

Carbon Isotope Discrimination and Gas Exchange in *Coffea arabica* during Adjustment to Different Soil Moisture Regimes*

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Abstract

Although carbon isotope discrimination (Δ) has been reported to decline in plants growing under reduced soil moisture, there is little information available concerning the dynamics of adjustments in Δ and gas exchange following a change in soil water availability. In this study Δ , photosynthetic gas exchange, and growth were monitored in container-grown coffee (*Coffea arabica* L.) plants for 120 days under three soil moisture regimes. At the end of 120 d, total leaf area of plants irrigated twice weekly was one half that of plants irrigated twice daily, although their assimilation rates on a unit leaf area basis were nearly equal throughout the experiment. This suggested that maintenance of nearly constant photosynthetic characteristics on a unit leaf area basis through maintenance of a smaller total leaf area may constitute a major mode of adjustment to reduced soil moisture availability in coffee. Intrinsic water-use efficiency (WUE) predicted from foliar Δ values was highest in plants irrigated weekly, intermediate in plants irrigated twice weekly and lowest in plants irrigated twice daily. When instantaneous WUE was estimated from independent measurements of total transpiration per plant and assimilation on a unit leaf area basis, the reverse ranking was obtained. The lack of correspondence between intrinsic and instantaneous WUE was attributed to adjustments in canopy morphology and leaf size in the plants grown under reduced water supply which enhanced transpiration relative to assimilation. Values of Δ predicted from the ratio of intercellular to ambient CO₂ partial pressure determined during gas exchange measurements were not always consistent with measured foliar Δ . This may have resulted from a patchy distribution of stomatal apertures in plants irrigated weekly and from a lag period between adjustment in gas exchange and subsequent alteration in Δ of expanding leaves. The importance of considering temporal and spatial scales, and previous growth and environmental histories in comparing current single leaf gas exchange behaviour with foliar Δ values is discussed.

Introduction

Discrimination (Δ) against the naturally occurring stable isotope ¹³C occurs during photosynthetic CO₂ fixation in plants. This discrimination has a biochemical component and a diffusional component, which together cause the relative ratio of ¹³C/¹²C (δ^{13} C) in plant tissue to be lower than that in the bulk atmospheric pool of CO₂ (Farquhar *et al.* 1989). In C₃ plants, the magnitude of Δ and the resulting carbon isotope composition of leaf tissue are determined largely by the ratio of intercellular to atmospheric partial pressure of CO₂ (p_i/p_a) that prevails when the tissue carbon is assimilated (Farquhar *et al.* 1982). The ratio p_i/p_a is also directly related to the ratio of the instantaneous rates of CO₂ assimilation and stomatal conductance, a measure of intrinsic water-use efficiency (WUE).

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The latter relationship has been exploited to establish correlations between genotypic variation in Δ and both WUE and yield for several crop (Farquhar and Richards 1984; Hubick *et al.* 1986; Martin and Thorstenson 1988; Hubick and Farquhar 1989; White *et al.* 1990; Meinzer *et al.* 1991) and native species (Ehleringer *et al.* 1985, 1987; Goldstein *et al.* 1988; Johnson *et al.* 1990). Foliar Δ values have also been used as an integrated measure of responses of photosynthetic gas exchange to changes in external environmental variables such as water availability (Hubick *et al.* 1988; Meinzer *et al.* 1990a), salinity (Farquhar *et al.* 1982; Guy *et al.* 1986) and irradiance (Zimmerman and Ehleringer 1990).

It is important to recognise that Δ reflects an intrinsic or potential WUE rather than the ratio of the actual fluxes of CO₂ and water vapour at the leaf level. The problems involved in scaling up from Δ measurements at the plant level to WUE at the canopy level have been outlined by Farquhar *et al.* (1988, 1989). Scaling from foliar Δ values to instantaneous WUE for isolated individuals of the same species growing in pots or in the field has been considered to be less problematic. Nevertheless, variations in canopy morphology, leaf size and orientation, and in stomatal conductance (g), may affect leaf energy balance and therefore the leaf-to-air vapour pressure gradient. This in turn may alter the actual flux of water vapour corresponding to a given value of Δ . Thus, even in genotypes of the same species growing in the same environment, the relative ranking with respect to Δ may not reflect the actual ranking in terms of instantaneous WUE.

Plants growing under reduced soil moisture are known to produce leaves with lower Δ values (Martin and Thorstenson 1988; Hubick and Farquhar 1989; Hall *et al.* 1990; Meinzer *et al.* 1990a). However, there is little information available concerning the dynamics of adjustment in Δ following a change in water availability or other environmental variables. A conventional approach used in many studies has been to relate spot measurements of current gas exchange to Δ values of leaves whose carbon was assimilated during an unknown period prior to the gas exchange measurements. Evergreen woody species having leaves with relatively long lifespans present a special problem in relating current physiological performance to leaf Δ values. The carbon isotope composition of leaf cellulose will reflect the combined influence of predictable phenological rhythms in gas exchange characteristics and of unpredictable seasonal variations in environmental variables such as moisture availability. The current behaviour of a leaf may thus bear little resemblance to the behaviour of the rest of the plant during the period in which the leaf's cellulosic carbon was assimilated.

