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NATURAL ABUNDANCES OF CARBON ISOTOPES (^{14}C, ^{13}C) IN LICHENS AND CALCIUM OXALATE PRUINA: IMPLICATIONS FOR ARCHAEOLOGICAL AND PALEOENVIRONMENTAL STUDIES

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ABSTRACT. Radiocarbon ages of calcium oxalate that occurs naturally on rock surfaces have been used recently in archaeological and paleoenvironmental studies. Oxalate rock coatings are found globally, with most appearing to be residues from epilithic lichens. To explore the source(s) of carbon used by these organisms for the production of oxalate we measured the natural abundances of ^{14}C and ^{13}C in 5 oxalate-producing lichen species, 3 growing on limestone in southwestern Texas and 2 on sandstone in Arkansas. We also examined the distribution of the isotopes between the calcium oxalate and lichen tissues by separating these components and measuring the $^{13}C/C$ independently. The results demonstrate that the limestone species were slightly enriched in ^{14}C, by 1.7‰, relative to the sandstone species, which suggests that "dead" carbon from the limestone substrate does not constitute a significant source of carbon for the production of oxalate. The calcium oxalate produced by the lichens is also enriched in ^{13}C by 6.5‰ compared to the lichen tissues, demonstrating that there is a large carbon isotope discrimination during oxalate biosynthesis. These results support the reliability of ^{14}C ages of calcium oxalate rock coatings used for archaeological and paleoclimate studies.

INTRODUCTION

It is becoming increasingly evident that the calcium oxalate minerals whewellite ($CaC_2O_4 \cdot H_2O$) and weddellite ($CaC_2O_4 \cdot (2 + x)H_2O$) are common on rock surfaces worldwide (Table 1). The oxalate generally occurs as thin ($\lesssim$1 mm) rock patinas with most appearing to be deposits from epilithic lichens (Del Monte et al. 1987; Edwards et al. 1993; Russ et al. 1996; Hofmann and Bernasconi 1998), although unlichenized microbes (Bonaventura et al. 1999) and organic acid aerosols (Watchman 1991) have been proposed as sources of some oxalates on rock surfaces. Recently, radiocarbon dates of oxalates that cover, encapsulate, or are incorporated within prehistoric rock paintings (pictographs) have been used to constrain or estimate the ages of the artifacts (Watchman 1993; Hedges et al. 1998; Russ et al. 1999; Watchman et al. 2000; Steelman et al. 2001). Oxalate ^{14}C ages have also been used in paleoclimate reconstructions that are based on the assumption that temporal variations in oxalate production can be correlated to fluctuations in lichen productivity in response to climate change (Russ et al. 1996; 2000).

The source(s) of carbon that leads to the formation of oxalate coatings is unknown, although assumed to be ambient CO_2. Here we report a study of the relative abundances of ^{14}C and stable carbon isotopes in living, oxalate producing (pruinose) lichens that allowed us to address (1) whether lichens incorporate significant levels of limestone (carbonate) carbon for the production of oxalate and (2) the distribution of carbon isotopes between the lichen tissues and oxalate coating (pruina).

Lichens are symbiotic associations between fungi (mycobiont) and photosynthetic microbes (photobiont) integrated within the fungal matrix (thallus). Calcium oxalate is the most common lichen byproduct, which generally occurs as a coating on the upper/outermost surface of the organism. The production of oxalate might benefit lichens by removing excess calcium ions and/or providing an external source of water stored in the calcium oxalate crystal lattice (Wadsten and Moberg 1985). After the death of the organism, the oxalate pruina can remain stable on the rock surface for millennia (Watchman 1993; Russ et al. 1996).

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Table 1 Summary of occurrences of calcium oxalate rock coatings

