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Oxygen isotope ratio stratification in a tropical moist forest

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Summary. Oxygen isotope ratios were determined in leaf cellulose from two plant species at Barro Colorado (Republic of Panama) in 4 different plots, two of which were undergoing an irrigation treatment during the dry season. There is a gradient in $\delta^{18}O$ values of leaf cellulose from the understory to canopy leaves, reflecting the differences in relative humidity between these two levels of the forest. This gradient is most pronounced in irrigated plots. For irrigated plots there was a highly significant correlation between $\delta^{18}O$ and $\delta^{13}C$ values, which was not observed in control plots. This relationship can be explained by humidity controlling stomatal conductance. Low humidity affects $\delta^{18}O$ values of leaf water during photosynthesis, which isotopically labels cellulose during its synthesis. Low humidity also decreases stomatal conductance, which affects discrimination against carbon-13 by photosynthetic reactions, thus affecting the $\delta^{13}C$ values of photosynthates. WUE values calculated by using plant carbon and oxygen isotope ratios were similar to those observed with gas exchange measurements in other tropical and temperate areas. Thus the concurrent analysis of carbon and oxygen isotope ratios of leaf material can potentially be useful for long term estimation of assimilation and evapotranspiration regimes of plants.

Key words: Stable isotope – Water use efficiency – Humidity – Tropical forests – Microenvironment

Stratification of carbon isotope ratios of plant organic matter in tropical and temperate forests has been well documented (Vogel 1978; Medina and Minchin 1980; Medina et al. 1986; Francey et al. 1985; Ehleringer et al. 1986; Schleser and Jayasekera 1985; Sternberg et al. 1989). Understory plants of forests are depleted in carbon-13 relative to plants in the canopy. This shift in carbon isotope ratios is due to the higher discrimination against ^{13}C during photosynthesis at the forest understory caused by lower light intensities (Francey et al. 1985; Ehleringer et al. 1986) as well as the increased input of isotopically depleted respired carbon dioxide at the forest understory (Medina et al. 1986; Sternberg et al. 1989). Gradients in carbon dioxide concentration due to the input of respired carbon dioxide in forests have been well documented by several investigators (Odum et al. 19780; Medina et al. 1986; Allen et al. 1972; Wofsy et al. 1988; Sternberg et al. 1989). Respired carbon dioxide having a $\delta^{13}C$ value of about $-28‰$ mixes with atmospheric carbon dioxide having a $\delta^{13}C$ value of $-7.8‰$ (Medina et al. 1986). We have previously observed that at lower levels of a tropical moist forest this mixture has $\delta^{13}C$ values as low as $-13‰$ and may be composed of up to 18% respired carbon dioxide (Sternberg et al. 1989).

Gradients in humidity in tropical forest have also been well documented (Allen et al. 1972; Lemon et al. 1970; Odum et al. 1970). Although carbon isotope ratios may be affected by relative humidity at different levels of a forest, it is difficult to determine whether changes in $\delta^{13}C$ values of plant matter reflect changes in humidity, in other environmental factors causing changes in the intercellular to external carbon dioxide concentration ratio, or in the input of respired carbon dioxide. Unlike carbon isotope ratios, oxygen isotope ratios of cellulose are more directly determined by humidity during photosynthesis (Burk and Stuiver 1981; Edwards et al. 1985; Fehrii and Letolle 1977). With the measurement of carbon and oxygen isotopic ratios of cellulose, it may be possible to assess on an integrative basis the relation between transpiration and assimilation. In this study we measured oxygen isotope ratios of leaf cellulose from plants at different levels of a tropical moist forest, and examined the possibility of using oxygen isotope ratios to determine (1) the relative humidity gradient from lower levels to the upper canopy of a tropical moist forest, (2) the effect of irrigation, and with carbon isotope ratios, (3) the photosynthetic water use efficiency.

