ratio analyses were collected for each species as a single, bulked sample. The sample consisted of approximately five leaves from each of three to five individuals located in different areas of a garden, wash or riparian area. Species identification followed Welsh et al. (1989).

Atmospheric samples for CO₂ were collected in Lost, Ribbon, Double, and Death Camus Gardens during 20–22 April 1990 and again in Ribbon Garden during 21–22 September 1996. Air was pulled through tubing (Bev-a-line IV) into a 2-L (1990 observations) or a 100-ml (1996 observations) glass flask by a pump (TD-3LL Pump, Brailsford & Co. Inc., Rye, N.Y., USA). The inlet to the tubing was located approximately 0.5 m above ground in the center of a garden in 1990 and at 0.25 and 1.0 m height in 1996. After flushing the flask with air for approximately 20 min, the pump was turned off and the high vacuum stopcocks on the flask were closed. The flasks were returned to the laboratory for extraction of the CO₂ and measurement of its isotope ratio (Flanagan et al. 1996).

Isotope ratio and nitrogen analyses

Plant leaf tissue samples were dried and ground to a fine powder. The carbon isotope ratio of leaf tissue was determined by preparing the material with an in-vial combustion procedure (Ehleringer and Osmond 1989; Ehleringer 1991), using either a Finnigan-MAT delta E, delta S, or MAT 252 gas isotope ratio mass spectrometer. In addition, some leaf samples were analyzed using an elemental analyzer coupled directly to a dual-inlet mass spectrometer (model delta S). The 1996 analyses were all performed using the MAT 252 in a continuous flow mode and set so that $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and [N] could be obtained from the same sample run.

Atmospheric carbon dioxide was purified cryogenically from flasks and then injected into the mass spectrometer for analysis (Flanagan et al. 1996).

Isotopic compositions are expressed using delta notation in parts per thousand (‰):

$$\delta = \left[\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right] \times 1000$$

where R is the molar ratio of the heavy to light isotope ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$). The carbon isotope ratios of atmospheric CO₂ and plant organic samples are expressed relative to the PDB standard. The nitrogen isotope ratios are expressed relative to atmospheric air as the standard.

Photosynthetic gas exchange

Measurements of CO₂ and water vapor flux were made on intact foliage samples with a portable photosynthesis system (Li-Cor 6200, Li-Cor Inc., Lincoln, Neb., USA). During 20–22 April 1990 several measurements were made on selected species at midday in Lost, Ribbon and Double Gardens. Photosynthetic gas exchange measurements were also made at midday on pairs of three species located in both a shaded and a sunlit area of Ribbon Garden during June 15, 1990. Gas exchange measurements are expressed on an area basis. Leaf area was measured with a leaf area meter (Li-3100, Li-Cor Inc., Lincoln, Neb., USA).

Results

The environmental conditions in an enclosed shaded garden (Ribbon Garden) contrasted significantly with that observed for a relatively open garden (Lost Garden). The central area of Ribbon Garden received virtually no direct sunlight at any time of the day (Fig. 2). As a consequence, air temperatures were lower and relative humidity higher (Fig. 2) than at a location immediately adjacent, but outside the garden.

The 1990 $\delta^{13}\text{C}$ values for atmospheric CO₂ collected at 0.5 m height in the different hanging gardens were

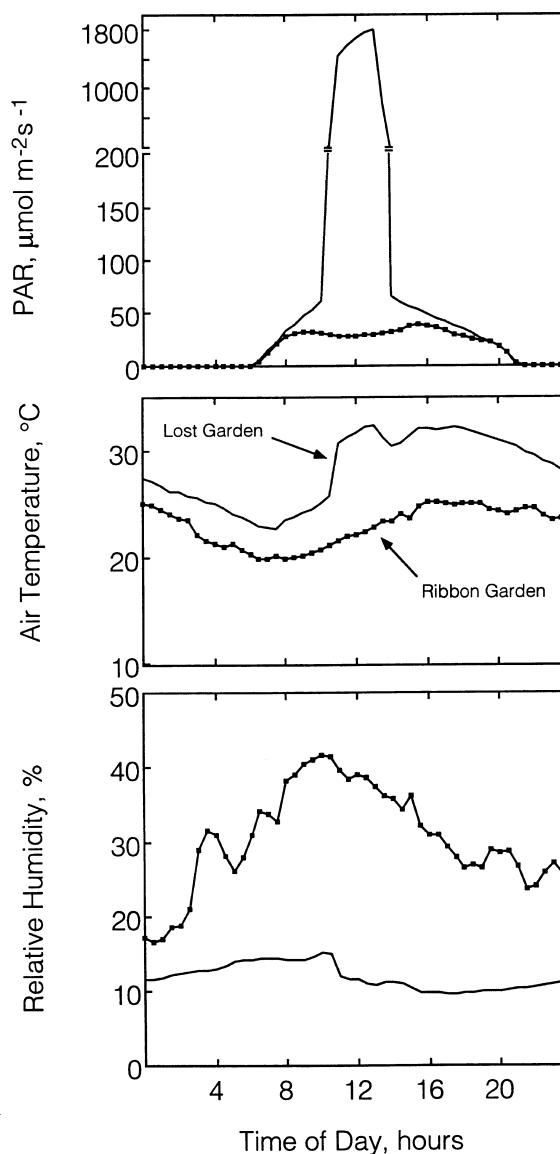


Fig. 2 Diurnal variation in meteorological conditions on 13 June 1990 in two hanging gardens contrasting in their solar exposure. Lost Garden (*solid line*) is an exposed, sunny garden and Ribbon Garden (*dotted line*) is an extremely shady, enclosed garden