Location	Association	Proposed origin	$\delta^{13}C$ (‰)	Reference
Italy	Coatings on ancient monuments, old buildings & rock surfaces	Lichens	NA	Del Monte et al. 1987
Australia	Natural coatings on pictographs & rock surfaces	Organic acids in rain	NA	Watchman 1991; Watchman et al. 2000.
Italy	Encrustations on Renaissance frescoes	Lichens	NA	Edwards et al. 1993, 1997
California (USA)	Natural coatings on pictographs & rock surfaces	None proposed	NA	Scott and Hyder 1993
Utah (USA)	Natural coating on a single pictograph	None proposed	NA	Chaffee et al. 1994
SW Texas (USA)	Natural coating on pictographs & rock surfaces	Lichens	−10.6 ± 1.9 (18)[a]	Russ et al. 1996; 2000
Northern Mediterranean	Coatings on ancient monuments, buildings & rock surfaces	Various mechanisms	NA	Various authors 1996
Argentina	Component in pictograph paint	Cacti used in paint recipe	−10.3 (1)[a]	Hedges et al. 1998
Argentina	Natural rock coating	None proposed	−26 (2?)[a]	Hedges et al. 1998
Arizona (USA)	Natural rock coating	Lichens	−11.8 (1)[a]	Hofmann and Bernasconi 1998
Switzerland	Natural rock coating	Lichens	−11.7 (1)[a]	Hofmann and Bernasconi 1998
Italy	Stone monuments	Microbes	NA	Bonaventura et al. 1999
Brazil	Natural coating on pictograph	None proposed	−11.67 (1)[a]	Steelman et al. 2002

[a]Number of individual analyses

The reliability of ^{14}C ages and stable carbon isotope ratios ($\delta^{13}C$) from oxalate deposits from lichens depends largely on whether or not inorganic carbon (carbonate or bicarbonate ions) from the basal rock is incorporated in the oxalate. Native carbon in ancient carbonate rocks such as limestones is completely depleted in ^{14}C, and enriched in ^{13}C by ~8‰ compared to atmospheric CO_2 (Craig 1953; Degens 1969). If such "dead" carbon from the substrate is included in the oxalate, either via biosynthesis, exchange reactions, or reactions of oxalic acid at the rock surface, then oxalate ^{14}C ages would be anomalously old and $\delta^{13}C$ values would represent a ^{13}C enrichment independent of metabolic processes.

Indirect evidence that lichen mycobionts can metabolize carbonate/bicarbonate ions was demonstrated by Lapeyrie et al. (1987; Lapeyrie 1988) by showing that oxalate ion production by the fungus *Paxillus involutus* was greater when grown on calcareous soil compared to acidic soil, and that this particular fungus incorporated bicarbonate ions from a growth medium for the biosynthesis of oxalic acid. Additional evidence of a possible bicarbonate effect is that calcium oxalate rock coatings are significantly enriched in ^{13}C compared to living lichens (Table 1). For example, Hofmann and Bernasconi (1998) reported $\delta^{13}C$ values of −11.8‰ and −11.7‰ from analyses of oxalate rock crusts in Arizona (USA) and Valais, Switzerland, respectively, while Steelman et al. (2001) reported an oxalate $\delta^{13}C$ value of −11.67‰ from a coating in Toca do Bastina, Brazil. Furthermore, 18 calcium oxalate crust samples from 12 sites in southwestern Texas yielded a mean $\delta^{13}C$ value of −10.6 ± 1.9‰ (Russ et al. 2000). Reported $\delta^{13}C$ values of living lichens, on the other hand, range from −35‰ to −14‰ (Lange et al. 1988), indicating a significantly lower ^{13}C content compared to the oxalate coatings. This isotope discrepancy might be due to utilization of carbonate in the rock substrate that would cause ^{14}C measurements to be unreliable, or other metabolic processes for which corrections can be made. The latter is the case for the intracellular

calcium oxalate in cacti which is enriched in ^{13}C by ~5‰ compared to the cactus tissues (Rivera and Smith 1979).

To investigate whether lichens incorporate substrate carbonate and/or bicarbonate ions for the production of oxalate we measured the relative abundances of the carbon isotopes ($\Delta^{14}C$ and $\delta^{13}C$ values) in 5 living, pruinose lichen species collected from limestone and sandstone surfaces in Texas and Arkansas, respectively (Table 2). We sampled areas on the specimens that appeared to have recent growth—edges and areas with fruiting bodies—and so expected $\Delta^{14}C$ values that reflected contemporary atmospheric CO_2. Then, if substrate carbon was incorporated in the oxalate the $^{14}C/C$ of the limestone species would be lower (smaller $\Delta^{14}C$ values) and the $^{13}C/C$ higher (less negative $\delta^{13}C$ values) compared to the lichens growing on the sandstone. However, as Bench et al. (2001, 2002) recently demonstrated, there is considerable internal carbon recycling and/or carbon turnover in the 2 lichens they studied. Such processes would cause a discrepancy between the contemporary atmospheric ^{14}C record and the ^{14}C content of the organisms, and limit our ability to predict the relative amount of carbonate carbon, if any, included for the production of oxalate.