Interpretation of leaf ^{18}O composition

There are three potential sources of oxygen for photosynthates in plants: oxygen from carbon dioxide and water may influence the $\delta^{18}O$ values of carbohydrates during photosynthesis, and atmospheric oxygen could influence the $\delta^{18}O$ values of carbohydrates during photorespiration (Sternberg 1989). Initially it was assumed that 2/3 of the oxygen isotopic signature for plant cellulose came from the oxygen isotope ratios of carbon dioxide, and 1/3 came from the oxygen isotope ratios of water available for photosynthesis (Epstein et al. 1977). The work of DeNiro and Epstein (1979), however, showed that oxygen isotope ratios of carbon dioxide are of little consequence to the oxygen isotope ratios of plant cellulose. Thus, water seems to be the primary labeling agent of cellulose. It is suspected that carbonyl oxygens from carbohydrates and other intermediates exchang-

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ing with water, may be the chemical step determining $\delta^{18}O$ values of cellulose (DeNiro and Epstein 1981; Sternberg and DeNiro 1983; Sternberg et al. 1986b; Sternberg 1989). Further, studies indicate that $\delta^{18}O$ values of plant cellulose are 27‰ (with minor variations) higher than the oxygen isotope ratios of the water available for photosynthesis (DeNiro and Epstein 1981; Sternberg et al. 1984; Sternberg et al. 1986a). The possibility that $\delta^{18}O$ values of cellulose are influenced by photorespiratory oxygen has not been fully considered, but studies by Berry et al. (1978) showed that when plants were exposed to highly enriched oxygen, double labeled intermediates of the Calvin Cycle were not observed. Although much work is still necessary to determine the exact mechanism of ^{18}O incorporation in cellulose, in this study, as in other studies (Burk and Stuiver 1981; DeNiro and Epstein 1981; Edwards et al. 1985; Sternberg et al. 1986b), we will use the empirical evidence that $\delta^{18}O$ values of cellulose are 27‰ higher than the water available for cellulose synthesis. This relationship can be expressed by the following equation:

$$\delta^{18}O_{cell} = \delta^{18}O_{lw} + 27‰ \quad (1)$$

where $\delta^{18}O_{cell}$ and $\delta^{18}O_{lw}$ are the $\delta^{18}O$ values of leaf cellulose and average value of leaf water respectively.

Oxygen isotope ratios of leaf water will not be the same as that of ground water. Although there are no isotopic fractionations associated with uptake of water (Wershaw et al. 1966; White et al. 1985; Sternberg and Swart 1987), there are fractionations associated with evapotranspiration of leaf water. Because $H_2{}^{16}O$ will evaporate faster than $H_2{}^{18}O$, there will be an accumulation of $H_2{}^{18}O$ in the leaf water until steady is reached; i.e. when the $\delta^{18}O$ value of transpired water is the same as that of soil water. Two different plant species exposed to the same humidity, however, will not always have leaf water with identical $\delta^{18}O$ values since there may be compartments in the leaf (e.g. vascular system) with water not undergoing evapotranspiration nor isotopic enrichment (Leaney et al. 1985). Thus the $\delta^{18}O$ values of leaf water will be determined by the mixture of leaf water undergoing evapotranspiration (mesophyll) and the fraction not undergoing evapotranspiration (vascular). The proportion of these fractions can vary from species to species.

The $\delta^{18}O$ value of the leaf water fraction evaporating under steady state and at the same temperature as ambient air is predictable by the equation developed by Dongman et al. (1974):

$$\delta^{18}O_{lw} = \delta^{18}O_s(1-h) + h\,\delta^{18}O_{amb} + \varepsilon^* + \varepsilon_k(1-h). \quad (2)$$

Where ε^* and ε_k are equilibrium and kinetic fractionation factors respectively; $\delta^{18}O_{lw}$, $\delta^{18}O_s$, $\delta^{18}O_{amb}$ are $\delta^{18}O$ values of leaf water, soil or stem, and ambient vapor respectively, and h is the relative humidity. Since we are relating $\delta^{18}O$ values of cellulose, which is a long term integrator of plant isotopic fractionation, to relative humidities, henceforth the values for Eq. 2 defined above will imply average rather than spontaneous values. The value ε^* is calculated by the following equation:

$$\varepsilon^* = 2.644 - 3.206\,(10^3/T) + 1.534\,(10^6/T^2) \quad (3)$$

where T is the ambient temperature in degrees Kelvin (Bootinga and Craig 1969). The value ε_k is the kinetic isotope fractionation factor determined to be 16‰ (Burk and Stuiver 1981). In moist tropical areas soil water has the $\delta^{18}O$ value of the weighted yearly average rainfall (Gat 1981). Thus