Members of the genus *Coffea* evolved as understorey shrubs in Ethiopian forests at elevations between 1700 and 2000 m. Coffee exhibits features typical of shade-adapted plants. Its maximum photosynthetic rates are low and photosynthesis saturates at low photon flux densities (Kumar and Tieszen 1980). In its native habitats annual rainfall is 1500–2000 mm and exhibits a pronounced seasonal distribution in which several consecutive months are nearly rainless. This probably has contributed to the substantial drought resistance shown by coffee (Meinzer *et al.* 1990b) and to its requirement for a period of reduced water availability to trigger phenological events such as floral bud release (Alvim 1960; Crisosto *et al.* 1992). In a previous study we described patterns of carbon isotope discrimination and gas exchange in diverse coffee cultivars following 1 month of progressive soil drying (Meinzer *et al.* 1990a). The objectives of the present study were to use a series of constant soil moisture regimes to characterise the longer-term dynamics of adjustments in carbon isotope discrimination, gas exchange and growth of coffee to reduced water availability, and to examine the relationship between instantaneous WUE and inherent WUE derived from Δ values. The resulting information permitted conclusions to be drawn concerning appropriate uses of carbon isotope discrimination and gas exchange measurements to assess integrated and current responses to a change in soil water supply in an evergreen woody species having leaves with a relatively long lifespan.

Materials and Methods

Plant Material and Growing Conditions

Coffee plants (*Coffea arabica* L.; cv. 'Guatemalan') were established from seed in 11 L pots in a mixture of soil, potting mix, and volcanic cinders (2:1:1 by volume) and were maintained in a screenhouse on the island of Oahu, Hawaii (lat. 21°21'N, long. 155°02'W, elev. 100 m). The walls of the screenhouse consisted of relatively coarse mesh screen which permitted free exchange of air with the external environment. A transparent plastic roof permitted maximum values of photosynthetic photon flux density (PPFD) inside the screenhouse to attain values >75% of those under full sunlight. Plants were irrigated automatically for 5 min twice daily through drip emitters installed in each container. The amount of water applied was sufficient to cause the pots to drain freely after each irrigation. The plants were approximately 1.2 m tall at the beginning of the experiment. On 1 March 1990 the irrigation frequency for five of the plants was decreased to 10 min twice weekly (2W) and to 10 min weekly (W) for another five plants. The remaining 5 plants continued to receive irrigation twice daily (2D). All pots were fertilised monthly with 16-16-16 (N-P-K) slow release pellets throughout the entire experiment. When the experiment was terminated at 120 d, foliar N content was 2.6, 3.2, and 3.8% in the 2D, 2W, and W plants, respectively. These values were within the range considered to be adequate for cultivated coffee (Mitchell 1988).

Growth Characteristics

At the beginning of the experiment the number of leaves and visible nodes were counted on two plagiotropic and two orthotropic branches per individual. Leaves and nodes on these branches were counted at approximately 14 d intervals during 120 d following imposition of the 2W and W irrigation regimes. In addition the youngest fully expanded pair of leaves was periodically removed from one unmarked branch on each individual for determination of its area, dry weight and leaf mass per area. These leaves were subsequently used for the stable carbon isotope analyses described below. At each sampling time the node to which the youngest pair of fully expanded leaves was attached was designated as node 1. Successively older nodes were designated by increasing node number. Additional leaf samples were excised from nodes 1, 3, 5 and 7 at 0 and 120 d for determination of area, dry weight, leaf mass per area and carbon isotope composition. All of the plants were completely defoliated at the end of the experiment and the total leaf area per plant was determined with an area meter (model 3100; LI-COR, Inc., Lincoln, NE, USA).

Carbon Isotope Discrimination

Stable carbon isotope composition of the youngest fully expanded leaves of each plant was determined at 0, 30, 60, 90 and 120 d after imposition of the 2W and W irrigation regimes. Leaves were oven-dried at 70°C, and finely ground. Subsamples were sent to the Stable Isotope Laboratory at Boston University where they were combusted and the relative abundance of ¹³C and ¹²C in the CO₂ produced was analysed by mass spectrometry. Carbon isotope composition was expressed as the ¹³C/¹²C ratio relative to that of the PeeDee belemnite standard with an error of less than 0.1‰ between repeated analyses of subsamples from the same leaf pair. The resulting δ¹³C values were used to calculate isotopic discrimination as

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p), \quad (1)$$

where δ_p is the isotopic composition of the plant material and δ_a is the isotopic composition of the air (Farquhar *et al.* 1982). Since the plants were maintained in a freely ventilated screenhouse, the δ¹³C value of the air was taken as -8‰, the value at Mauna Loa, Hawaii (Anonymous 1984).

Gas Exchange

Assimilation (*A*) and stomatal conductance (*g*) of fully expanded leaves attached at nodes 1 through 3 were determined with a portable photosynthetic gas exchange system (LI-6200; LI-COR, Inc., Lincoln, NE). Gas exchange was measured on a total of 19 days during the experiment with intervals between days ranging from 1 to 15 d. Two leaves per plant were selected, resulting in a sample size of 10 leaves per soil moisture regime on a given day. At least three measurements were made on each leaf between 0900 and 1500 hours, when light levels were typically above saturation for photosynthesis. On selected days, the daily course of gas exchange was characterised in more detail by performing six to eight sets

of measurements. Leaf chamber CO₂ concentration was allowed to return to the ambient level between measurements. The ratio of intercellular to ambient CO₂ partial pressure (p_i/p_a) was calculated using the corrections described by von Caemmerer and Farquhar (1981). These estimates of p_i/p_a were used to predict Δ from the equation

$$\Delta = a + (b - a)(p_i/p_a), \quad (2)$$

where a is the discrimination resulting from slower diffusion of ¹³CO₂ than ¹²CO₂ in air (4.4‰), and b is the discrimination associated with carboxylation by Rubisco ($\approx 27\%$) (Farquhar and Richards 1984).