Table 2 Radiocarbon ($\Delta^{14}C$) and stable carbon isotope ($\delta^{13}C$) results from pruinose lichens in southwestern Texas and northeastern Arkansas

Species	Location	Substrate	AMS lab nr	$\Delta^{14}C$	$\delta^{13}C$ (‰)
Flavoparmelia baltimorensis	NE Arkansas	Sandstone	50964	115.3 ± 5.0	−23.65
Dirinaria frostii	NE Arkansas	Sandstone	50970	142.7 ± 4.9	−22.13
Caloplaca saxicola	SW Texas	Limestone	50966	206.6 ± 5.6	−17.07
Caloplaca saxicola	SW Texas	Limestone	50967	219.1 ± 5.7	−17.76
Caloplaca saxicola	SW Texas	Limestone	50968	189.9 ± 5.5	−20.33
Lecania Sp.	SW Texas	Limestone	50969	162.3 ± 5.4	−17.92
Lecania Sp.	SW Texas	Limestone	AA42664	192.5 ± 7.1	−18.22
Lecidea Sp.	SW Texas	Limestone	AA42662	163.6 ± 6.3	−18.93

We also explored the distribution of the stable carbon isotopes between the lichen tissues and oxalate pruina by separating these components and measuring the $\delta^{13}C$ values of each. There is greater variability in $\delta^{13}C$ values reported for lichens than for higher plants—including both C_3 and C_4 plants—despite all lichen photobionts using the C_3 metabolic pathway. The $\delta^{13}C$ of lichens is governed primarily by moisture conditions, specifically the amount and phase of water required to activate and maintain photosynthesis. Three categories of lichens have been identified based on carbon isotope compositions, and which is related to the water requirements of the different photobionts whether cyanobacteria (cyanobionts), green algae (phycobionts) or a combination of both (photosymbiodemes). Phycobionts, for example, can initiate photosynthesis and reach maximum activity with lower water contents and when the water source is vapor alone (high humidity, dew or fog). Cyanobionts, on the other hand, require considerably more water and in the liquid phase (Lange et al. 1986; Lange et al. 1988). Fractionation of the carbon isotopes is induced by diffusion resistance of CO_2 through water-filled membranes (Lange et al. 1988) and/or structural changes in the photobiont and mycobiont cells caused by hydration and dehydration processes (Scheidegger et al. 1995). The presence of a carbon concentrating mechanism (CCM) employed by cyanobionts and some phycobionts has also been deduced, and that influences the overall isotopic composition of these particular organisms. Thus, lichens with cyanobionts (and phycobionts with a CCM) are "C_4-like" with $\delta^{13}C$ values $\gtrsim -23$‰; phycobionts without a CCM are more "C_3-like" with $\delta^{13}C$ values $\lesssim -24$‰; and photosymbiodemes have the lightest isotopic composition, with $\delta^{13}C$ values ~ −33‰ (Máguas et al. 1993, 1995; Smith and Griffiths 1996).

MATERIALS AND METHODS

Lichen samples were collected from southwestern Texas (29°53′N, 100°54′W) and northeastern Arkansas (92°45′N, 36°12′W) by removing a portion of the basal rock with the lichens intact. Subsamples were sent for identification to B Ryan (Arizona State University). To prepare for the analyses the sample surfaces were rinsed using E-pure (18.2 Mega Ohm/cm) water to remove loose detritus then dried in a 90 °C oven. A small (~1 mg) aliquot of the specimen was removed and analyzed using Fourier transform infrared analysis (FTIR) to establish the presence of oxalate.

AMS $\Delta^{14}C$ and $\delta^{13}C$ Analyses

^{14}C and stable carbon isotope ratio analyses were performed on 5 lichen species, 3 collected from limestone surfaces in southwestern Texas and 2 growing on sandstone in northeast Arkansas (Table 2). Samples were prepared by removing ~2 cm^2 of the lichen from the substrate with a dental pick, followed by grinding using an agate mortar and pestle. Approximately 150 mg of the powdered sample was placed in a Teflon beaker and 40 mL of dilute phosphoric acid (pH ~2.4) added to remove carbonates. The solution was maintained at pH <3.0 by drop-wise addition of concentrated phosphoric acid, and allowed to stand for ~48 hr with intermittent stirring. The sample was filtered using a micropore glass filter (10–15 μm) and the filtrate consisting of the lichen tissue and calcium oxalate pruina dried at 90 °C. Each sample was split, with one aliquot used for the accelerator mass spectrometry (AMS) ^{14}C measurement and the other for the stable carbon isotope analysis.