$$\delta^{18}O_s = \delta^{18}O_r, \quad (4)$$

where $\delta^{18}O_r$ is the yearly average rainfall. In order to calculate the average $\delta^{18}O$ value of ambient vapor in the forest understory ($\delta^{18}O_{amb}$) in Eq. 2, the assumption is made that the higher relative humidity in forest microenvironments relative to open areas is principally because of steady state evapotranspiration in leaves. Thus ambient vapor is a mixture of two pools of water vapor. One pool, that which is the source of rain, is approximated by the following relationship:

$$\delta^{18}O_{atm} = \delta^{18}O_r - \varepsilon^*, \quad (5)$$

where $\delta^{18}O_{atm}$ is the $\delta^{18}O$ value of the atmospheric vapor (Allison et al. 1985; Gat 1981; Burk and Stuiver 1981; Edwards et al. 1985). The other pool is derived from steady state evapotranspiration in the leaves and thus having the same $\delta^{18}O$ value of soil water. The proportion of each of these pools is h'/h for the atmospheric vapor pool, and $(h-h')/h$ for the excess water vapor derived from evapotranspiration, where h' is the relative humidity in clear open areas and h is the relative humidity within the forest. Using a mass balance equation for the fraction of water from each pool and Eqs. 4 and 5, $\delta^{18}O$ values of atmospheric vapor at different microenvironments can be expressed by the following equation:

$$\delta^{18}O_{amb} = \left[h'\left(\frac{\delta^{18}O_s - \varepsilon^*}{h}\right)\right] + \frac{h-h'}{h}(\delta^{18}O_s). \quad (6)$$

This equation simplifies to

$$\delta^{18}O_{amb} = \frac{h\,\delta^{18}O_s - h'\varepsilon^*}{h}. \quad (7)$$

Inserting this equation into Eq. 2 and using the relationship expressed by Eq. 1 the following equation relating the average relative humidity during photosynthesis and the $\delta^{18}O$ value of cellulose is derived:

$$h = 1 - \frac{\delta^{18}O_{cell} - 27‰ - \delta^{18}O_s - \varepsilon^*(1-h')}{\varepsilon_k}. \quad (8)$$

Therefore given the $\delta^{18}O$ value of soil water which approximates that of the weighted average rainwater (Gat 1981), and leaf cellulose, average relative humidity in cleared areas, and temperature, it should be possible to estimate the integrative relative humidity of the microenvironment the leaf was exposed to during the course of photosynthesis.

Materials and methods

All leaf samples were collected at Barro Colorado Island (Republic of Panama). Detailed description of BCI can be found in Croat (1978) and Leigh et al. (1982). Climate in Barro Colorado is characterized by a wet season (May to mid-December) and a dry season (mid-December to beginning of May, Croat 1978), with a mean annual temperature of 27° C. Relative humidities in the forest are usually 10 to 20% greater than in clearings (Croat 1978).

Understory relative humidities used for comparison with the isotopically-derived values were measured during the

1988 dry season in irrigated and control plots between 08.00–16.00 h using a thermocouple psychrometer. Measurements were collected 4–5 days each week between early January and late April at least 3 times per hour at 1.3 m above the soil. Means derived from these data were weighted such that the mean from each one hour period was accorded equal importance. Relative humidities from 1987 are not reported here because values were determined for only a few days during early and mid dry season. Relative humidities determined at midday in the laboratory clearing on BCI did not differ between the dry seasons of 1987 and 1988 (Smithsonian Environmental Sciences Program weather station data). Similarly, relative humidities measured with a sling psychrometer at four different locations in each control and irrigated plot at midday did not differ between 1987 and 1988.

Isotopically-derived values of canopy relative humidity are compared to the relative humidities measured in the laboratory clearing at midday on BCI as reported by Croat (1978) for the months of December through May.

Leaf samples were collected at the end of the 1986–87 dry season at 1 m for shaded plants, and between 9 and 25 m for leaves exposed to direct sun from the canopy as in Sternberg et al. (1989). Carbon isotope ratios of these leaf samples were previously reported (Sternberg et al. 1989). Samples from two species *Hirtella triandra* and *Tetragastris panamensis* were collected from four different plots, two of which were undergoing an artificial irrigation treatment during the dry season. A more complete description of this experiment can be found in Sternberg et al. (1989).