Total transpiration (E) per plant was measured as mass flow through the stem using a heat balance method as described by Baker and Van Bavel (1987). Briefly, sap flow gauges (Dynamax Inc., Houston, TX) consisting of a heating element, a thermopile and individual thermocouples were attached near the bases of the stems below the first branch. The heating elements were operated at constant power and the radial and vertical heat fluxes were evaluated with the thermopile and thermocouples, respectively. A datalogger (CR21X, Campbell Scientific Corp., Logan, UT) equipped with a 32-channel multiplexer (AM32, Campbell Scientific) permitted eight gauges to be operated simultaneously.

Root Hydraulic Conductance

After 120 d the plants were decapitated at approximately 20 cm above soil level. The upper portion was saved for determination of total leaf area as described above. The lower portion consisting of the intact root system growing in its original container was sealed inside a large pressure vessel with the stump protruding. The root system was pressurised with compressed air at a constant pressure of 0.7 MPa and the xylem exudate was collected and weighed at regular intervals to determine flow rate. Steady state flow rates were used to calculate total root system hydraulic conductance ($\text{mmol plant}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) from the ratio of flow to pressure applied. All plants were thoroughly irrigated the day prior to hydraulic conductance determinations.

Results

Growth characteristics of coffee plants differed markedly under the three soil moisture regimes imposed (Fig. 1). After the first 15 d, during which no new nodes were observed, the rate of node production of all plants increased and remained relatively constant throughout the rest of the experiment (Fig. 1A). After 109 d the W plants had produced approximately half as many new nodes as the 2D plants. The number of leaves per branch increased linearly with time in the 2D plants after 15 d (Fig. 1B). Increased rates of leaf senescence and abscission in the 2W and W plants prevented the total number of leaves per branch from increasing linearly with time. In addition to producing fewer leaves than the 2D plants, the 2W and W plants also produced substantially smaller leaves (Fig. 1C). These adjustments in leaf number and leaf size resulted in a fourfold difference in total leaf area between the 2D and W plants at the end of the experiment.

Carbon isotope discrimination in leaves decreased under all soil moisture regimes during the experiment (Fig. 2). At 120 d the magnitude of the decline in Δ ranged from approximately 1‰ in the 2D plants to 4.5‰ in the W plants. The decline in Δ was nearly linear in the 2W and W plants throughout the entire experiment, indicating that more than 120 d were required for adjustment in Δ to be completed. The magnitude of the decline in Δ under each soil moisture regime was correlated ($P < 0.05$) with the initial value of Δ at 0 d when all plants were still being irrigated twice daily (Fig. 3). The range in initial values of Δ was c. 3.2‰ among the 15 individuals studied. This was an estimate of genotypic variation in Δ , since all individuals had been maintained under the same irrigation regime since planting.

The steady production of new leaves with progressively lower Δ values (Fig. 2) resulted in a pattern of sharply increasing Δ with increasing leaf age when leaves from different positions along the branch were sampled at 120 d (Fig. 4). At 0 d there was a 0.7‰ variation in Δ among leaves at the four positions sampled even though the plants had previously been maintained under constant irrigation regime (Fig. 4).

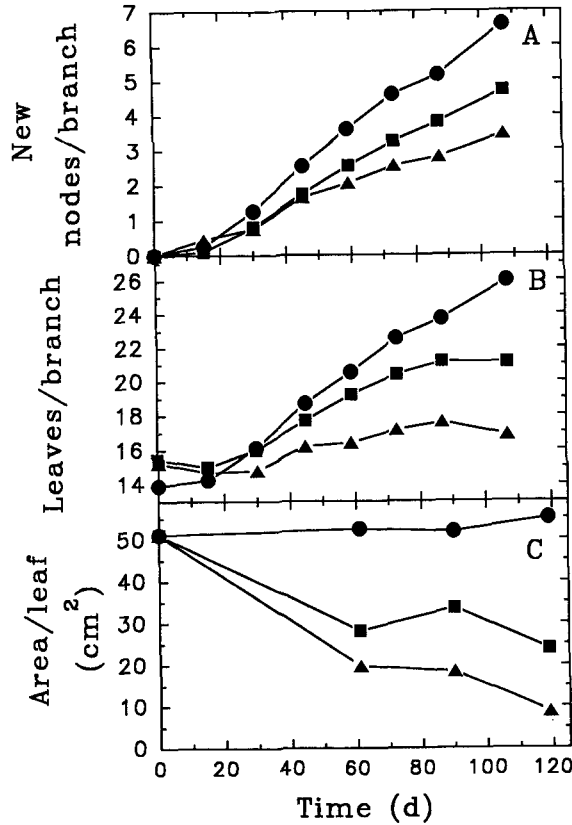


Fig. 1. Growth characteristics of coffee plants irrigated twice daily (●), twice weekly (■), and weekly (▲). The weekly and twice weekly irrigation regimes were imposed at 0 d. All plants were previously irrigated twice daily. (A) Average number of new nodes per branch. (B) Average number of new leaves per branch. (C) Area of the youngest fully expanded leaf.