The AMS measurements were performed at either the Center for Accelerator Mass Spectrometry (CAMS) at Lawrence Livermore National Laboratories or the University of Arizona NSF-Arizona AMS Facility. Samples were further processed for the AMS assay by combusting the powder to CO_2 at 950 °C in the presence of CuO, and graphite targets produced using standard protocol (Vogel et al. 1987). The $\delta^{13}C$ analyses were performed using a Finnigan Delta Plus isotope ratio mass spectrometer (IRMS) interfaced with a Carlo Erba EA-1108 elemental analyzer; samples were combusted to CO_2 in the elemental analyzer, and the isotopic composition of the CO_2 was determined by continuous flow IRMS. $\delta^{13}C$ values are expressed relative to the V-PDB standard, and precision was $\leq 0.1‰$.

$\delta^{13}C$ Analysis of Separated Oxalate Pruina and Lichen Tissues

As above, the lichens were removed from the substrate using a dental pick and ground in an agate mortar and pestle. Approximately 100 mg of the powdered lichen was placed in a Teflon beaker along with 30 mL of 1.5 N HCl to remove carbonates and dissolve the calcium oxalate. The solution was stirred at 80 °C for 2 hr, then allowed to stand overnight to completely dissolve the calcium oxalate. The acid insoluble tissue, mainly thallus, was isolated from the solution by filtering through a micropore (10–15 μm) glass filter, and the solid residue dried at 90 °C.

We precipitated the calcium oxalate from the solution under a stream of N_2 to prevent contamination from atmospheric CO_2 by first neutralizing the mother liquor with boiling 3 N NaOH then adding 3 mL of saturated $CaCl_2$ solution. The calcium oxalate precipitate was filtered using a micropore (4– 8 μm) glass filter and dried at 90 °C. The stable carbon isotope ratios of each component was measured as described above. This method was tested via 5 separate trials of a single homogenized lichen (*Lecania* sp.) sample, measurements of 3 different areas of a single *Lecania* specimen, and experiments using a calcium oxalate standard.

RESULTS AND DISCUSSION

All lichens used in this study had calcium oxalate pruina, as established using FTIR. Another common feature was that each species contained green algal photobionts.

$\Delta^{14}C$ Values of Lichens Growing on Limestone and Sandstone

The AMS ^{14}C results show that the mean $\Delta^{14}C$ value of the sandstone species ($\Delta^{14}C$ = 129.0 ± 19.4‰) is similar to that of contemporary atmospheric CO_2 (Nydal and Lövseth 1983; Levin and Kromer 1997), while the mean $\Delta^{14}C$ value of the lichens growing on limestone ($\Delta^{14}C$ = 189.0 ± 22.8‰) is enriched in ^{14}C by 60‰ compared to the sandstone species (Table 2). Moreover, the ^{14}C values from the limestone samples are consistently greater than values obtained by Bench et al. (2001; 2002) from analyses of *Caloplaca trachyphylla* (mean ^{14}C = 175.8 ± 70.5‰) growing on sandstone and *Rhizocarpon geographicum* (mean $\Delta^{14}C$ = 152.6 ± 19.8‰) growing on siliceous rocks (Table 3). Thus, the limestone specimens have more atmospheric carbon that must have been incorporated during an earlier period when the atmospheric $^{14}CO_2$ concentration was higher (due to bomb ^{14}C production). This could be due to inclusion of older portions of the lichens during the sampling/scraping process or that the recent growth includes considerably more recycled carbon, a phenomenon demonstrated by Bench et al. (2001; 2002) for the 2 lichen species they studied.

Table 3 Average radiocarbon ($\Delta^{14}C$) data from lichens analyzed in this study and reported by Bench et al. (2001, 2002).

Substrate	Nr of species	Nr of measurements	Mean $\Delta^{14}C$ (‰)	Standard deviation (‰)
Limestone (this study)	3	6	189.0	22.8
Sandstone (this study)	2	2	129.0	19.4
Siliceous rocks (Bench et al. 2001)	1	24	152.6	19.8
Sandstone (Bench et al. 2002)	1	44	175.8	70.5

The comparison of the stable carbon isotope ratios between limestone and sandstone species, however, gives evidence that the $\delta^{13}C$ values for lichens growing on limestone are greater than those for lichens growing on sandstone. One interpretation is that the evident enrichment in ^{13}C for the lichen growing on limestone could be due to incorporation of carbonate from the substrate, but the ^{14}C results indicate this interpretation is unlikely. Instead, the difference in the $\delta^{13}C$ values might be attributed to differences in the moisture conditions in which these lichens were collected. Lichens growing in more moist conditions are depleted in ^{13}C compared to lichens growing in relatively drier environments (Shomer-Ilan et al. 1979; Teeri 1981). Because the collection sites in Arkansas (mean annual rainfall ~1200 mm/yr) are considerably wetter than the sites in Texas (mean annual rainfall ~ 450 mm/yr), this difference in the $\delta^{13}C$ values could be expected.