–7.9‰ (Lost), –7.9‰ (Ribbon), –8.0‰ (Double), and –8.0‰ (Death Camus). These values are very similar to those reported for the isotopic ratios of atmospheric CO₂ collected at similar latitudes in remote, marine locations (Keeling et al. 1989), indicating that potential variations in the carbon isotope ratios of leaf tissues in the hanging garden were not affected significantly by CO₂ released from the soil, but should be primarily controlled by leaf photosynthetic gas exchange characteristics. We again measured the concentration and $\delta^{13}\text{C}$ values for atmospheric CO₂ at Ribbon Garden in September 1996. Atmospheric CO₂ concentration varied between 356 and 363 $\mu\text{l l}^{-1}$ over a 24-h period within the herbaceous vegetation layer (0.25 m height); there

Table 1 Comparison of the average carbon isotope ratio ($\delta^{13}\text{C}$, ‰) of common plant species occurring in several hanging gardens in Glen Canyon, Utah. The hanging gardens are ranked from the most exposed, sunniest garden (Death Camus) to the most enclosed, shadiest garden (Ribbon). Plants were collected in April 1990 and are separated into those occurring on the face or the foot slope of the hanging garden. Dashes indicate that the species was not present in that hanging garden

	Sunny, exposed			Shady, enclosed	
	Death Camus	Three	Double	Lost	Ribbon
Face					
Herbaceous					
<i>Adiantum capillus-veneris</i>	-26.7	-27.2	-28.1	-28.1	-29.1
<i>Mimulus eastwoodiae</i>	-	-30.6	-	-	-32.1
Foot slope					
Herbaceous					
<i>Aquilegia micrantha</i>	-	-	-	-28.4	-29.7
<i>Cirsium rydbergii</i>	-27.9	-28.4	-28.2	-29.9	-31.9
<i>Panicum acuminatum</i>	-25.8	-27.2	-27.3	-26.7	-
<i>Petrophytum caespitosum</i>	-28.4	-27.7	-28.4	-28.9	-30.5
<i>Primula specuicola</i>	-	-29.7	-28.8	-29.6	-
<i>Solidago sparsiflora</i>	-29.7	-30.1	-29.8	-29.9	-33.0
Shrub-tree					
<i>Cercis occidentalis</i>	-28.0	-25.7	-	-	-29.2
<i>Quercus gambelli</i>	-	-28.2	-	-26.9	-29.1
<i>Rhamnus betulaeifolia</i>	-	-	-27.9	-26.8	-30.4
<i>Rhus trilobata</i>	-26.7	-26.8	-27.2	-27.0	-

was little variation with height and the CO_2 concentration at 1.0 m was always within $1 \mu\text{l l}^{-1}$ of the 0.25 m height value. Atmospheric $\delta^{13}\text{C}$ values during the day averaged -7.9‰ .

Carbon isotope ratios were more negative than typically seen in C_3 species, especially when compared with carbon isotope ratios values that have been reported for arid regions (Ehleringer 1989, 1993). In 1990 there was over a 7‰ range in the leaf $\delta^{13}\text{C}$ values for the common plant species occurring in the hanging gardens (Table 1). In that year, *Cercis occidentalis*, growing in Three Garden, had the carbon isotope ratio most enriched in ^{13}C , while *Solidago sparsiflora* growing in Ribbon Garden had the isotope ratio most depleted in ^{13}C . In a com-

parison of individual species growing in different gardens, *Cirsium rydbergii* showed the largest variation (4‰) in leaf $\delta^{13}\text{C}$ values (Table 1). When the hanging garden vegetation growing in Lost Garden and in Ribbon Garden was resampled in 1996, the leaf $\delta^{13}\text{C}$ values were somewhat more negative than in 1990, with the most depleted value at -34.8‰ associated with *Mimulus eastwoodiae* (Fig. 3). The correlation between leaf $\delta^{13}\text{C}$ values between years was statistically significant ($r = 0.825$, $P < 0.0001$), suggesting that there was little variation in the relative ranking of leaf carbon isotope ratio values between years.