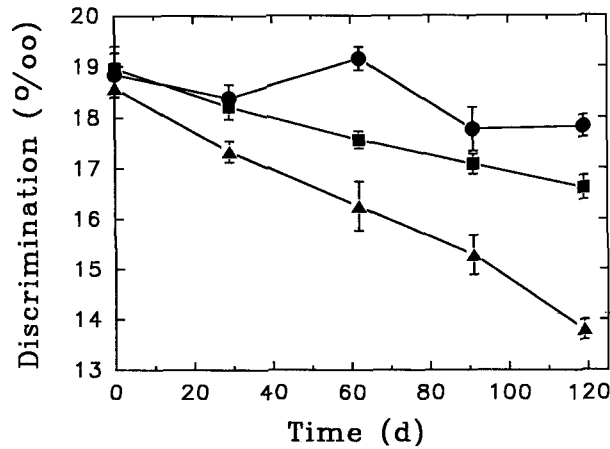


Fig. 2. Carbon isotope discrimination in the youngest fully expanded leaf of coffee plants growing under three different moisture regimes. Symbols and treatments as in Fig. 1. All plants were subjected to the same irrigation regime until 0 d. Points are means (\pm s.e.) of determinations from five plants.

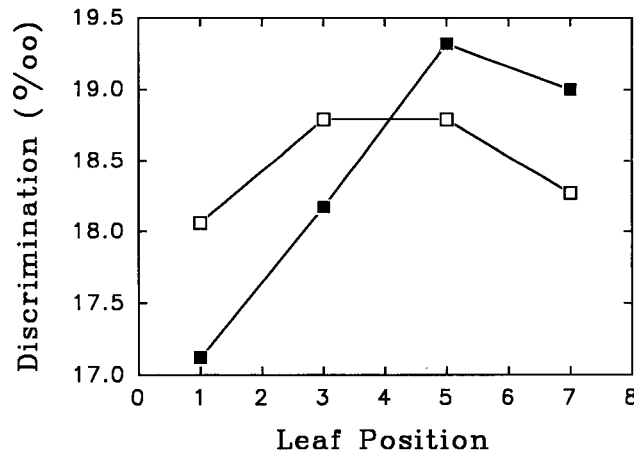
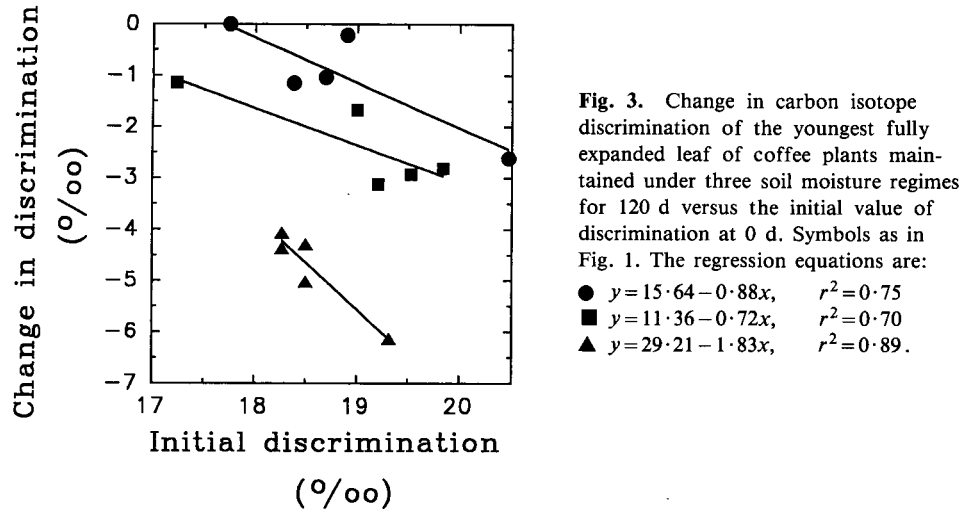


Fig. 4. Carbon isotope discrimination in leaves in relations to leaf age and position on plagiotropic branches. Node 1 is the point of attachment for the youngest fully expanded pair of leaves. Increasing node numbers represent successively older pairs of leaves. Samples were taken immediately before (□) and 120 d after (■) decreasing irrigation frequency from twice daily to twice weekly.

There was a significant negative correlation ($P < 0.001$) between Δ of the youngest fully expanded leaf and leaf mass per area (Fig. 5A). Leaf mass per area (LMA) was highest in the W plants and not significantly different among the 2D and 2W plants. In apparent contrast with the pattern shown in Fig. 5A, Δ increased with LMA ($P < 0.005$) in leaves sampled from positions 1, 3, 5, and 7 at 120 d (Fig. 5B). This pattern was attributed to the combined effect of LMA continuing to increase with leaf age beyond the fully expanded stage and to the decline in Δ associated with decreasing leaf age (Fig. 4).