Leaf samples were dried at 50° C, deveined and ground on a Wiley mill to mesh 20 (about 1 mm^2 orifice size). Cellulose was extracted according to the method of Wise (1944) using sodium chlorite and acetic acid. This extracted cellulose was used for oxygen isotope analysis. Oxygen isotope analysis was done by the method of Burk (1979) except that isoquinoline was used in the place of quinoline. Oxygen isotope ratios were determined on the carbon dioxide extracted from this procedure with aprecision of ± 0.5‰. $\delta^{18}O$ values reported here are expressed relative to SMOW (standard mean ocean water). Samples were analyzed in a PRISM (V.G.) mass spectrometer at the Department of Geology, University of Miami, Coral Gable, FL 33124.

Results and discussion

Stratification of $\delta^{18}O$ values

$\delta^{18}O$ values of leaf cellulose at 1 m were significantly lower than the corresponding species at 9 m or above for both species in irrigated plots (Fig. 1). It is unlikely that this effect is due to differences between isotope ratios of irrigation and ground water. First the two species tested here have relatively shallow roots regardless of the developmental stage and therefore the irrigation effect would also be found in canopy plants. Second, irrigation water was from nearby lake Gatum. If it is different than ground water, it would be more enriched in oxygen-18 than ground water because of evaporation. Therefore the effect of irrigation would be to increase the $\delta^{18}O$ values of leaf cellulose in irrigated plots relative to control plots, and yet the opposite was observed. For control plots $\delta^{18}O$ values of understory leaves were lower than canopy leaves, but differences were not pronounced. Using the previously reported $\delta^{18}O$ value

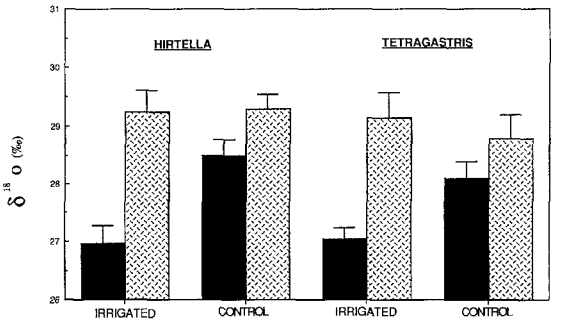

Fig. 1. Average $\delta^{18}O$ values of leaf cellulose for *Hirtella* and *Tetragastris* at heights of 1 m (*solid bars*) and heights at or above 9 m (*stippled bar*) for irrigated and control plots. Significant differences exists between $\delta^{18}O$ values of understory and canopy leaves for irrigated plots ($F=23.57$, $P<0.01$, df=1,10 for *Hirtella*; and $F=19.55$, $P<0.01$, df=1,10 for *Tetragastris*), while differences are less apparent in control plots ($F=4.33$, $P<0.07$, df=1,11 for *Hirtella*; and $F=2.00$, $P<0.19$, df=1.13 for *Tetragastris*). $\delta^{18}O$ value of understory leaves in irrigated plots are significantly lower than that of understory leaves in control plots ($F=12.13$, $P<0.01$, df=1,10 for *Hirtella*; $F=7.20$, $P<0.02$, df=1, 13 for *Tetragastris*). Errors bars are 2 SEM

of the weighted average yearly rainfall value for Panama as the value of soil water (−5.64‰, Gat 1981) and Eq. 7, the average relative humidity estimated for each treatment and forest level are shown on Table 1, these values compare favorably with average values measured in the field during the dry season. The relative humidity values calculated from $\delta^{18}O$ values of leaf cellulose from canopy plants may be lower than the actual daytime relative humidity, since the comparison on Table 1 is with the midday average (Croat 1978). The calculated relative humidities reported here are consistent with the observation that irrigation carried out at Barro Colorado had a significant effect in the relative humidity at the lower levels of the forest (Wright and Cornejo 1988).