The average carbon isotope ratios of plants among hanging gardens (average $\delta^{13}\text{C}$ value for all the species in a garden) were significantly different (Table 2). Most likely this resulted because each garden had a different azimuthal orientation, resulting in contrasting solar radiation levels, although this factor was not quantified. There was a significant seasonal effect on average $\delta^{13}\text{C}$ value (Table 2, Fig. 4). The carbon isotope ratios of plants were more negative later in the growing season (October) than in the spring (April). On average, plants in Death Camus Garden (the most exposed garden) had the highest leaf $\delta^{13}\text{C}$ values. In contrast, Ribbon Garden

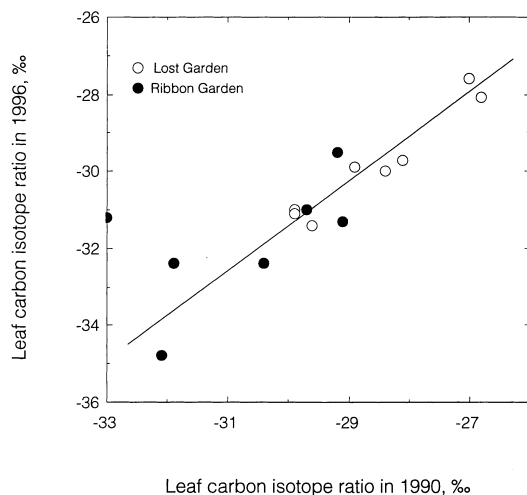


Fig. 3 Correlation between the leaf carbon isotope ratios measured on a species in 1990 and 1996 in plants from both Lost Garden and Ribbon Garden in southern Utah

Table 2 Analysis of variance for leaf carbon isotope ratio ($\delta^{13}\text{C}$) in plants from hanging gardens. Replicates for the analysis are species values recorded in a particular garden

Source	df	Mean square	F	P
Garden	4	50.93	25.32	0.0001
Sample Time	2	9.60	4.77	0.0098
Garden \times Sample time	8	1.35	0.67	0.7151
Residual	149	2.01		

(the most shaded and likely not to receive direct solar radiation at any time of the year) had average $\delta^{13}\text{C}$ values that were significantly more negative than values observed in the other gardens at any time of the year (Table 2, Fig. 4).

The overall range of carbon isotope ratios of plants in Lost Garden, a hanging garden with exposure to full

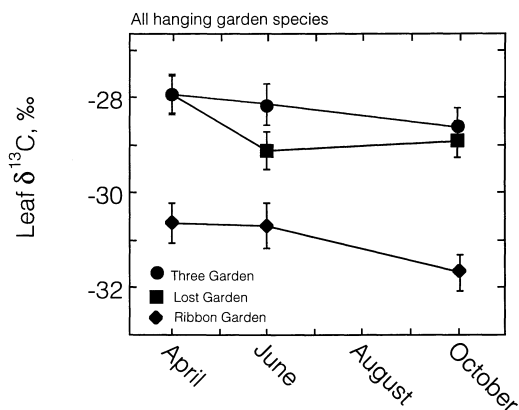


Fig. 4 Seasonal variation in the average carbon isotope ratio of plant species in hanging gardens differing in their solar exposure: *solid square* Lost, *solid diamond* Ribbon, *solid circle* Three. Statistical analyses of the data are shown in Table 2

Table 3 Comparison of the species composition and carbon isotope ratio ($\delta^{13}\text{C}$, ‰) of C_3 plants in communities adjacent to hanging gardens in Glen Canyon. The ephemeral wash is the arid zone vegetation immediately adjacent to Lost Garden and the riparian zone is the vegetation in close proximity to Ribbon Garden

Species	Ephemeral wash	Riparian zone
Herbaceous		
<i>Abronia fragans</i>	-26.4	
<i>Eriogonum inflatum</i>	-26.9	
<i>Eriogonum microthecum</i>	-28.1	
<i>Oenothera longissima</i>	-26.6	
<i>Oryzopsis hymenoides</i>	-23.9	
<i>Solidago sparsiflora</i>	-29.7	
<i>Sphaeralcea grossulariaefolia</i>	-28.0	
Shrub		
<i>Bursera sp.</i>	-23.8	
<i>Chrysothamnus nauseosus</i>	-26.7	
<i>Encelia virginensis</i>	-26.2	
<i>Ephedra viridis</i>	-23.0	
<i>Gutierrezia microcephala</i>	-25.7	
<i>Rhus trilobata</i>	-26.8	
Herbaceous		
<i>Equisetum sp.</i>		-24.9
<i>Solidago sparsiflora</i>		-30.4
Shrub-tree		
<i>Baccharis sp.</i>		-28.0
<i>Ceanothus sp.</i>		-26.1
<i>Celtis reticulata</i>		-25.8
<i>Fraxinus anomala</i>		-26.6
<i>Populus tremuloides</i>		-29.0
<i>Quercus gambelli</i>		-26.3
<i>Rhus trilobata</i>		-26.5
<i>Tamarix sp.</i>		-25.3