The patterns of Δ observed during adjustment to reduced soil moisture availability (Fig. 2) indicated that the prevailing p_i/p_a in leaves of the 2W and W plants decreased continuously throughout the experiment. Average values of p_i/p_a determined from gas

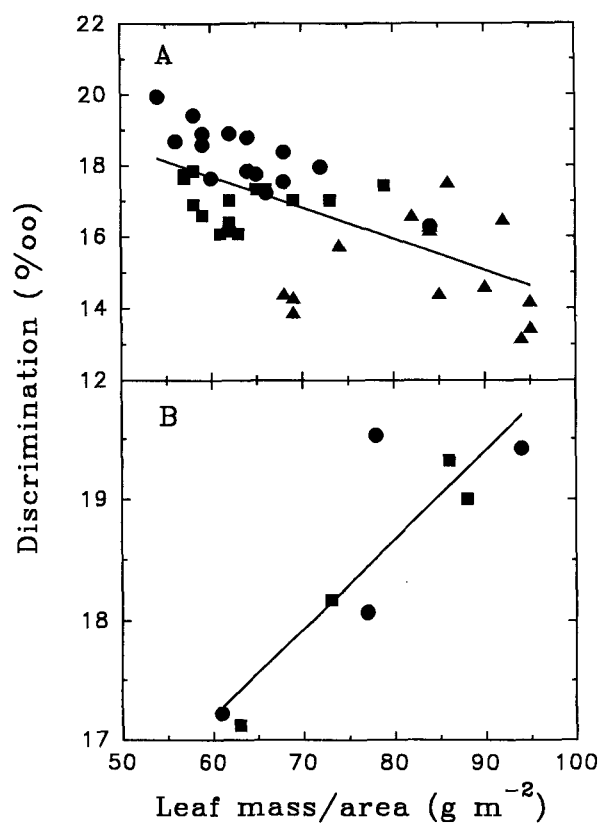


Fig. 5. Relationship between carbon isotope discrimination in leaves and leaf mass per area. Symbols as in Fig. 1. (A) Youngest fully expanded leaf sampled at 61, 90, and 120 d after imposing new soil moisture regimes. (B) Leaves sampled from nodes 1, 3, 5, and 7 at 120 d. Symbols as in Fig. 1. The regression equations are:

$$A. y = 22.81 - 0.09x, \quad r^2 = 0.42$$

$$B. y = 12.76 + 0.07x, \quad r^2 = 0.77.$$

Table 1. Comparison of predicted Δ with measured Δ in leaves of coffee plants growing under three soil moisture regimes

Predicted Δ was calculated from equation (2) using p_i/p_a determined between 60 and 90 d after decreasing irrigation frequency from twice daily to twice weekly or weekly. Measured Δ is the average of leaf samples obtained at 90 and 120 d after decreasing irrigation frequency

Irrigation frequency	p_i/p_a	Δ (‰)	
		Predicted	Measured
Twice daily	0.58	17.5	17.8
Twice weekly	0.54	16.6	16.8
Weekly	0.52	16.2	14.4

exchange measurements between 60 and 90 d after imposition of the 2W and W soil moisture regimes were used to calculate Δ from equation (2). Calculated values of Δ for the 2D and 2W plants compared favourably with those derived directly from isotopic analysis of leaf tissue (Table 1). When equation (2) was applied to p_i/p_a measurements obtained from the W plants, Δ was overestimated by 1.8%.

The relative role of stomatal and non-stomatal factors in regulating p_i/p_a was assessed by analysing patterns of A and g obtained from instantaneous gas exchange measurements. Maximum values of A and g and the relationship between them were found to vary both with a change in soil moisture regime and with time elapsed since the beginning of the experiment independently of a change in soil moisture regime (Fig. 6). Near the beginning of the experiment, between 6 and 14 d, the highest values of A and g observed were c. $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $0.08 \text{ mol m}^{-2} \text{s}^{-1}$, respectively (Fig. 6A) and a single, nearly linear function could be used to describe the relationship between A and g for plants growing under all moisture regimes. Approximately 30 d later maximum values of A and g had risen to $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $0.14 \text{ mol m}^{-2} \text{s}^{-1}$, respectively (Fig. 6B). A single curve described the relationship between A and g for all plants. Near the end of the experiment, two functions were required to describe the relationship between A and g because A frequently appeared to be nearly constant and independent of g in the 2D plants (Fig. 6C).

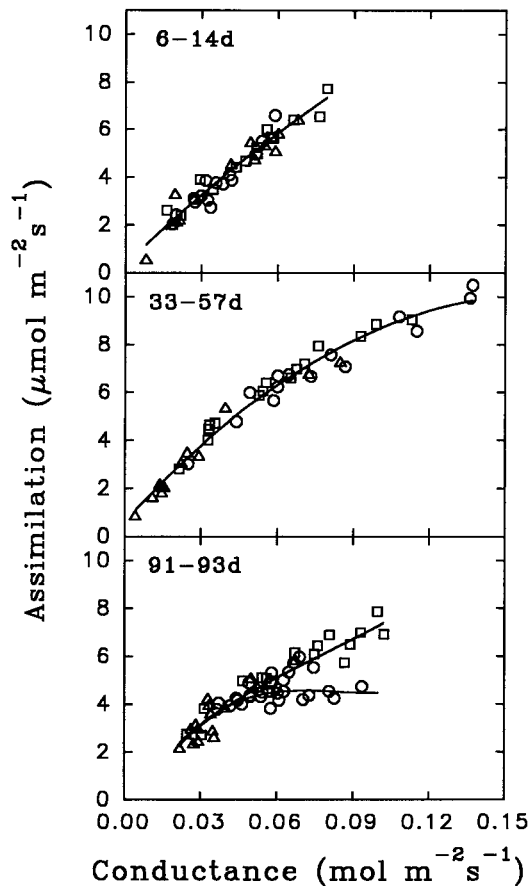


Fig. 6. Relationship between assimilation and stomatal conductance for coffee plants at different time intervals after imposing new irrigation regimes. Points are the two highest values measured for each plant on each of three days during the periods indicated.