Two-sample t-tests and Wilcoxon rank sum tests were performed to determine if a statistical difference is present in the $\Delta^{14}C$ and $\delta^{13}C$ values for lichen species growing on limestone and lichen species growing on sandstone. A summary of these results follows, with p-values and observed test statistics appearing in Table 4. It should be pointed out that the statistical power of these tests is hindered by the small sample sizes. When sample sizes are small, the size of the effect must be large for it to be evident.

First, the $\Delta^{14}C$ and $\delta^{13}C$ data from the *Caloplaca saxicola* and *Lecania* lichen species growing on limestone were compared. The results suggest there is not a statistically significant difference in the

$\Delta^{14}C$ and $\delta^{13}C$ values among different lichen species growing on the same substrate. This proposition is further supported by comparing the $\delta^{13}C$ values for the different limestone species contained in Table 5. The data provides no evidence of a significant difference between the $\delta^{13}C$ values for the *Caloplaca* and *Lecania* species when comparing their isolated calcium oxalate pruina separated from the lichen tissues, the lichen tissues themselves, nor the $\delta^{13}C_{tis} - \delta^{13}C_{ox}$ differences for the 2 species. This suggests we can pool the $\Delta^{14}C$ and $\delta^{13}C$ values from the different lichen species growing on the same substrate and focus our attention on comparing the limestone values to those from other substrates.

Table 4 Results of statistical analyses: Two-sample t-tests and Wilcoxon rank sum test

		Test statistic		p-value
Comparisons between different lichen species growing on limestone	Location of data	t-test Wilcoxon	Degrees of freedom	t-test Wilcoxon
$\Delta^{14}C$	Table 2	t = 1.6062 W = 11	1.645 NA	0.275 0.4
$\delta^{13}C$	Table 2	t = –0.3157 W = 10	2.09 NA	0.781 0.8
$\delta^{13}C$ for isolated calcium oxalate	Table 5	t = –0.2378 W = 50	3.37 NA	0.826 0.7989
$\delta^{13}C$ for lichen tissues	Table 5	t = –0.3362 W = 50	7.40 NA	0.7460 0.7986
$\delta^{13}C_{tis}$ - $\delta^{13}C_{ox}$	Table 5	t = 0.0551 W = 55	9.57 NA	0.957 0.6711
		Test statistic		p-value
Comparisons after pooling values from different lichen species	Location of data	t-test Wilcoxon	Degrees of freedom	t-test Wilcoxon
$\Delta^{14}C$ limestone vs. $\Delta^{14}C$ sandstone	Table 2	t = –3.6253 W = 33	2.044 NA	0.9669 1
$\Delta^{14}C$ limestone vs. $\Delta^{14}C$ silica	Table 2 and Bench et al. (2001)	t = 3.60 —	7 —	0.009 —
$\Delta^{14}C$ limestone vs. $\Delta^{14}C$ sandstone	Table 2 and Bench et al. (2002)	t = 0.94 —	22 —	0.359 —
$\delta^{13}C$ limestone vs. $\delta^{13}C$ sandstone	Table 2	t = 5.076 W = 33	1.831 NA	0.0219 0.0357

The two-sample t-test and Wilcoxon rank sum test did not provide any statistical evidence that the $\Delta^{14}C$ values for lichens growing on limestone are less than the values for lichens growing on sandstone. In fact, the data contained in Table 2 suggest that the average $\Delta^{14}C$ values for lichen growing on limestone is at least 11.7‰ higher than the average $\Delta^{14}C$ for lichen growing on sandstone. A comparison of the $\Delta^{14}C$ values for the limestone species (Table 2) with those for *Rhizocarpon geographicum* growing on siliceous rocks (Bench et al. 2001) and *Caloplaca trachyphylla* growing on sandstone (Bench et al. 2002) was carried out utilizing two-sample t-tests with a Bonferroni correction (Bonferroni 1936; Miller 1981). The results suggest that the average $\Delta^{14}C$ values for lichen growing on limestone is at least 12.5‰ higher than the average $\Delta^{14}C$ for lichen growing on silica, and that there is no significant difference between the $\Delta^{14}C$ values for the limestone and sandstone substrates.