sunlight only in the afternoons, overlapped with those values observed for other plant species growing in an adjacent, relatively mesic ephemeral wash outside the hanging garden (Table 3). However, since the aridland vegetation outside that garden was primarily woody species, whereas that within the garden was primarily herbaceous species, the appropriate analysis was a community-level life-form comparison. Those results showed that average carbon isotope ratios of herbaceous species were more negative (Mann-Whitney $U' = 40.5$, $P = 0.038$) within the hanging garden community (-28.8‰) than in the ephemeral wash (-27.1‰). Similarly, the average carbon isotope ratios of woody species were more negative (Mann-Whitney $U' = 17.5$, $P = 0.024$) within the hanging garden community (-26.9‰) than in the ephemeral wash (-25.4‰). The carbon isotope ratios of plants from Ribbon Garden, a hanging garden shaded throughout the day, contrasted with an adjacent riparian vegetation (Table 3). The average leaf $\delta^{13}\text{C}$ value of woody species at Ribbon garden (-29.6‰) was significantly more negative than observed for plants growing in the riparian vegetation (-26.7‰) with an actively flowing stream not more than 200 m away (Mann-Whitney $U' = 24.0$, $P = 0.012$).

In a comparison of leaf photosynthetic characteristics among some commonly occurring plants in Lost, Ribbon and Double Gardens during April 1990, the plants in Ribbon Garden had significantly lower CO_2 assimilation rates at midday (Table 4). Both the low light intensity and moderate leaf temperatures observed in Ribbon Garden contributed to the lower CO_2 assimilation rates, although light intensity was probably the principle limiting factor. In contrast, leaf conductance was not significantly different among plants in Lost, Ribbon and Double Gardens. This resulted in a significantly higher leaf intercellular CO_2 concentration in the plants growing in Ribbon Garden (Table 5). The higher intercellular CO_2 concentrations observed using gas exchange techniques were consistent with the low leaf $\delta^{13}\text{C}$ values observed for plants in Ribbon Garden (Table 1, Fig. 4).

During the June 1990 trip to Ribbon Garden, leaf photosynthetic characteristics in 3 plant species were compared between individuals growing on the edge of the garden, which received full sun at midday, and individuals growing in the center of the garden, which was shaded from full sunlight throughout the day (Table 6). Assimilation of CO_2 was significantly lower, and intercellular CO_2 concentration was significantly higher in the shaded plants. There was no significant difference in leaf conductance, although rates tended to be lower in the shaded plants (Table 6). Since the majority of plants in Ribbon Garden received no direct sunlight, the low instantaneous CO_2 assimilation rate and high intercellular CO_2 concentration observed for the shaded plants are considered more typical photosynthetic gas exchange characteristics for plants in Ribbon Garden. We modeled the photosynthetic dependence on intercellular CO_2

Table 4 Comparison of mean photon flux density (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$), leaf temperature ($^{\circ}\text{C}$), CO_2 assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), leaf conductance (g , $\text{mmol m}^{-2} \text{s}^{-1}$), and intercellular CO_2 concentration (c_i , $\mu\text{mol} \cdot \text{mol}^{-1}$) at midday among plant species in Lost, Ribbon, and Double gardens during April 1990. Values for each species represent the average of 2 or 3 measurements for different plants within a garden. Analysis of variance was performed to compare the mean values for characteristics measured in the 3 different gardens (using the displayed species values as replicates). Within each column, mean values for a garden followed by the same letter are not significantly different based on the result of a Tukey-Kramer multiple comparison test after an ANOVA. The results of the ANOVA are shown in Table 5

Species	PAR	Leaf temp	A	g	c_i
Lost Garden					
<i>Aquilegia micrantha</i>	272	26.4	8.9	305	275
<i>Cirsium rydbergii</i>	754	24.8	17.7	898	275
<i>Panicum acuminatum</i>	325	27.6	13.1	315	254
<i>Rhamnus betulaeifolia</i>	1276	32.5	9.0	114	183
<i>Rhus trilobata</i>	406	27.9	11.4	305	256
<i>Solidago sparsiflora</i>	647	26.3	27.7	250	287
Mean \pm SE	613 \pm 153 ^a	27.6 \pm 1.1 ^a	14.6 \pm 2.9 ^a	365 \pm 111 ^a	255 \pm 15 ^a
Ribbon Garden					
<i>Aquilegia micrantha</i>	80	18.1	3.1	238	313
<i>Cirsium rydbergii</i>	85	16.6	4.4	443	315
<i>Epipactus gigantea</i>	56	18.2	2.3	203	319
<i>Rhamnus betulaeifolia</i>	72	19.4	3.3	243	312
<i>Rubus neomexicanus</i>	62	20.3	2.7	143	304
Mean \pm SE	71 \pm 5 ^b	18.5 \pm 0.6 ^b	3.1 \pm 0.4 ^b	254 \pm 51 ^a	313 \pm 2 ^b
Double Garden					
<i>Cirsium rydbergii</i>	1565	25.7	13.4	284	222
<i>Epipactus gigantea</i>	1706	31.4	11.6	209	224
<i>Panicum acuminatum</i>	2011	28.6	26.1	715	233
<i>Rhamnus betulaeifolia</i>	1757	28.7	16.9	348	213
<i>Solidago sparsiflora</i>	1891	27.6	33.5	1192	245
Mean \pm SE	1786 \pm 77 ^c	28.4 \pm 0.9 ^a	20.3 \pm 4.1 ^a	550 \pm 183 ^a	227 \pm 5 ^a