Covariation in $\delta^{18}O$ and $\delta^{13}C$ values

A highly significant relationship between $\delta^{18}O$ and $\delta^{13}C$ values of leaves from irrigated plots but not for leaves from control plots was observed (Figs. 2 and 3). Two possible hypothesis may explain this relationship. First, the covariation of relative humidity at different levels of the forest with input of isotopically depleted respired carbon dioxide could cause the observed correlation between $\delta^{18}O$ and $\delta^{13}C$ values. Lower levels of the forest have a high input of respired carbon dioxide and higher relative humidity, whereas, upper levels have a lower input of respired carbon dioxide and lower relative humidities, increasing the $\delta^{18}O$ and $\delta^{13}C$ values of leaf tissues. When the effect of respired carbon dioxide is eliminated and $\delta^{18}O$ values are plotted against the discrimination for carbon-13(Δ), however, the relationship between $\delta^{18}O$ and Δ values of leaf tissue remain high ($r=0.93$ and 0.71 for *Tetragastris* and *Hirtella* in irrigated plots respectively). The discrimination factor was calculated by subtracting the $\delta^{13}C$ values of shade leaves from $\delta^{13}C$ values of ambient CO_2 at 1 m ($\delta^{13}C=-10.6$‰, Sternberg et al. 1989) and the $\delta^{13}C$ values of sun leaves from $\delta^{13}C$ values of ambient CO_2 at 25 m ($\delta^{13}C=-8.9$‰, Sternberg et al. 1989). Further, if this hypothesis were correct the same correlation would be observed in control plots, and this is not the case.

Table 1. Average $\delta^{18}O$ and $\delta^{13}C$ values (± 1 SEM) for *Hirtella* and *Tetragastris* collected at understory and canopy levels for irrigated and non irrigated (control) plots. Also shown on table is the relative humidity during photosynthesis calculated by using measured $\delta^{18}O$ values and Eq. 7 as well as the directly measured relative humidity during the dry season of 1988 as explained in the text. Relative humidity from upper canopy samples are compared with average values reported in clearings during the dry season by Croat (1978)

Species	$\delta^{18}O$‰	$\delta^{13}C$‰	Treatment	Height	RH(calc)	RH(meas)
Hirtella	27.0 ± 0.3	-33.8 ± 0.2	Irrigation	Low	82.4%	82.6%[a]
Hirtella	28.5 ± 0.3	-32.5 ± 0.4	Control	Low	72.9%	72.2%[a]
Hirtella	29.2 ± 0.3	-30.0 ± 0.6	Irrigation	High	68.3%	68.6%[b]
Hirtella	29.3 ± 0.2	-29.4 ± 0.7	Control	High	67.6%	68.6%[b]
Tetragastris	27.0 ± 0.2	-32.8 ± 0.6	Irrigation	Low	82.4%	82.6%[a]
Tetragastris	28.1 ± 0.3	-33.3 ± 0.3	Control	Low	75.4%	72.2%[a]
Tetragastris	29.2 ± 0.4	-28.9 ± 0.5	Irrigation	High	68.3%	68.6%[b]
Tetragastris	28.8 ± 0.4	-28.7 ± 0.3	Control	High	70.8%	68.6%[b]

[a] Relative humidities taken from irrigated and control sites during the dry season of 1988. See Materials and methods for explanation
[b] Croat 1978

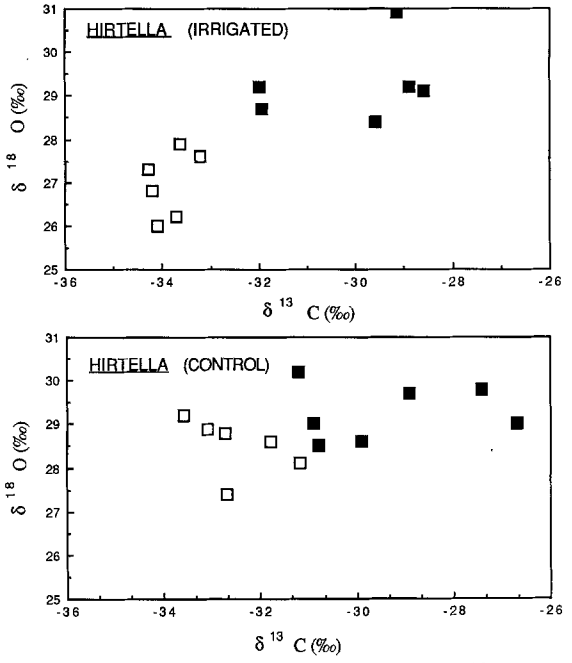

Fig. 2. Relationship between $\delta^{18}O$ values and $\delta^{13}C$ values for *Hirtella* in irrigated ($r = 0.80$, $P < 0.01$) and control plots $\delta^{18}O$ ($r = 0.29$, $P > 0.05$). Open squares are for isotopic values of understory plants and closed squares are for those of canopy leaves