- Irrigation twice daily
- Irrigation twice weekly
- △ Irrigation weekly.

The patterns of A and g in Figs 6B and 6C suggested that at least two mechanisms contributed to the reduced p_i/p_a and Δ observed in the 2W and W plants. When the relationship between A and g is curvilinear as in Fig. 6B, stomatal restriction of gas exchange to the steeper portion of the curve will increase A/g and reduce p_i/p_a . This was observed in the W plants and to a lesser extent in the 2W plants. If the relationship between A and g is linear with a zero intercept, p_i/p_a and Δ will remain constant as g varies. A second mechanism leading to a reduction in p_i/p_a and Δ is a higher photosynthetic capacity at a given level of g , which was observed in the 2W plants near the end of the experiment (Fig. 6C). Average values of A and g in the 2D and 2W plants determined between 60 and 90 d suggest that the latter mechanism predominated in causing reduced Δ in the 2W plants (Table 2). Assimilation rates were nearly equal in the two groups of plants while g was 25% lower in the 2W plants.

Table 2. Total final leaf area and gas exchange characteristics of coffee plants growing under three different soil moisture regimes for 120 d

Average daily values (\pm s.e.) of assimilation (A), stomatal conductance (g), transpiration (E) and instantaneous water use efficiency (A/E) are shown. Number of replicates is 5 for leaf area, 30 for A and g , and 12 for E . E was determined as sap flow using a heat balance method as described in the text

Irrigation frequency	Leaf area (m ²)	A (μ mol m ⁻² s ⁻¹)	g (mol m ⁻² s ⁻¹)	E (mmol m ⁻² s ⁻¹)	A/E
Twice daily	2.24 \pm 0.08	4.50 \pm 0.25	0.060 \pm 0.004	0.43 \pm 0.01	10.5
Twice weekly	1.25 \pm 0.07	4.22 \pm 0.28	0.045 \pm 0.004	0.70 \pm 0.05	6.5
Weekly	0.53 \pm 0.06	2.22 \pm 0.27	0.023 \pm 0.003	0.89 \pm 0.13	2.5

The patterns of gas exchange and of Δ observed (Table 1; Fig. 2) indicated that inherent water-use efficiency (WUE) was highest in the W plants and lowest in the 2D plants. To determine whether this ranking according to inherent or potential WUE corresponded to that of actual instantaneous WUE (A/E), independent measurements of transpiration on a whole-plant basis were compared with single leaf measurements of A scaled up to an entire plant basis using total leaf area. Total E per plant was nearly identical in the 2D and 2W plants (Fig. 7), even though total leaf area of the 2D plants was nearly twice that of the 2W plants (Table 2). Total E of the W plants was also disproportionately high in relation to their total leaf area (cf. Fig. 7, Table 2). Transpiration rates on a unit leaf area basis were thus highest in the W plants and lowest in the 2D plants (Table 2), which was the reverse of their ranking according to g measured in single leaves (Table 2). Scaling of A from a unit leaf area basis to an entire plant basis by multiplying A by total leaf area indicated that total A per plant was highest in the 2D plants and lowest in the W plants (Fig. 7). In this procedure for scaling A it was assumed that A was the same for all leaves on a given individual. The ratio of A and E , an estimate of instantaneous WUE, was highest in the 2D plants and lowest in the W plants (Fig. 7). The relationship between actual WUE and soil moisture regime was thus the inverse of that between soil moisture and inherent WUE derived from leaf Δ measurements.

The relationship between inherent WUE and the relative ability of the root system to supply the shoot with water when coffee plants were grown under different soil moisture regimes was evaluated by plotting Δ against root hydraulic conductance (Fig. 8). Carbon isotope discrimination was largely independent of root hydraulic conductance when soil moisture was abundant, but declined sharply over the range of root hydraulic conductance associated with the soil moisture regimes experienced by the 2W and W plants. Root hydraulic conductance as defined here may have been largely a function of the size of the root system which was probably smaller in plants grown under reduced soil moisture

availability, since final leaf area of these plants was smaller (Table 2). It was thus not possible to determine whether Δ and root hydraulic conductance were mechanistically linked with each other or whether both responded directly to soil water status.