Table 5 Results of an analysis of variance ($df = 2, 13$) for comparison of midday values of photon flux density (PAR), leaf temperature, and photosynthetic gas exchange characteristics among plants in Lost, Ribbon, and Double Gardens during April 1990. Mean species values were used as replicates for the ANOVA. Data for assimilation were log transformed, and data for intercellular CO_2 were power (x^2) transformed for the ANOVA (Sokal and Rohlf 1981)

Characteristic	F	P
PAR	61.16	0.0001
Leaf temperature	33.32	0.0001
CO_2 assimilation rate	30.47	0.0001
Leaf conductance	1.34	0.297
Intercellular CO_2	28.07	0.0001

Table 6 Comparison of midday values of photon flux density (PAR), leaf temperature, and photosynthetic gas exchange characteristics in open and shaded areas in Ribbon Garden during June 1990. Two measurements were made on each of 3 species (*Aquilegia micrantha*, *Cirsium rydbergii*, *Rubus neomexicanus*) in each area during mid-day. Values shown are the mean \pm SE, $n=6$. The column labelled significance indicates the result of an ANOVA ($df = 1,10$) comparing measurements made in the open and shaded areas. The values for assimilation were log-transformed for the ANOVA (Sokal and Rohlf 1981)

	Open	Shaded	Significance
PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	2001 \pm 25	52 \pm 12	* *
Leaf temperature ($^{\circ}\text{C}$)	37.6 \pm 0.7	29.3 \pm 0.7	* *
CO_2 assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	7.3 \pm 0.6	1.7 \pm 0.1	* *
Leaf conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	242 \pm 18	163 \pm 46	NS
Intercellular CO_2 ($\mu\text{mol mol}^{-1}$)	248 \pm 6	301 \pm 3	* *

** $P \leq 0.0001$, NS not significant

concentration for a hanging garden plant under high and low light levels (Fig. 5). Given the field observations of a constant leaf conductance, the model predicts that under low light levels the intercellular CO_2 concentration will be as high as observed; this value is consistent with the long-term intercellular CO_2 concentration estimated from leaf carbon isotope ratio analyses.

Leaf nitrogen levels measured on plants from Lost Garden and Ribbon Garden ranged between 12.0 and 30.2 mg g^{-1} , with an average of 18.5 mg g^{-1} (Table 7). These values are perhaps greater than expected given that these plants are growing on an aolian substrate. While these leaf N values are not high for C_3 plants (Schulze et al. 1994), they are in the upper range of values reported for shade plants (Boardman 1977). The mass to leaf area ratio differed among these species, largely because a few species, such as *Cirsium rydbergii* (167 g m^{-2}) and *Petrophytum caespitosum* (204 g m^{-2}), had thick leaves. Otherwise, the mass to leaf area ratio of hanging garden plants tended to be in the 24–65 g m^{-2} range (Table 7). The range of leaf nitrogen values, expressed on a leaf area basis, was not significantly correlated with the mass to leaf area ratio ($r = -0.138$, n.s.). Nor was there any significant relationship between leaf carbon isotope ratio and the mass to leaf area ratio ($r = 0.089$, n.s.).

Given the generally low soil nitrogen values that characterize these arid regions and the rarity of N-fixing higher plants in the region, we examined leaf nitrogen isotope ratios to see if the *Nostoc* found on hanging garden walls might be serving as a nitrogen-input source for this community (Table 8). Leaf nitrogen isotope ratios averaged -2.3‰ (range of -0.7 to -6.1‰), consistent with the notion that most of the nitrogen was derived from a biological fixation source (i.e., *Nostoc*).

Table 7 Comparison of mass to leaf area ratio (g m^{-2}) and leaf nitrogen content expressed on both mass-to-mass (mg g^{-1}) and mass-to-leaf-area (mmol N m^{-2}) bases. Samples were collected from mature leaves in Ribbon Garden and in Lost Garden during September 1996

	Mass to leaf area g m^{-2}	Leaf nitrogen content mg g^{-1}	Leaf nitrogen content mmol N m^{-2}
Lost Garden			
<i>Adiantum capillus-veneris</i>	43.6	22.1	69
<i>Aquilegia micrantha</i>	46.5	23.5	78
<i>Cirsium rydbergii</i>	167.4	15.7	188
<i>Petrophytum caespitosum</i>	204.2	10.5	153
<i>Primula specuicola</i>	65.1	12.0	56
<i>Rhamnus betulaeifolia</i>	96.8	17.2	119
<i>Rhus trilobata</i>	109.4	15.9	124
<i>Smilacina stellata</i>	45.6	30.2	98
<i>Solidago sparsiflora</i>	109.2	21.0	164
Ribbon Garden			
<i>Adiantum capillus-veneris</i>	33.6	23.0	55
<i>Aquilegia micrantha</i>	41.4	15.4	46
<i>Cercis occidentalis</i>	47.6	22.5	76
<i>Cirsium rydbergii</i>	100.5	13.7	98
<i>Mimulus eastwoodiae</i>	24.4	18.6	32
<i>Rhamnus betulaeifolia</i>	48.7	13.7	68
<i>Rubus neomexicana</i>	52.5	13.6	51
<i>Solidago sparsiflora</i>	65.5	19.0	89