The mean $\Delta^{14}C$ value for the lichens growing on limestone is consistent with atmospheric CO_2 as the sole carbon reservoir if the lichen material was produced within the last 20–25 yr. However, we can-

not rule out that even older material was present in the samples, produced when the atmospheric ^{14}C abundance was even greater, and that this was combined with "dead" carbon from carbonate substrate to yield the observed $\Delta^{14}C$ values.

$\delta^{14}C$ Values of Isolated Calcium Oxalate Pruina and Acid-Insoluble Lichen Tissues

One purpose for separating the pruina from living lichens and measuring the $\delta^{13}C$ of the calcium oxalate and lichen tissues independently was to explore why the $\delta^{13}C$ values of calcium oxalate rock coatings are enriched in ^{13}C compared to the values reported in the literature for living lichens. The separation procedure was tested using a calcium oxalate standard, with a mean $\delta^{13}C$ value = $-17.42 \pm 0.02‰$. The mean $\delta^{13}C$ value of the processed standard ($-17.72 \pm 0.06‰$) indicated a 0.30‰ shift in the isotope composition of the treated calcium oxalate.

The results of the analysis of 5 aliquots from a single homogenized *Lecania* sample (oxalate pruina mean $\delta^{13}C$ value = $-15.62 \pm 0.08‰$; lichen tissue mean value = $-22.50 \pm 0.37‰$) demonstrated the method was reproducible (Table 5). The oxalate $\delta^{13}C$ values of the second *Lecania* sample, in which 3 separate areas from the same lichen specimen were removed and analyzed, also confirms the reproducibility of the method (oxalate pruina mean $\delta^{13}C$ value $-14.83 \pm 0.03‰$; lichen tissue mean value = $-22.50 \pm 0.14‰$).

The calcium oxalate pruina proved to be consistently enriched in ^{13}C compared to the lichen tissues. Specifically, the oxalate pruina (mean $\delta^{13}C = -15.19 \pm 0.92‰$) was estimated (with 95% confidence) to be enriched in ^{13}C by between 5.7‰ and 7.3‰ relative to the lichen tissue (mean $\delta^{13}C = -21.66 \pm 1.15‰$). This is even greater than the 5‰ difference between the tissues and intercellular calcium oxalate in cacti reported by Rivera and Smith (1979). It also shows that the $\delta^{13}C$ values of calcium oxalate rock coatings ($\delta^{13}C \sim -11.5‰$) are consistent with lichen sources with $\delta^{13}C$ values $\sim -18‰$, well within the $\delta^{13}C$ range reported for lichens (from $-35‰$ to $-14‰$; Lange et al. 1988).

Table 5 Stable carbon isotope ratios of lichen oxalate pruina and tissue separates

Species	Sample location	Substrate	Oxalate $\delta^{13}C$ (‰)	Tissue $\delta^{13}C$ (‰)	$\delta^{13}C_{ox} - \delta^{13}C_{tiss}$ (‰)
Lecania	SW Texas	Limestone	-15.62 ± 0.08[a]	-22.50 ± 0.37[a]	6.88
Lecania	SW Texas	Limestone	-14.83 ± 0.03[b]	-22.50 ± 0.14[b]	7.67
Lecania	SW Texas	Limestone	−15.05	−23.42	8.37
Lecania	SW Texas	Limestone	−15.45	−21.06	5.61
Lecania	SW Texas	Limestone	−14.96	−22.61	7.65
Lecania	SW Texas	Limestone	−16.04	−19.52	3.48
Lecania	SW Texas	Limestone	−16.37	−20.44	4.07
Lecania	SW Texas	Limestone	−15.00	−21.90	6.90
Caloplaca	SW Texas	Limestone	−15.22	−21.16	5.94
Caloplaca	SW Texas	Limestone	−14.98	−21.61	6.63
Caloplaca	SW Texas	Limestone	−13.37	−20.36	6.99
Caloplaca	SW Texas	Limestone	−17.30	−22.90	5.60
Verrucaria	SW Texas	Limestone	−13.99	−21.57	7.58
Verrucaria	SW Texas	Limestone	−14.30	−22.20	7.90
Unidentified	SW Texas	Limestone	−15.45	−20.00	4.55
Lecanora	Arizona	Quartzite breccia	−15.03	−22.87	7.84
			-15.19 ± 0.92	-21.66 ± 1.19	6.48 ± 1.47

[a] Average ±1 σ of 5 aliquots from 1 homogenized lichen sample

[b] Average ±1 σ of 3 different regions of a single lichen sample treated independently

CONCLUSIONS

We measured the ^{14}C content of what appeared to be recent growth of 5 oxalate-producing lichen species, 3 of which were growing on limestone in southwestern Texas and 2 on sandstone in northeastern Arkansas. The $\Delta^{13}C$ values of the sandstone species were consistent with contemporary atmospheric $^{14}CO_2$ levels, while the limestone species were enriched in ^{14}C by approximately 60‰ compared to those growing on the sandstone. This result is opposite of what was expected if substrate carbonate was a significant source of carbon.