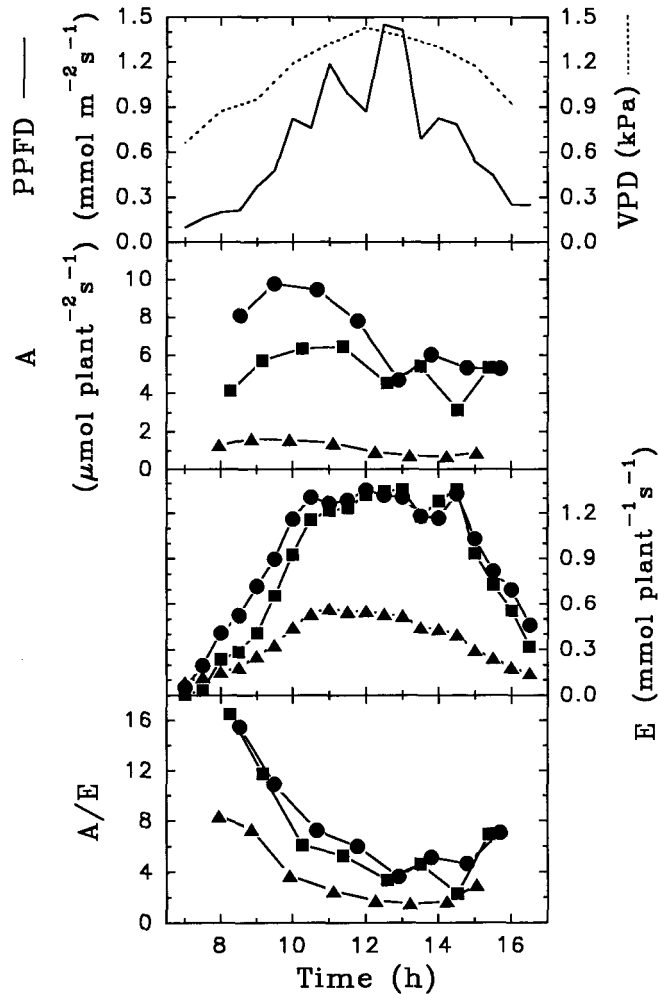


Fig. 7. Daily course of photosynthetic photon flux density (PPFD), vapour pressure deficit (VPD), assimilation (A) and transpiration (E) on a per plant basis, and instantaneous water use efficiency (A/E) at 93 d after imposing new irrigation regimes. Symbols as in Fig. 1.

Discussion

Carbon isotope discrimination, gas exchange and growth of coffee plants exhibited continuous adjustments during a 120 d period following a change in the soil moisture regime. Even in plants maintained under a constant irrigation regime, adjustments in Δ , gas exchange and growth that were apparently phenological in origin were observed. At the end of 120 d, the total leaf area of plants irrigated twice weekly was one-half that of plants irrigated twice daily although their assimilation rates per unit leaf area had been maintained nearly equal throughout the experiment (Table 2). This suggests that a major mode of

adjustment to reduced soil moisture availability in coffee is maintenance of nearly constant photosynthetic activity on a unit leaf area basis through a reduction in the rate of increase in total leaf area per plant. In long lived woody species, homeostasis of properties on a unit leaf area basis through reduced rates of leaf expansion may be a common form of adjustment to limited availability of water, nutrients and other resources (Pereira 1990). Interpretation of short-term single-leaf gas exchange measurements in field-grown individuals of species exhibiting this form of phenotypic adjustment to resource limitation may be problematic if their total leaf area is not taken into account. Even if taken into account, size differences among individuals with similar single leaf gas exchange rates could not be correctly attributed to differences in age or in resource availability unless additional information were available.

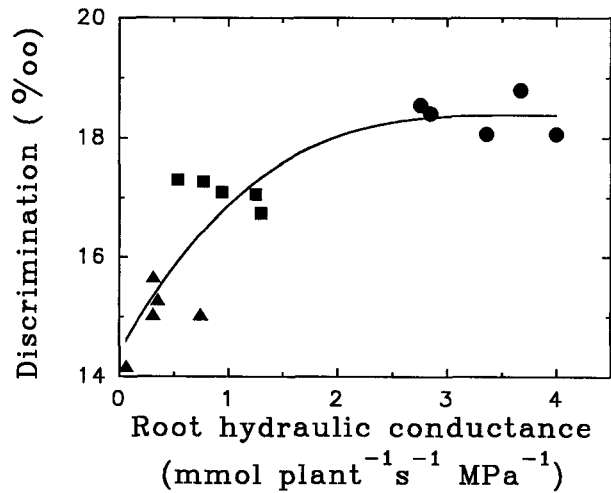


Fig. 8. Relationship between carbon isotope discrimination in leaves and root hydraulic conductance. Values of discrimination are averages of samples collected at 60, 90, and 120 d after imposing new irrigation regimes. Symbols as in Fig. 1.

The positive correlation between foliar Δ values at 0 d and the magnitude of adjustment in Δ after 120 d (Fig. 3) was probably a manifestation of the positive correlation between Δ and g previously reported for coffee (Meinzer *et al.* 1990a). The results obtained in the present study support our previous proposal that coffee genotypes with higher foliar Δ values when water is non-limiting become water-limited more rapidly under reduced water supply because of their higher values of g and therefore higher rates of water use. Positive correlations between Δ and g (Condon *et al.* 1987; DeLucia *et al.* 1988; Ehleringer 1990) may reflect an underlying correlation between gas exchange and root growth (White *et al.* 1990).