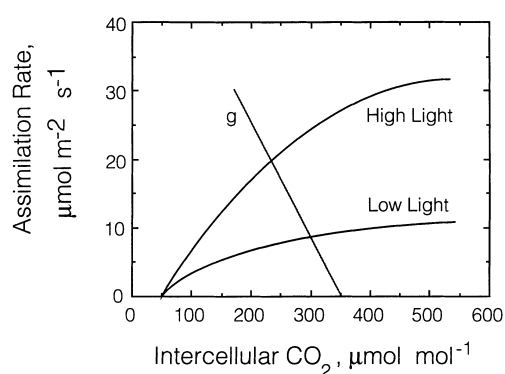


Fig. 5 Modelled effect of low light intensity on photosynthetic capacity and leaf intercellular CO_2 concentration, assuming a constant stomatal conductance. The line labelled g represents conductance to CO_2 (supply function). Assimilation rates were calculated as a function of intercellular CO_2 concentration (demand function) using a photosynthesis model (Farquhar and von Caemmerer 1982) with the following biochemical characteristics: maximum carboxylation capacity of Rubisco (V_{cmax}) = $110 \mu\text{mol m}^{-2} \text{s}^{-1}$; maximum electron transport capacity (J_{max}) = $240 \mu\text{mol m}^{-2} \text{s}^{-1}$. Environmental characteristics were a leaf temperature of 25°C and a light intensity of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (high light) or of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (low light). With a constant stomatal conductance, an increase in assimilation, associated with higher light intensity, results in a lower intercellular CO_2 concentration

These values contrast with leaf nitrogen isotope ratios of 5–9‰, which have been previously reported for arid zone plants in nearby ecosystems (Evans and Ehleringer 1994).

Discussion

Hanging gardens are unusual mesic refugia distributed across a vast aridland landscape on the Colorado Plateau. Given the obvious differences in water availability

Table 8 Comparison of leaf nitrogen isotope ratios ($\delta^{15}\text{N}$, ‰) from mature leaves on plants occurring in several hanging gardens in Glen Canyon, Utah

	Nitrogen isotope ratio (‰)		
	Lost Garden	Three Garden	Ribbon Garden
<i>Adiantum capillus-veneris</i>	-2.7	–	-3.3
<i>Aquilegia micrantha</i>	-1.7	–	-2.5
<i>Cercis occidentalis</i>	–	-0.7	-0.7
<i>Cirsium rydbergii</i>	-1.3	-1.5	-1.0
<i>Lobelia cardinalis</i>	–	-1.6	–
<i>Mimulus eastwoodiae</i>	–	-0.7	-2.3
<i>Petrophytum caespitosum</i>	-6.1	–	–
<i>Primula specuicola</i>	-2.4	–	–
<i>Rhamnus betulaeifolia</i>	-3.9	–	-2.6
<i>Rhus trilobata</i>	-3.6	–	–
<i>Rubus neomexicana</i>	–	–	-3.6
<i>Smilacina stellata</i>	-0.7	–	–
<i>Solidago sparsiflora</i>	-4.0	–	-1.6

between these refugia and the surrounding desert shrublands, it is perhaps not surprising that leaf carbon isotope ratios of hanging garden plants were substantially more negative than has been previously observed for aridland species (Ehleringer 1989, 1993). What is perhaps unusual is that leaf $\delta^{13}\text{C}$ values of hanging garden plants with only moderate water supplies could be more negative than observed for adjacent riparian species with more than adequate water supplies. Reduced solar exposure, related to orientation of individual hanging gardens, is the most likely explanation for this difference, given that it is commonly observed that plants in low-light environments often have more negative leaf $\delta^{13}\text{C}$ values (Farquhar et al. 1989). While more negative leaf $\delta^{13}\text{C}$ values have been observed in plants growing in the understory of forest ecosystems, it is often the case that a low $\delta^{13}\text{C}$ value is in part because

in these forest environments the isotopic composition of source CO_2 is significantly depleted in ^{13}C ($\delta^{13}\text{C} = -10\text{‰}$ to -14‰ ; Broadmeadow et al. 1992; Broadmeadow and Griffiths 1993; Buchmann et al. 1997) relative to the well mixed atmosphere, which has a $\delta^{13}\text{C}$ value of approximately -8.0‰ (Keeling et al. 1989). The hanging garden plants were exposed to an atmospheric source CO_2 with an average carbon isotopic composition of approximately -8‰ , and so the low leaf $\delta^{13}\text{C}$ values were not caused by exposure to source CO_2 with very low ^{13}C contents.