While it might be reasonable to expect that little or no "dead" carbon from the limestone was incorporated by the lichens growing on this substrate, these results do not provide definitive evidence that this is the case. Without knowing the true age of the sampled areas, and thus the actual amount of atmospheric ^{14}C incorporated by organisms, the amount of carbon from the limestone, if any, remains ambiguous.

The stable carbon isotope composition of the calcium oxalate pruina produced by these lichens is 6.5‰ enriched in ^{13}C compared to lichen tissues. Calcium oxalate rock coatings thought to be byproducts of past lichen activity have $\delta^{13}C$ values that range from −6.8 to −13.7‰ (Table 1), and thus produced by lichens with $\delta^{13}C$ values that range from −13.3 to −20.2‰. These values are consistent with the stable carbon isotope composition of lichens that have cyanobacterial photobionts, or green algal photobionts that employ a CO_2 concentrating mechanism.

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REFERENCES

Bench G, Clark BM, Mangelson NF, St Clair LL, Rees LB, Grant PG, Southon JR. 2001. Accurate lifespan estimates cannot be obtained from ^{14}C profiles in the crustose lichen Rhizocarpon geographicum (L.) DC. *Lichenologist* 33:539–42.

Bench G, Clark BM, Mangelson NF, St Clair LL, Rees LB, Grant PG, Southon JR. 2002. Use of $^{14}C/C$ ratios to provide insights into the magnitude of carbon turnover in the crustose saxicolous lichen Caloplaca trachyphylla. *Lichenologist* 34:169–80.

Bonaventura MPD, Maddalena DG, Cacchio P, Ercole C, Lepidi A. 1999. Microbial formation of oxalate films on monument surfaces: bioprotection or biodeterioration? *Geomicrobiology Journal* 16:55–64.

Bonferroni CE. 1936. Teoria statistica delle classi e calcolo delle probabilita. Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze 8:3–62.

Chaffee SD, Hyman M, Rowe MW, Coulam NJ, Schroedl A, Hogue K. 1994. Radiocarbon dates on the All American Man pictograph. *American Antiquity* 59:769–81.

Craig H. 1953. The geochemistry of the stable carbon isotopes. *Geochemica et Cosmochemica Acta* 3:53–92.

Degens ET. 1969. Biogeochemistry of the stable carbon isotopes. In: Eglinton G, Murphy M, editors. *Organic geochemistry*. New York: Springer Verlag. p 304–29.

Del Monte M, Sabbioni C, Zappia G. 1987. The origin of calcium oxalates on historical buildings, monuments and natural outcrops. *The Science of the Total Environment* 67:17–39.

Edwards HGM, Farwell DW, Seaward MRD. 1993. Raman spectra of oxalates in lichen encrustations on Renaissance frescoes. *Spectrochimica Acta* 47A:1531–9.

Edwards HGM, Russel NC, Seaward MRD. 1997. Calcium oxalate in lichen biodeterioration studied using FT-Raman spectroscopy. *Spectrochemica Acta* Part A 53:99–105.

Hedges REM, Ramsey CB, Van Klinken GJ, Petitt PB, Nielsen-Marsh C, Etchegoyen A, Niello JOF, Boschin MT, Llamazares AM. 1998. Methodological issues in the ^{14}C dating of rock paintings. *Radiocarbon* 40(1): 35–44.

Hofmann BA, Bernasconi SM. 1998 Review of occurrences and carbon isotope geochemistry of oxalate minerals: implications for the origin and fate of oxalate in diagenetic and hydrothermal fluids. *Chemical*

Geology 149:127–46.

Lange OL, Kilian E, Zielger H. 1986. Water vapor uptake and photosynthesis of lichens: performance differences in species with green and blue-green alae as phycobionts. *Oecologia* 71:104–10.

Lange OL, Green TGA, Ziegler H. 1988. Water status related photosynthesis and carbon isotope discrimination in species of lichen genus Pseudocyphellaria with green or blue-green photosymbiodemes. *Oecologia* 75:494–501.