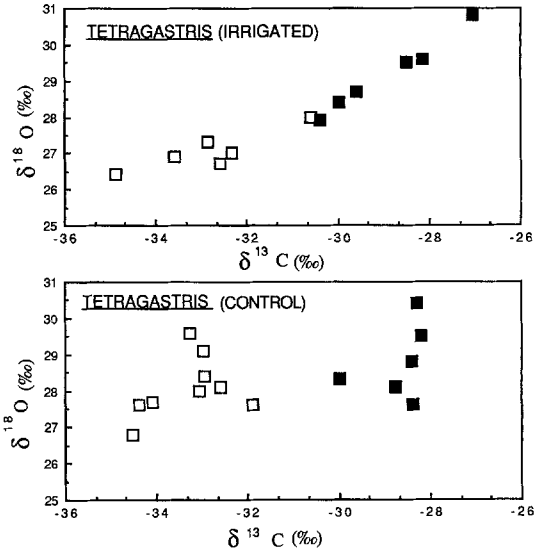

Fig. 3. Relationship between $\delta^{18}O$ and $\delta^{13}C$ values for *Tetragastris* in irrigated ($r = 0.96$, $P < 0.01$) and control plots ($r = 0.21$, $P > 0.05$). Symbols are as described in Fig. 2

The second hypothesis is that environmental factors responsible for changes in ^{13}C discrimination covaries with changes in relative humidity which would affect the $\delta^{18}O$ values of cellulose. One of these factors could be the variation in light intensity. At upper sites in irrigated plots higher light intensities and lower humidities cause a lower discrimination against carbon-13 because of high assimilation rates and a higher evaporative regime respectively, giving the leaf tissue a relatively high $\delta^{13}C$ and $\delta^{18}O$ value. At lower sites, low light intensities and higher humidity cause higher discrimination against carbon-13 and a low evaporative regime respectively, giving the leaf relatively low $\delta^{13}C$ and $\delta^{18}O$ values. For control plots stomata may be variably opened or closed depending on the water status of the plant (Schulze 1986); adding an additional variable to carbon-13 discrimination during photosynthesis, and thus no such correlation is observed.

Humidity itself could be an environmental factor affecting discrimination against carbon-13 during photosynthesis. Control of the internal to external CO_2 concentration ratios in leaves by the vapor pressure gradient has been previously observed in a Eucalypt forest stand (Wong and Dunin 1987). In irrigated plots the primary control of stomatal opening may be humidity, while for plants in control plots, stomatal conductance may be controlled by other factors such as root or leaf water potential (Schulze 1986) and leaf temperature (Ball et al. 1988). Thus in irrigated plots when the humidity is low, cellulose will have a relatively high $\delta^{18}O$ value (Eq. 7). Further, low humidity will cause stomatal closure decreasing the conductance to carbon dioxide, increasing the carbon dioxide gradient, and thus decreasing the discrimination against carbon-13 by photosynthesis, giving this same leaf a relatively high $\delta^{13}C$ value (Farquhar et al. 1982).

Water use efficiency

The simplest definition of water use efficiency is given by the formula

$$\text{WUE (mmoles/mole)} = \frac{c_a - c_i}{\Delta w\, 1.6} \quad (9)$$

where c_a and c_i are the atmospheric and intercellular carbon dioxide concentration (mmoles/mole) respectively, and Δw is water vapor concentration gradient (moles/mole). If leaf temperature is the same as ambient temperature, this equation can be rewritten in the form

$$\text{WUE} = \frac{c_a\left(1 - \frac{c_i}{c_a}\right)}{V(1-h)\, 1.6}, \quad (10)$$

where V is the vapor concentration (mole/m^3) of water at saturation for a particular temperature.