Instead the low leaf $\delta^{13}\text{C}$ values in hanging garden plants are caused by unusually high leaf intercellular CO_2 concentrations. The carbon isotope ratios of C_3 plants are dependent on two variables: source atmospheric $\delta^{13}\text{C}$ values and the intercellular CO_2 concentration (Fraquhar et al. 1989). Light intensity has been shown to influence the operational intercellular CO_2 concentration, with values increasing toward atmospheric values as light levels decrease (Ehleringer et al. 1986; Zimmerman and Ehleringer 1990; see Fig. 5). The gas exchange data revealed that hanging garden plants had higher intercellular CO_2 concentrations than typically observed in C_3 plants (Fraquhar and Sharkey 1982). Recall that the average carbon isotope ratio of plants in Ribbon Garden was lower than the plants in other hanging gardens during all sample times. These very low $\delta^{13}\text{C}$ values observed in Ribbon Garden were primarily associated with the continuously low light intensities. Thus, in contrast to Lost Garden, where a fraction of the daily carbon gain would be acquired under high light levels, all of the carbon acquired by Ribbon Garden plants was acquired under shade conditions.

The $\delta^{13}\text{C}$ values observed for some plants in Ribbon Garden (particularly *Solidago sparsiflora* and *Mimulus eastwoodiae*) are among the most negative values reported for any terrestrial plant species growing under natural environmental conditions (O'Leary 1988; Farquhar et al. 1989). When these leaf carbon isotope ratios are converted to carbon isotope discrimination values (Δ) (Farquhar et al. 1989), they attain Δ values as high as 27.8‰ which are among the highest, if not highest, carbon isotope discrimination values reported for naturally-grown plant materials.

Our observation of a relatively high rate of stomatal conductance and a high intercellular CO_2 concentration in plants from the Ribbon Garden is consistent with results from other studies of photosynthesis and carbon isotope discrimination in shaded environments. Previous studies with forest understory species by Percy (1987) and Percy and Pfitsch (1991) have suggested two major benefits of a high intercellular CO_2 concentration. First, quantum yield or light-use efficiency increases substantially with an increase in intercellular CO_2 concentration in the range $290\text{--}350 \mu\text{mol mol}^{-1}$ (Ehleringer and Björkman 1977). Second, a relatively high stomatal conductance (and therefore high intercellular CO_2 concentration) relative to the photosynthetic rate reduces the stomatal limitation of carbon gain, especially during

sunflecks. Reducing stomatal limitation of photosynthesis during a short sunfleck in a forest understory is of obvious advantage for maximizing carbon gain (Percy 1987; Percy and Pfitsch 1991). While reducing stomatal limitations to carbon gain could also be beneficial to plants in a hanging garden exposed to short-term light changes, the advantage is likely to be lost when leaves are exposed to full sunlight for more than a few minutes.

There was a significant seasonal change in the average $\delta^{13}\text{C}$ values for plants in the hanging gardens. The pattern of change toward more negative leaf $\delta^{13}\text{C}$ values at the end of the growth season is similar to the pattern observed for maple tree foliage in an eastern, deciduous-coniferous forest (Lowdon and Dyck 1973). In contrast, studies involving the surrounding arid zone vegetation have shown that most species showed an increase in foliage ^{13}C content during the growing season associated with progressive seasonal soil drought (Smith and Osmond 1987; Smedley et al. 1991; Ehleringer et al. 1992). We have no clear explanation for the small seasonal decrease in leaf $\delta^{13}\text{C}$ values observed in our study.

Several of the plant species sampled are endemic to hanging garden habitats in southern Utah (e.g., *Aquilegia micrantha*, *Circium rydbergi*, *Mimulus eastwoodiae*, *Primula specuicola*; Malanson 1980; Welsh 1989), but are closely related to species with wide distributions across North America. Other non-endemic species common to hanging gardens have extensive distributions in diverse habitats within North America, but are not typically found in aridland habitats (e.g., *Adiantum capillus-veneris*, *Pterophytum caespitosum*, *Solidago sparsiflora*). Thus, we may be dealing with a flora that is relictual, but systematically unique. While the species composition and the carbon isotopic composition of plants in hanging gardens are distinct from typical plant assemblages in adjacent aridland ecosystems, other gas exchange characteristics of hanging garden plants are consistent with the relatively moderate environmental conditions experienced by other plant species in low light environments (Percy 1990). Welsh (1989) considered hanging garden plants to be opportunists from distant mesic habitats, taking advantage of the localized mesic conditions in the gardens to invade the otherwise harsh aridland environment of southern Utah. In doing so, these plants express unusually low $\delta^{13}\text{C}$ values that clearly distinguish hanging garden plants from the surrounding arid zone vegetation.

Acknowledgements Research was supported by the University of Utah and the Ecological Research Division, Office of Health and Environmental Research, U.S. Department of Energy. L.B.F. was also supported by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada.