Lapeyrie F, Chilvers GA, Bhem CA. 1987. Oxalic acid synthesis by the mycorrhizal fungus Paxillus involutus. *New Phytology* 106:139–46.

Lapeyrie F. 1988. Oxalate synthesis from soil bicarbonate by the mycorrhizal fungus Paxillus involutus. *Plant and Soil* 110:3–8.

Levin I, Kromer B. 1997. Twenty years of atmospheric $^{14}CO_2$ observations at Schauinsland Station, Germany. *Radiocarbon* 39(2):205–18.

Máguas C, Griffiths H, Broadmeadow MSJ. 1995. Gas exchange and carbon isotope discrimination in lichens: evidence for interactions between CO_2-concentrating mechanisms and diffusion limitations. *Planta* 196: 95–102.

Máguas C, Griffiths H, Ehleringer J, Serôdio J. 1993. Characterization of photobiont associations in lichens using carbon isotope discrimination techniques. In: Ehleringer J, Hall A, Farquhar G, editors. *Stable isotopes and plant-water relations*. New York: Academic Press. p 201–12.

Miller RG. 1981. *Simultaneous statistical inference*. 2nd ed. Springer Verlag. p 6–8.

Nydal R, Lövseth K. 1983. Tracing bomb ^{14}C in the atmosphere 1962–1980. *Journal of Geophysical Research* 88:3621–3642.

Rivera ER, Smith BN. 1979. Crystal morphology and $^{13}C/^{12}C$ composition of solid oxalate in cacti. *Plant Physiology* 64:966–70.

Russ J, Palma RL, Loyd DH, Boutton TW, Coy MA. 1996. Origin of the whewellite-rich rock crust in the Lower Pecos Region of southwest Texas and its significance to paleoclimate reconstructions. *Quaternary Research* 46:27–36.

Russ J, Kaluarachchi WD, Drummond L, Edwards HGM. 1999. The nature of a whewellite-rich rock crust associated with pictographs in southwestern Texas. *Studies in Conversation* 44:91–103.

Russ J, Loyd DH, Boutton TW. 2000. A paleoclimate reconstruction for southwestern Texas using oxalate residue from lichen as a paleoclimate proxy. *Quaternary International* 67:29–36.

Scheidegger C, Schroeter B, Frey B. 1995. Structural and functional processes during water vapour uptake and desiccation in selected lichens with green algal photobionts. *Planta* 197:399–409.

Scott DH, Hyder WD. 1993. A study of the California Indian rock art pigments. *Studies in Conservation* 38: 155–73.

Shomer-Ilan A, Nissenbaum A, Galun M, Waisel Y. 1979. Effect of water regime on carbon isotope composition of lichens. *Plant Physiology* 63:201–5.

Smith EC, Griffiths H. 1998. Intraspecific variation in photosynthetic responses of trebouxoid lichens with reference to the activity of a carbon-concentrating mechanism. *Oecologia* 113:360–9.

Steelman KL, Rickman RD, Rowe MW, Boutton TW, Russ J, Guidon N. 2001. AMS radiocarbon ages of an oxalate accretion and rock paintings at Toca do Serrote da Bastiana, Brazil. In: Jakes K, editor. *Archaeological chemistry V*. Washington, DC: American Chemical Society. p 22–35.

Teeri JA. 1981. Stable carbon isotope analysis of mosses and lichens growing in xeric and moist habitats. *The Bryologist* 84:82–4.

Various authors. 1996. In: Realini M, Toniolo L, editors. *The Oxalate films in the conservation of works of art*. Milan: Centro CNR. 539 p.

Vogel JS, Nelson DE, Southon JR. 1987. ^{14}C background levels in an accelerator mass spectrometer system. *Radiocarbon* 29(3):323–33.

Wadsten T, Moberg R. 1985. Calcium oxalate hydrates on the surface of lichens. *Lichenologist* 17:239–45.

Watchman AL. 1991. Age and composition of oxalate-rich crusts in Northern Territory, Australia. *Studies in Conservation* 36:24–32.

Watchman AL. 1993. Evidence of a 25,000-year-old pictograph in Northern Australia. *Geoarchaeology* 8: 465–73.

Watchman AL, David B, McNiven IJ, Flood JM. 2000. Micro-archaeology of engraved and painted rock surface crusts at Yiwarlarlay (the Lightning Brothers site), Northern Territory, Australia. *Journal of Archaeological Science* 27:315–25.