Foliar Δ values indicated that intrinsic WUE was highest in coffee plants irrigated weekly, intermediate in plants irrigated twice weekly, and lowest in plants irrigated twice daily (Table 1; Fig. 2). However, the reverse ranking was obtained when instantaneous WUE was calculated from independent measurements of whole-plant transpiration rates and of A on a unit leaf area basis scaled to an entire plant basis using the total leaf area (Fig. 7; Table 2). This pattern contrasts with that observed by Wright *et al.* (1988), who reported the expected negative correlation between WUE and foliar Δ for field-grown peanut genotypes. The apparent positive correlation between Δ and instantaneous WUE of coffee plants grown under different soil moisture regimes may have been attributable to adjustments

in canopy morphology and leaf size which enhanced E relative to A . Visual observations indicated that, owing to large differences in total leaf area, the extent of self-shading in the canopies of plants irrigated twice daily was much greater than in either plants irrigated twice weekly or weekly. In coffee, a species native to shaded habitats, an increase in prevailing irradiance per unit leaf area would probably increase E more than A . Kumar and Tieszen (1980) observed saturation of A at a PPFD value of approximately $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ in sun-grown coffee plants. Similarly, in the present study there was little evidence of increases in A with increasing PPFD above $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 7). An increase in prevailing irradiance would thus be expected to increase leaf temperature and therefore the leaf to air vapour pressure gradient without increasing A substantially. Increased leaf temperature associated with increased irradiance may also have inhibited A over the range of temperatures observed in the present study (30–35°C; Kumar and Tieszen 1980). Reduced leaf size with declining soil moisture availability (Fig. 1) would have increased coupling between leaves and the environment by decreasing the boundary layer resistance (Jarvis and McNaughton 1986). Boundary layer resistance may also have been reduced by increased wind penetration through the canopies of plants grown under reduced water supply. This would be expected to enhance E more than A because the relative change in the resistance of the CO_2 diffusion pathway would be smaller. A potential uncertainty in our estimates of instantaneous WUE was that actual fluxes of CO_2 in entire plants were determined by scaling up from measurements of individual leaves enclosed in a cuvette while fluxes of water vapour were determined as sap flow in unenclosed plants. However, use of a leaf chamber should have had a much smaller influence on scaling of A than on scaling of E because of the distribution of resistances in the pathways for diffusion of CO_2 and water vapour. In addition the close agreement between predicted and measured foliar Δ values for plants irrigated twice daily and twice weekly (Table 1) suggested that the influence of the boundary layer on p_i/p_a was similar in the cuvette and in unencumbered leaves.

Values of Δ predicted from gas exchange measurements using equation (2) were not always consistent with measured foliar Δ values. In coffee plants irrigated weekly, Δ predicted from gas exchange measurements was substantially higher than measured Δ (Table 1). This may have resulted from a patchy distribution of stomatal apertures in these plants. Stomatal patchiness, characterised by groups of uniformly open stomata and groups of closed stomata, would cause p_i/p_a to be overestimated in conventional gas exchange measurements (Farquhar *et al.* 1987; Downton *et al.* 1988). However, foliar Δ values would still reflect the assimilation-weighted average of prevailing p_i/p_a . Stomatal patchiness is most frequently observed in leaves of plants experiencing relatively severe water deficits. Another factor influencing the relationship between predicted and measured Δ in coffee was the lag period for alterations in foliar Δ values of expanding leaves and the stability of Δ in pre-existing leaves following phenological and water deficit-induced adjustments in gas exchange. This resulted in pronounced variation in Δ in leaves at different nodes along a branch (Fig. 4), even when their current gas exchange behaviour was similar. In an earlier study with droughted coffee plants (Meinzer *et al.* 1990a), good agreement between predicted and measured Δ was obtained because Δ was measured in expanding leaves containing carbon assimilated by older leaves in which gas exchange was measured. Vitousek *et al.* (1990) concluded that lack of correspondence between measured $\delta^{13}\text{C}$ values in *Metrosideros polymorpha* and $\delta^{13}\text{C}$ predicted from gas exchange was attributable to internal resistance associated with high LMA. However, we believe that the explanations offered above are sufficient to account for the patterns of actual and predicted Δ in coffee.

Conclusions

In comparing measurements of current gas exchange with foliar Δ values in species having leaves with relatively long lifespans, consideration should be given to the conditions that prevailed when a particular leaf's cellulosic carbon was actually assimilated and to the time

scales governing adjustments in p_i/p_a and subsequent changes in foliar Δ values. Similar considerations apply to measurements at different spatial scales. Spot measurements of gas exchange in single leaves may not reveal differences in total carbon assimilation at the whole plant level resulting from variations in plant size. The causes of size-related differences in total plant carbon assimilation cannot be correctly partitioned between age and environmental influences unless something is known of a plant's previous growth and environmental history.

Even for genetically similar individuals of the same species growing in the same habitat, foliar Δ values represent only intrinsic WUE or the potential ratio of A and E rather than the actual ratio of fluxes. Adjustments in leaf and canopy characteristics may cause realised instantaneous WUE to be different from that predicted with Δ values. Regardless of the relationship between Δ and instantaneous WUE, there is increasing evidence from coffee and other species (Küppers 1984; White *et al.* 1990) that intrinsic WUE is correlated with the hydraulic capacity of the roots and soil to supply the leaves with water.

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