References

- Boardman NK (1977) Comparative photosynthesis of sun and shade plants. *Annu Rev Plant Physiol* 28:355–377

- Broadmeadow MSJ, Griffiths H (1993) Carbon isotope discrimination and the coupling of CO₂ fluxes within forest canopies. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego, pp 109–130
- Broadmeadow MSJ, Griffiths H, Maxwell C, Borland AM (1992) The carbon isotope ratio of plant organic material reflects temporal and spatial variations in CO₂ within tropical forest formations in Trinidad. *Oecologia* 89:435–441
- Buchmann N, Guehl J-M, Barigah T, Ehleringer JR (1997) Inter-seasonal comparison of CO₂ concentrations, isotopic composition, and carbon cycling in an Amazonian rainforest (French Guiana). *Oecologia* 110:120–131
- Ehleringer JR (1989) Carbon isotope ratios and physiological processes in aridland plants. In: Rundel PW, Ehleringer JR, Nagy KA (eds) Stable isotopes in ecological research. Springer, Berlin Heidelberg New York, pp 41–54
- Ehleringer JR (1991) ¹³C/¹²C fractionation and its utility in terrestrial plant studies. In: Coleman DC, Fry B (eds) Carbon isotope techniques. Academic Press, New York, pp 187–200
- 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 – water relations. Academic Press, San Diego, pp 155–172
- Ehleringer JR, Björkman O (1977) Quantum yields for CO₂ uptake in C₃ and C₄ plants. *Plant Physiol* 59:86–90
- Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Percy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological ecology. Field methods and instrumentation. Chapman and Hall, London, pp 281–300
- Ehleringer JR, Field CB, Lin ZF, Kuo CY (1986) Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. *Oecologia* 70:520–526
- Ehleringer JR, Philips SL, Comstock JP (1992) Seasonal variation in the carbon isotopic composition of desert plants. *Funct Ecol* 6:396–404
- Evans RD, Ehleringer JR (1994) Water and nitrogen dynamics in an arid woodland. *Oecologia* 99:233–242
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* 33:317–345
- Farquhar GD, Caemmerer S von (1982) Modelling photosynthetic response to environmental conditions. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Encyclopedia of plant physiology, new series vol 12B. Springer, Berlin Heidelberg New York, pp 548–588
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Mol Biol* 40:503–537
- Flanagan LB, Brooks JR, Varney GT, Berry SC, Ehleringer JR (1996) Carbon isotope discrimination during photosynthesis and the isotope ratio of respired CO₂ in boreal forest ecosystems. *Global Biogeochem Cycl* 10:629–640
- Keeling CD, Bacastow RB, Carter AF, Piper SC, Whorf TP, Heimann M, Mook WG, Roeloffzen H (1989) A three-dimensional model of atmospheric CO₂ transport based on observed winds. 1. Analysis of observational data. *Geophys Monogr* 55:165–236
- Lowdon JA, Dyck W (1973) Seasonal variations in the isotope ratios of carbon in maple leaves and other plants. *Can J Earth Sci* 11:79–88
- Malanson GP (1980) Habitat and plant distributions in hanging gardens of the Narrows, Zion National Park, Utah. *Great Basin Nat* 40:178–182
- O’Leary MH (1988) Carbon isotopes in photosynthesis. *BioScience* 38:328–336
- Percy RW (1987) Photosynthetic gas exchange responses of Australian tropical forest trees in canopy, gap and understory micro-environments. *Funct Ecol* 1:169–178
- Percy RW (1990) Sunflecks and photosynthesis in plant canopies. *Annu Rev Plant Physiol Mol Biol* 41:421–453
- Percy RW, Pfitsch WA (1991) Influence of sunflecks on the δ¹³C of *Adenocaulon bicolor* plants occurring in contrasting forest understory microsites. *Oecologia* 86:457–462
- Schulze E-D, Kelliher FM, Kömer C, Lloyd J, Leuning R (1994) Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: a global ecology scaling exercise. *Annu Rev Ecol Syst* 25:629–660
- Smedley MP, Dawson TE, Comstock JP, Donovan LA, Sherrill DE, Cook CS, Ehleringer JR (1991) Seasonal carbon isotope discrimination in a grassland community. *Oecologia* 85:314–320
- Smith SD, Osmond CB (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*. Morphology, stomatal conductance and water-use efficiency in field populations. *Oecologia* 72:533–541
- Sokal RR, Rohlf FJ (1981) Biometry, 2nd ed. Freeman, New York
- Welsh SL (1989) On the distribution of Utah’s hanging gardens. *Great Basin Nat* 49:1–30
- Welsh SL, Toft CA (1981) Biotic communities of hanging gardens in southeastern Utah. *Nat Geogr Res Rep* 13:663–681
- Welsh SL, Atwood ND, Goodrich S, Higgins LC (1987) A Utah flora (Great Basin naturalist memoirs, 9). Brigham Young University Press Provo, Utah 894 pp.
- Zimmerman J, Ehleringer JR (1990) Carbon isotope ratios are correlated with irradiance levels in the Panamanian orchid *Catasetum viridiflavum*. *Oecologia* 83:247–249