For understory WUE, we used leaf temperatures collected during the dry season of 1986–87 from a common understory shrub (*Psychotria limonensis*) growing in shaded, sunflecked sites similar to understory sites where leaves for ^{18}O analysis were collected (27.2 ± 1 SEM °C for irrigated sites; 28.0 ± 0.1° C for control sites). These temperatures were usually only a few tenths of a degree different from ambient air temperature. Leaf temperatures of canopy leaves were estimated to be 1.6° C above temperatures in the understory control sites (29.6° C) as indicated by air temperature data taken at 1 m and above the canopy by the Smithsonian Environmental Sciences Program at BCI. Thus for canopy leaves, there may be some error in the calculation of WUE because leaf temperatures may not vary solely as a function of air temperature over this gradient. Equation 8 can be used to express the value of $(1-h)$ in Eq. 10 in terms of oxygen isotope ratios. The term $[1-(c_i/c_a)]$ can be expressed by using the equation

$$\Delta = a + (b-a)\cdot\frac{c_i}{c_a}, \quad (11)$$

where Δ is the photosynthetic discrimination factor (approximately equal to $\delta^{13}C_{\text{atmospheric}} - \delta^{13}C_{\text{plant}}$), b and a are the carbon-13 discrimination by ribulose bis-phosphate carboxylase (27‰) and stomatal resistance (4.4‰) respectively (Farquhar et al. 1982). The following equation is derived

$$\text{WUE} = \frac{[c_a]\left\{1 - \frac{\Delta - 4.4}{22.6}\right\}}{V\left\{\frac{\delta^{18}O_{\text{cell}} - 27‰ - \delta^{18}O_s - \varepsilon^*(1-h')}{\varepsilon_k}\right\} 1.6}. \quad (12)$$

The average isotopically derived W.U.E. values for *Hirtella* and *Tetragastris* in control and irrigated plots was 6.2 mmole/mole (Fig. 4). This average is the same as the average value previously reported by Yoshie (1986) for plants from a temperate forest (6.2 mmole/mole). These values are also similar to WUE of *Nicotiana glauca* and *Corylus avellana* under high humidities (Farquhar et al. 1980). Further, Ehlerlinger et al. (1986) observed WUE values as high as 7 mmoles/moles for plants in environments having high humidity using carbon isotope ratios and measured relative humidity. Surprisingly, no significant difference in water use efficiency was observed between leaf samples from the understory and canopy in control plots (Fig. 4). This constancy in WUE may be explained by microenvironmental effects causing a positive relationship between CO_2 assimilation and evaporative water loss at different levels of the forest. However, further validation of this observation will require careful long term studies of gas exchange of plants exposed to different humidities and comparing WUE values derived from gas exchange with those derived from carbon and oxygen isotope ratios.

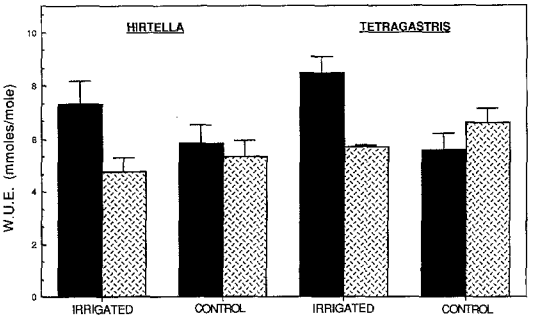

Fig. 4. Average WUE of leaves from *Hirtella* and *Tetragastris* in irrigated and control treatements at 1 m (*solid bars*) and heights greater than 9 m (*stipled bars*). In irrigated sites, WUE in the understory is significantly higher than WUE in the canopy ($F = 7.08$, $P < 0.03$, df = 1,10 for *Hirtella*; $F = 22.29$, $P < 0.01$, df = 1,10 for *Tetragastris*). There is no significant difference for canopy and understory leaves of *Hirtella* in control sites ($F = 0.50$, $P < 0.50$, df = 1,11), while leaves of *Tetragastris* show a trend in control sites ($F = 3.20$, $P < 0.10$, df = 1,13). Error bars are 2 SEM

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